RECHERCHE DES DÉTERMINANTS DE LA SPÉCIFICITÉ PARASITAIRE DANS LE MODÈLE *LAMELLODISCUS* (DIPLECTANIDAE, MONOGENEA)-SPARIDAE (TELEOSTEI) EN MÉDITERRANÉE

par

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présentée et soutenue à l'Université de Perpignan par

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RÉSUMÉ

L'objectif de cette thèse était de mieux comprendre ce qui contrôle la spécificité parasitaire dans le système hôte-parasite constitué par les poissons de la famille des Sparidae et leurs monogènes (Plathelminthes ectoparasites) spécifiques du genre *Lamellodiscus*. En d'autres termes, il s'agissait de comprendre les causes amenant une espèce parasite à utiliser une seule ou plusieurs espèces hôtes. On suppose que la spécificité est soumise à des contraintes écologiques et évolutives. L'hypothèse d'une augmentation de la diversité taxonomique avec la spécificité a également été testée. Cela est basé sur l'idée que les espèces généralistes, plus tolérantes à leur environnement, sont supposées être moins sujettes à subir des spéciations après un changement d'hôte, et ainsi être moins diversifiées que les espèces spécialistes. Comme l'histoire évolutive des parasites peut être influencée par celle de leurs hôtes à travers des phénomènes de coévolution, il était nécessaire d'obtenir une phylogénie des hôtes et des parasites. L'étude de la coévolution hôte-parasite dans ce système avait pour but de déterminer si le profil d'association hôte-parasite (et donc la spécificité) est contrôlé par des interactions coévolutives. Des phylogénies ont été élaborées pour les hôtes et les parasites à partir de données moléculaires obtenues par séquençage d'ADN. Cette analyse moléculaire a permis de reconsidérer le statut taxonomique de plusieurs espèces de monogènes : sur la base des séquences obtenues, *Lamellodiscus virgula* et *L. obeliae* s'avèrent être une seule espèce (*L. virgula*), alors que *Furnestinia echeneis* fait partie du genre *Lamellodiscus*. Plusieurs méthodes d'étude de la coévolution ont été utilisées dans ce travail. L'une d'elles, ParaFit, a été mise au point pendant la thèse. Toutes les méthodes indiquent que ce système complexe ne semble pratiquement pas être soumis à des phénomènes de cospéciation. Aucun lien entre la diversification taxonomique et la spécificité n'a pu être mis en évidence chez les *Lamellodiscus* et la famille qui les contient, les Diplectanidae. Par contre, un tel lien a été mis en évidence au niveau des groupes principaux de parasites. Les déterminants écologiques et phylogénétiques de la spécificité ont ensuite été recherchés à l'aide d'analyses statistiques multivariables. Les variables considérées étaient des caractéristiques des hôtes considérées comme des déterminants écologiques potentiels de la spécificité. La phylogénie des parasites a été prise en compte dans ces analyses à l'aide de méthodes

comparatives, comme la méthode des contrastes indépendants. La spécificité apparaît être fortement contrainte par la phylogénie, ce qui suggére l'existence de déterminants génétiquement transmissibles. Les analyses révèlent également que les parasites spécialistes ont tendance à utiliser les hôtes les plus grands. Cela est interprété comme une spécialisation sur une ressource prédictible.

Mots-clés: Spécificité, Monogène, Poisson, Coévolution, Phylogénie moléculaire, *Lamellodiscus*, Sparidae, Analyse comparative, Diversification taxonomique

ABSTRACT

The objective of this thesis was to obtain a better understanding of the factors controlling host specificity in the host-parasite system formed by fish from the family Sparidae and their specific monogeneans parasites from the genus *Lamellodiscus* (Platyhelminthes). In other words, the goal was to understand the factors determining the number of hosts used by a parasite species. We assumed that specificity is under ecological and evolutionary constraints. The hypothesis of an increase of taxonomic diversity with specificity was also tested. It is based on the idea that generalist species are more tolerant to their environment, and therefore less subject to speciation after a host change event, and are then less diversified than specialist species. Since the evolutionary history of parasites can be influenced by the history of their hosts via coevolutionary interactions, it was necessary to obtain phylogenies for the hosts and parasites. The aim of the study of host-parasite coevolution in this system was to assess if the pattern of host-parasite association (and consequently, specificity) was determined by coevolutionary interactions. Phylogenies were obtained for hosts and parasites from the analysis of DNA sequences. This analysis, carried out at the molecular level, led us to reconsider the taxonomic status of several monogenean species. On the basis of the DNA sequences obtained, *Lamellodiscus virgula* and *L. obeliae* appear to form a single species (*L. virgula*), while *Furnestinia echeneis* is transferred to the genus *Lamellodiscus*. Several analytical methods were used to study host-parasite coevolution in this system. Among them, ParaFit was designed during this thesis. All methods agreed that this host-parasite system does not exhibit a general cospeciation pattern. No link between taxonomic diversity and specificity has been found in *Lamellodiscus*, nor in their family, the Diplectanidae. However, such a link was found when the main groups of parasites were considered. Ecological and phylogenetic determinants of specificity were investigated via multivariate statistical methods. The variables included in the analyses were potential host-related ecological determinants of specificity. The parasite phylogeny was taken into account through comparative methods, including the independent contrasts method. Specificity appears to be strongly constrained by the phylogeny, suggesting the existence of genetically transmitted determinants. The

analyses also revealed that *Lamellodiscus* monogeneans tend to specialize on larger hosts. This is interpreted as a specialization on a predictable resource.

Key-Words: Specificity, Monogenean, Fish, Coevolution, Molecular Phylogeny, *Lamellodiscus*, Sparidae, Comparative Analysis, Taxonomic Diversification

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À Lamia.
Je lui dois au moins ça
et bien plus encore.

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I. INTRODUCTION

A. PROBLÉMATIQUE

1. Contexte général

Le contrôle de la spécialisation n'est pas quelque chose de parfaitement clair en écologie. Certains organismes sont inféodés à un type de ressource et/ou d'habitat, alors que d'autres semblent être capables de s'adapter à une grande variété de conditions. La spécialisation est le processus dynamique qui conduit un organisme à la spécificité pour une ressource donnée (voir Futuyma & Moreno, 1988) ; les deux notions étant intimement liées, l'un ou l'autre terme sera utilisé dans ce travail. On pourrait penser que plus un organisme est polyvalent, plus sa "réussite évolutive" devrait être grande. Pourquoi, comme le mentionnent Timms & Read (1999), n'existe-t-il pas d'organisme capable d'exploiter toutes les niches écologiques? La nature regorge d'exemples de spécialisation étroite voire extrême (Rosenzweig, 1995). Le fait d'être spécialiste, comme celui d'être généraliste, a ses avantages et ses inconvénients. Un organisme très spécialisé est totalement dépendant de son unique ressource et est très sensible à ses fluctuations. La disparition de cette ressource entraîne celle du spécialiste. En revanche, on peut supposer que ce dernier est tellement bien adapté à la ressource qu'il peut l'exploiter d'une façon optimale. Le généraliste, lui, a un choix de ressources à sa disposition, ce qui lui évite d'être trop dépendant de l'une d'entre elles, mais il n'exploite chacune que d'une façon imparfaite. C'est une situation typique de compromis ("trade-off"). Mais quelles sont les conditions qui favorisent l'un ou l'autre comportement? Qu'est-ce qui entraîne l'apparition voire le maintien de l'une ou l'autre de ces stratégies?

Plusieurs études ont tenté de répondre à ces questions d'un point de vue théorique, en utilisant la modélisation. À l'aide d'un modèle mathématique simple, Ward (1992) a suggéré que les organismes tendent à se spécialiser pour une ressource prédictible, c'est-à-dire stable dans le temps et l'espace et donc minimisant les risques d'extinction. Templeton et Rothman (1974) ont suggéré que les différences d'habitat doivent être larges par rapport à la tolérance du phénotype pour que la spécialisation soit favorisée, ce qui est aussi souligné par Wilson & Yoshimura (1994) et van Tienderen (1997). Comme le notent Futuyma & Moreno (1988), tous ces modèles sont basés sur la conception qu'il y a un

compromis entre exploiter beaucoup de ressources avec une intensité moyenne (stratégie généraliste) et en exploiter une de façon optimale (stratégie spécialiste), comme par exemple dans le travail de Wilson & Yoshimura (1994). La question qui se pose pourrait être celle-ci : vaut-il mieux faire beaucoup de choses moyennement ou une seule de façon très efficace? Ce concept explique l'apparition de la spécialisation par compétition interspécifique où, dans le cas d'une ressource unique, les génotypes spécialistes plus efficaces peuvent exclure les généralistes (MacArthur & Levins, 1964). Cette notion de compromis a néanmoins été remise en question par Fry (1996). En effet, ces compromis sont rarement observés dans la nature. Les théories et modèles se basent en effet parfois sur des prémisses davantage présumées que vérifiées expérimentalement (voir Cornell et al., 1999). Plusieurs modèles ont suggéré que dans un environnement fluctuant, les stratégies généralistes étaient favorisées car les génotypes devaient être capables de s'adapter à toute une variété de conditions (e.g., Roughgarden, 1972 ; Lynch & Gabriel, 1987 ; Rosenzweig, 1987).

Le rôle de la compétition n'est pas facile à mettre en évidence et la compétition elle-même est difficile à déceler et à mesurer. Parfois, l'abondance de la ressource et le nombre de niches inutilisées a conduit certains auteurs (Rohde, 1979 ; Colwell, 1986) à suggérer que la spécialisation pouvait être due au fait que les populations de faible densité se rassemblaient sur une ressource unique afin de pouvoir se reproduire. Cette hypothèse a été contestée par certains auteurs (Adamson & Caira, 1994) qui estiment que les arguments présentés par Rohde (1979) peuvent s'expliquer sans faire appel à une agrégation à but reproductif.

La compétition interspécifique dépend de la structure de la distribution de la ressource (Begon et al., 1996). Si celle-ci est répartie en taches éloignées les unes des autres, les spécialistes sont favorisés, alors que les conséquences ne sont pas les mêmes si la distribution est plus continue. Cela conduit au problème de la dispersion des organismes, qui peut être vu comme un facteur important du déterminisme de la spécialisation. La restriction de l'habitat (spécialisation) peut s'expliquer par de faibles capacités de dispersion (Timms & Read, 1999).

Smiley (1978) a montré que, pour une association plante-insecte phytophage, l'apparition de la monophagie (i.e. spécialisation) est sans doute davantage due à des facteurs écologiques (prédation ou abondance de l'hôte) qu'à la compatibilité avec l'hôte. C'est seulement ensuite qu'évoluent les adaptations (biochimiques et métaboliques) qui font perdre à l'insecte (qui joue ici le même rôle qu'un parasite) la possibilité d'exploiter d'autres espèces de plantes. En effet, il arrive que les insectes n'utilisent pas certaines espèces de plantes pourtant disponibles et "convenables" du point de vue chimique, ce qui indique que cette compatibilité plante-insecte n'est pas un déterminant, ou du moins n'est pas le seul déterminant (cause) de la spécificité. Cela rejoint l'opinion de Futuyma & Moreno (1988) qui voient les traits morpho-physiologiques plus comme des conséquences que comme des causes de la spécialisation, qui serait davantage le fait d'un changement comportemental. Dans la même optique, Bernays (1998) propose que la spécialisation est favorisée dans le cas d'une réponse rapide du consommateur (parasite, phytophage, ...) au signal de la ressource, ce qui suggère qu'un déterminant essentiel de la spécificité réside dans la biologie comportementale du consommateur, avant ses adaptations morphologiques ou physiologiques. Les contraintes neurales liées au comportement apparaissent dans ce contexte comme une cause importante de la spécialisation.

Futuyma & Moreno (1988) insistent sur le fait que les causes de la spécialisation ne peuvent être pleinement comprises que dans un contexte phylogénétique. Seule une telle approche peut permettre de distinguer la spécialisation due à des contraintes phylogénétiques de celle purement due à des effets écologiques (voir Brooks & McLennan, 1991 ; Harvey & Pagel, 1991).

2. Cas des relations hôte-parasite

Les associations hôte-parasite sont de bons modèles pour l'étude des phénomènes évolutifs en général (Price, 1980 ; De Meeûs et al., 1998 ; Paterson & Banks, 2001) et des déterminants de la spécialisation en particulier. En effet, la niche écologique du parasite est en général beaucoup plus facile à définir que celle des organismes libres : son environnement principal, son habitat et sa nourriture sont représentés par l'hôte. La spécificité dans ce cas est plus facile à définir. Il existe bien sûr des cas de parasitisme un peu différents, comme le parasitisme protélien (seulement à l'état larvaire), ou des cas de

cycles complexes dans lesquels les phases libres sont nombreuses, mais la majorité des parasites sont principalement influencés par leurs hôtes. En outre, il est possible de disposer d'une hypothèse sur l'évolution de l'environnement du parasite à l'aide d'une phylogénie de l'hôte. Les techniques d'optimisation de caractères peuvent même permettre de proposer des hypothèses de traits de vie des hôtes ancestraux (voir Brooks & McLennan, 1991 ; Cunningham et al., 1998). Enfin, dans le cas des associations hôte-parasite, certains facteurs écologiques sont absents, comme la prédation (en général), ce qui permet de limiter le nombre de facteurs à étudier.

a. La spécificité

i. Définition

Je n'aborderai que le cas des relations hôte-parasite, mais cela peut être facilement étendu aux relations plantes-phytophages ou à toutes situations où un organisme peut être spécialiste ou généraliste d'un milieu bien défini.

La spécificité peut s'entendre pour le *site* ou pour l'*hôte* :

- *Site* : Les parasites sont souvent très spécifique pour certaines localisations sur leurs hôtes (voir Adamson & Caira, 1994). Certaines espèces habitent plusieurs espèces d'hôtes, mais se retrouvent toujours dans le même tissu. Sur la base de données moléculaires, Littlewood et al. (1997) ont suggéré, dans le cas des Polystomes (monogènes Polyopisthocotylea), que les espèces qui occupaient les mêmes sites sur des hôtes différents étaient plus proches entre elles que les espèces habitant les mêmes hôtes mais sur des localisations différentes.
- *Hôte* : De nombreuses espèces de parasites ont une gamme d'hôtes réduite (Euzet & Combes, 1980). Les vrais généralistes (i.e. exploitant des hôtes taxonomiquement très différents) sont rares parmi les parasites. On peut ainsi mesurer la spécificité d'un parasite par le nombre d'hôtes qu'il possède (Lymbery, 1989) : moins il possède d'hôtes, plus son degré de spécificité est élevé. Un parasite qui n'utilise qu'un seul hôte est appelé *spécialiste* (e.g., Euzet & Combes, 1980 ; Ludwig, 1982). Par opposition, les parasites utilisant plusieurs hôtes sont dits *généralistes*. Les concepts de généralistes et de spécialistes sont bien sûr relatifs (e.g., Kitahara & Fuji, 1994).

La spécificité d'un parasite reflète son degré d'adaptation à un ou plusieurs hôtes. On considère parfois comme spécialistes les parasites qui n'utilisent qu'un genre, voire une famille d'hôtes ; cela dépend du type d'organisme et du niveau taxonomique de l'étude (Ludwig, 1982). On peut aussi mesurer la spécificité par des indices qui prennent en compte la prévalence et l'intensité de l'infestation parasitaire (Rohde, 1982, 1994).

La spécificité varie dans l'espace et au cours du temps. Suivant les zones ou les périodes considérées, les mêmes espèces d'hôtes ne sont pas toujours parasitées par les mêmes communautés de parasites (voir Norton & Carpenter, 1998).

On peut définir trois échelles de spécificité (en terme de nombre d'hôtes parasités) qui peuvent aider à mieux comprendre ces différences :

- *Spécificité locale* : C'est le nombre d'hôtes utilisés par une espèce parasite dans une zone géographique locale donnée. Le problème est de définir ce qu'on entend par "locale", ce qui est fonction de la taille des populations et des capacités de dispersion des espèces considérées. Fox & Morrow (1981) ont suggéré que la spécificité pour l'hôte devait n'être considérée qu'au niveau local. Cela est également l'opinion de Thompson (1994).

- *Spécificité totale* : C'est l'union des spécificités locales pour une espèce de parasite. Cela représente donc la somme de tous les hôtes connus pour cette espèce. Il est possible qu'aucune population de cette espèce de parasite n'utilise effectivement tous ces hôtes.

- *Spécificité potentielle* : C'est la spécificité totale à laquelle on ajoute les hôtes qui ne sont jamais parasité dans la nature mais que le parasite a été capable d'exploiter en laboratoire (e.g., Jaenike, 1993 ; Solter & Maddox, 1998). Ces hôtes peuvent être séparés des parasites dans la nature par une barrière écologique qui rend impossible la colonisation.

Comme pour le cas général de la spécialisation en écologie, on suppose que la spécificité parasitaire est le résultat d'un processus évolutif et/ou adaptatif (Brooks & McLennan, 1991 ; Begon et al., 1996). Pour l'étude de la spécificité, ou de tout autre trait

de vie ou variable phénotypique, il est donc important de prendre en compte l'histoire évolutive des parasites (Brooks & McLennan, 1991 ; Harvey & Pagel, 1991). Cela permet de déterminer si la situation en matière de spécificité est le résultat d'influences actuelles (compétition interspécifique, ...) ou passées (coévolution, contraintes phylogénétiques...) (voir Brooks, 1985). Le fait de prendre en compte la phylogénie permet de réduire le nombre d'hypothèses *ad hoc* et de ne pas fonder l'interprétation des résultats d'une étude sur des suppositions plus ou moins arbitraires (Holmes & Price, 1980 ; Brooks, 1980a,b).

Certains taxons de parasites sont connus pour contenir des espèces plus spécialistes ou plus généralistes que d'autres. Par exemple, les crustacés copépodes parasites sont généralement assez généralistes alors que les monogènes sont très spécialistes (Baer, 1957 ; Kennedy, 1975 ; Rohde, 1982 ; Noble et al., 1989). En outre, à l'intérieur même d'un groupe de généralistes, on trouve parfois des espèces très spécialistes, ou inversement, ce qui peut permettre de penser que la spécificité est contrôlée par un mélange de causes passées et d'influences plus actuelles.

ii. Déterminants potentiels

De nombreux travaux ont été réalisés sur la spécificité parasitaire et permettent de dégager certains éléments pouvant l'influencer :

- *Écologie des parasites* (Rohde, 1978 ; Guégan & Agnèse, 1991). La compétition interspécifique peut notamment avoir un effet sur la spécificité (Holmes, 1973). Comme cela a été mentionné plus haut, Rohde (1979) a proposé que l'agrégation pouvait favoriser la reproduction. En plus des nombreux travaux de Klaus Rohde (e.g., 1979, 1994), le travail de Simkova et al. (2000) portant sur plusieurs espèces de *Dactylogyrus* (Monogenea, Dactylogyridae) suggère que la compétition n'est pas importante chez les monogènes branchiaux, bien que celle-ci puisse avoir de l'importance dans le cas (particulier) des monogènes vivant à l'intérieur de leurs hôtes (Jackson et al., 1998), où l'espace est plus limitant.
- *Complexité du cycle parasitaire* (Poulin, 1992 ; Morand, 1996). Les parasites ayant un cycle de vie complexe, utilisant plusieurs hôtes, sont en général moins spécifiques que ceux qui ont un cycle direct (voir aussi Sasal et al., 1998). Cela est en partie interprété par le fait que les parasites à cycle direct procèdent à une

recherche active de leur hôte, alors que le transfert des stades parasitaires des espèces à cycle complexe se fait principalement passivement, à travers les interactions de prédation. Cette recherche active est néanmoins parfois associée à une baisse de spécificité (Snyder & Janovy, 1996).

- *Stade du cycle parasitaire*. Les parasites peuvent avoir une spécificité différente selon la phase de développement de leur cycle dans laquelle ils se trouvent. Par exemple, les digènes sont connus pour être beaucoup plus spécifiques vis-à-vis de leur hôte intermédiaire que de leur hôte final. Certains nématodes montrent un profil inverse.

- *Morphologie de certaines structures anatomiques* (Tompkins & Clayton, 1999 ; Morand et al., 2000 ; Simkova et al., 2001). Les parasites spécialistes montrent une adaptation morphologique plus importante à leur hôte que les généralistes. Cela peut être interprété comme une cause et/ou une conséquence de la spécificité.

- *Facteurs immunologiques* (Adamson & Caira, 1994). La spécificité peut être maintenue par l'incapacité des parasites à survivre sur des hôtes inhabituels. Cela se vérifie spécialement pour les parasites interagissant de façon intensive avec le système immunologique de l'hôte. Les interactions immunologiques sont complexes chez certains types de parasites comme les monogènes (Buchmann, 1999).

- *Position phylogénétique* (Sasal et al., 1998). La spécificité parasitaire est relativement constante à l'intérieur des grands groupes de parasites (Digènes, Monogènes, Nématodes, Acanthocéphales, ...), ce qui laisse supposer une influence phylogénétique, au moins à grande échelle, sur la spécificité. Par "influence phylogénétique", on entend des contraintes transmissibles génétiquement, qui peuvent être de différentes natures (physiologiques, immunologiques, morphologiques, ...). Les différents facteurs mentionnés dans cette liste peuvent donc être à l'origine d'un effet phylogénétique sur la spécificité. Cela dépend de leur labilité au niveau évolutif. On suppose souvent que la spécificité parasitaire est en partie dépendante, et donc héritée, de la biologie de l' hypothétique ancêtre libre du parasite (Adamson & Caira, 1994).

- *Écologie de l'hôte* (Euzet & Combes, 1980 ; Adamson & Caira, 1994). Cela est particulièrement vrai pour les parasites à cycle complexe dont la transmission d'un hôte à l'autre dépend en grande partie de leur comportement alimentaire.
- *Taille de l'hôte* (Sasal et al., 1999). On a constaté que les parasites avaient tendance à se spécialiser sur les hôtes de grande taille, ce qui a été interprété comme une spécialisation sur une ressource prédictible.
- *Génotype de l'hôte* (Le Brun et al., 1992). Cette étude a montré que la proportion des gènes d'une espèce de poisson dans un hybride était corrélée à la prévalence d'infestation par le parasite de cette même espèce parente, l'autre n'étant jamais utilisée par ce parasite. Cela est interprété par le lien étroit entre génotype et caractères éthologiques.
- *Nombre des hôtes potentiels* (Poulin, 1992). Le nombre d'hôtes utilisés semble dépendre, pour certaines espèces de parasites, du nombre d'hôtes "convenables" disponibles, les hôtes convenables étant au moins en partie ceux qui sont phylogénétiquement proches de l'hôte d'origine. Cela indique que la spécificité peut dépendre des opportunités de colonisation qui s'offrent au parasite.
- *Abondance relative des hôtes potentiels* (Norton & Carpenter, 1998). Certains parasites semblent capables d'utiliser un seul hôte si celui-ci est suffisamment abondant, ou plusieurs si l'hôte original voit son effectif baisser. Dans les deux cas, tous les hôtes potentiels sont présents dans le milieu, seule leur abondance relative varie. Ce comportement correspond à un élargissement de la niche écologique.
- *Habitat de l'hôte*. Les variables du milieu extérieur, comme la température ou la salinité, peuvent modifier la spécificité du parasite (e.g., Zander, 1998). Cela peut-être dû à des modifications de la physiologie des parasites, ou à un changement des interactions compétitives avec les autres espèces parasites qui peuvent être moins tolérantes à ces variations environnementales.

Tous ces facteurs peuvent bien entendu agir en même temps et être liés les uns aux autres.

b. La coévolution hôte-parasite

Les hôtes représentant une partie essentielle de l'environnement des parasites, l'histoire évolutive de cette composante peut avoir une influence importante sur la phylogénie des parasites. Cela mène au concept de coévolution, qui implique que de nouvelles associations hôte-parasite se forment au cours du temps. Cela peut se faire de plusieurs façons :

- *Cospéciation* : transmission par descendance. Dans ce cas, lorsqu'il y a spéciation de l'espèce hôte, le lot de parasites hébergé par la nouvelle espèce va effectuer une spéciation à son tour. C'est ce que l'on appelle la cospéciation. La spéciation de l'espèce parasite peut se faire avec plus ou moins de décalage par rapport à celle de l'hôte (Hafner & Nadler, 1990 ; Page & Hafner, 1996). Les associations hypothétiques qui évolueraient uniquement ainsi suivraient exactement la règle de Fahrenholz (1913) : "la phylogénie des parasites reflète la phylogénie des hôtes" (voir Klassen, 1992). Il peut également y avoir spéciation de l'hôte sans que le parasite ne subisse de spéciation :

- *Capture* : transmission par colonisation, ou changement d'hôte ("host-switching"). Ici, le parasite colonise une nouvelle espèce-hôte par dispersion. Le parasite peut alors subir ou non une spéciation sur son nouvel hôte (Brooks & McLennan, 1991, 1993). Cette nouvelle espèce d'hôte n'est pas forcément phylogénétiquement liée à l'espèce originale, même si les contraintes phylogénétiques tendent à favoriser une nouvelle association avec une espèce proche (voir Morand et al., 1996), conséquence du fait que des espèces fortement liées ont davantage de chance d'avoir un développement similaire (Pagel & Harvey, 1988). Si une espèce-soeur est colonisée, puis que le parasite y subit une spéciation, on parle alors de pseudo-cospéciation (Hafner & Nadler, 1990 ; Norton & Carpenter, 1998), qui est difficile à distinguer de la cospéciation vraie.

Outre ces deux processus de bases, plusieurs événements évolutifs peuvent compliquer le profil de coévolution observé :

- *Duplication* : spéciation d'une espèce parasite sur l'hôte sans spéciation de l'hôte.

- *Disparition*: une espèce parasite peut être absente d'une des nouvelles espèces d'hôte formée après la spéciation de l'hôte original ("sorting event"). Cela peut être dû à l'extinction de cette espèce ou à son absence, à l'origine, de l'une des deux nouvelles espèces d'hôtes ("missing the boat").

Ces quatre événements coévolutifs sont notamment détaillés dans Ronquist (1997), Page & Charleston (1998) et Paterson & Banks (2001).

Les associations hôte-parasite peuvent ainsi présenter des profils coévolutifs complexes faits de ces quatre événements imbriqués, et plusieurs méthodes ont été mises au point pour proposer des scénarios évolutifs potentiels. Ces méthodes seront examinées plus en détail dans la partie Méthodologie.

On peut penser qu'une coévolution hôte-parasite stricte (évolution conjointe par cospéciations) est intimement liée au degré de spécificité parasitaire. En effet, on ne conçoit ce type d'évolution que si les parasites sont très inféodés à leurs hôtes, puisque ainsi la probabilité de transfert d'hôte est faible. Néanmoins, l'inverse n'est pas forcément vrai : des transferts d'hôtes fréquents peuvent être liés à une forte spécificité si les transferts sont accompagnés d'un événement de spéciation (Secord and Kareiva, 1996). Quoiqu'il en soit, l'étude de la cospéciation est importante dans l'étude de la spécificité, car dans le cas d'interactions coévolutives fortes entre les hôtes et les parasites, l'évolution des parasites et le choix de leurs hôtes vont être dirigés en grande partie par ces derniers. Dans ce cas, l'histoire évolutive des parasites dépend de celle de leurs hôtes et les déterminants écologiques de la spécificité devraient avoir une portée limitée. En revanche, en l'absence de cospéciations répandues dans l'association hôte-parasite, la question des déterminants du choix des hôtes reste entière.

3. Le cas des monogènes

De nombreux facteurs sont ainsi susceptibles d'agir sur la spécificité parasitaire. Il est intéressant d'étudier un groupe pour lequel certains de ces facteurs sont constants, afin de contrôler leur effet. Pour chercher à déterminer les causes potentielles de la spécificité, il faut aussi que la spécificité soit variable à l'intérieur de ce groupe.

Les monogènes possèdent de bonnes caractéristiques pour ce type d'étude : ces parasites ont un cycle direct, ce qui élimine ce facteur et permet de simplifier les hypothèses à poser; ils sont presque entièrement dépendants de leur(s) hôte(s), qui constitue donc ici leur environnement, et ils sont connus pour être particulièrement spécifiques (Baer, 1957 ; Kennedy, 1975 ; Rohde, 1979, 1982 ; Noble et al., 1989).

Barker (1991), Poulin (1992) et Kearn (1994) pensent que la forte spécificité des monogènes s'explique par le fait que ceux-ci ont probablement intimement co-évolué avec leurs hôtes, comme l'ont suggéré Tinsley & Jackson (1998) chez les monogènes Polystomatidae d'amphibiens. Humphery-Smith (1989) fait la liste des caractéristiques des parasites qui pourraient favoriser une évolution par cospéciations successives avec leurs hôtes. Les parasites très spécifiques et non pathogènes sont les mieux placés, ce qui est le cas des monogènes. D'autres auteurs, comme Brooks & McLennan (1991) pensent au contraire que les monogènes possèdent les caractéristiques idéales pour effectuer de nombreux transferts d'hôtes. Cela demande à être étudié de manière fine.

Sasal et al. (1999) ont suggéré que les monogènes spécialistes se trouvaient sur les espèces de poissons les plus grands. Simkova et al. (2001) suggèrent que les monogènes spécialistes sont plus étroitement apparentés à leurs hôtes que les généralistes, mettant en évidence l'importance, pour l'établissement de la spécificité, des structures anatomiques nécessaires à l'accrochage.

La compétition interspécifique ne semble pas être une cause importante de la spécificité chez les monogènes (Euzet & Combes, 1998 ; Simkova et al., 2000). En effet, la plupart des monogènes sont des ectoparasites de la peau ou des branchies, et ils vivent dans un milieu où le nombre de niches disponibles semble très élevé (Rohde, 1978). C'est cela qui a conduit Rohde (1979) à proposer l'hypothèse mentionnée plus haut d'une spécialisation dont le but est de provoquer l'agrégation des individus pour la reproduction. Il n'y a que chez les rares espèces de monogènes endoparasites (e.g., Polystomes) qu'une compétition pour l'espace semble exister (Jackson et al., 1998).

Les caractéristiques des monogènes en font ainsi de bons modèles pour l'étude des déterminants de la spécificité parasitaire.

La plupart des études sur la spécificité ont été réalisées à partir de modèles verbaux ou théoriques ; ou alors, quand ces études analysaient des données réelles, un ou quelques facteurs seulement étaient pris en compte. En outre, très peu d'études de la spécificité se sont faites dans un contexte phylogénétique (e.g., Poulin, 1992).

Pour les raisons décrites plus haut, j'ai choisi d'étudier dans cette thèse une association monogène–poisson : l'association entre les monogènes *Lamellodiscus* (Diplectanidae) et les Sparidae (Téléostéens) en Méditerranée (voir Chapitre II). Cette étude a été réalisée dans le Golfe du Lion (France), à une échelle qu'on peut qualifier de locale. La faune parasitaire de la Méditerranée est une des mieux connues du monde (Caro et al., 1997), ce qui limite les éventuels biais d'échantillonnage. En d'autres termes, on connaît probablement tous, ou la plupart des poissons et des parasites de l'association étudiée. Le but de ce travail est de comprendre les causes de la spécificité parasitaire dans ce système hôte-parasite. Pourquoi certains parasites ont-ils de nombreux hôtes, alors que d'autres se restreignent à peu, voire à un seul hôte? Est-ce que les déterminismes de la spécificité sont davantage écologiques, c'est-à-dire est-ce la situation environnementale "actuelle" qui contrôle le type de spécificité parasitaire observée, où est-ce que cela est majoritairement contrôlé par un héritage phylogénétique? En d'autres termes, est-ce qu'un parasite est généraliste parce que les conditions s'y prêtent (ou l'y obligent), ou parce que son ancêtre l'était? Quelle est la part de contrainte phylogénétique dans le déterminisme de la spécificité?

Je vais tenter de répondre à ces questions par une étude réalisée surtout au niveau intra-générique, c'est-à-dire à un niveau très fin pour éviter certains biais liés à un contrôle phylogénétique à un niveau trop élevé, où les classifications taxonomiques parfois relativement arbitraires comme celles des genres ou des familles peuvent introduire des erreurs.

B. OBJECTIFS

1. Objectif principal

L'objectif principal de cette thèse est de rechercher les déterminants de la spécificité des *Lamellodiscus* de Méditerranée.

2. Objectifs spécifiques

a. Rechercher les déterminants de la spécificité et étudier le lien entre spécificité et diversification aux niveaux supra-génériques.

b. Reconstruire la phylogénie des Diplectanidae afin de mieux définir les extra-groupes à utiliser pour étudier la phylogénie des *Lamellodiscus*. La phylogénie des monogènes est encore assez peu précise (Boeger & Kritsky, 1993, 1997 ; Olson & Littlewood, sous presse).

c. Augmenter la précision de la taxonomie des *Lamellodiscus* de Méditerranée. Celle-ci est actuellement basée sur les caractères morphologiques. Une approche moléculaire peut apporter des précisions quant au statut éventuel d'espèces cryptiques, ou au contraire d'un polymorphisme intra-spécifique conduisant à classer dans des espèces différentes des individus qui ne représentent que des variants d'une même espèce.

d. Reconstruire la phylogénie moléculaire des *Lamellodiscus* de Méditerranée. La phylogénie des monogènes est encore peu résolue au niveau spécifique (Poulin, 1996) et au niveau moléculaire (Littlewood, 1999a,b ; Mollaret et al., 2000 ; Jovelin & Justine, 2001). Cela passe par la recherche de bons marqueurs de l'évolution de ce groupe.

e. Reconstruire la phylogénie des Sparidae de Méditerranée. Une phylogénie moléculaire des Sparidae, basée sur l'analyse de séquences d'ADN mitochondrial 16S, a récemment été proposée par Hanel & Sturmbauer (2000), mais l'arbre proposé comporte des parties non résolues. Les hypothèses précédentes, basées sur des caractères morphologiques (e.g., Tortonese, 1975 ; De la Paz, 1981) ou

biochimiques (e.g., Basaglia, 1991 ; Reina et al., 1994 ; Garrido-Ramos et al., 1995) s'avèrent très incertaines.

f. Étudier la coévolution hôte-parasite chez une association monogène-poisson, ce qui n'a été que peu étudié (e.g., Klassen & Beverley-Burton, 1987 ; Boeger & Kritsky, 1997), particulièrement à un niveau intra-générique.

g. Identifier et quantifier les fractions phylogénétiques et écologiques dans le déterminisme de la spécificité chez les *Lamellodiscus*, et rechercher les adaptations morphologiques qui sont les causes ou les conséquences de la spécificité.

C. HYPOTHÈSES

Les hypothèses d'influences sur la spécificité parasitaire sont classées un peu artificiellement en influences "passées" et "présentes" par souci de clarté. Comme cette thèse est réalisée sous forme d'articles scientifiques, le découpage en hypothèses ne suit pas tout à fait l'ordre des publications composant la thèse, car chaque article peut traiter simultanément de différents aspects de la problématique. Le ou les article(s) correspondant(s) à l'étude de chaque hypothèse est mentionné après celle-ci.

1. Influences phylogénétiques (passées)

a. Diversification taxonomique

Nous considérons ici l'hypothèse de Brooks & McLennan (1991, 1993) qui suggèrent qu'un parasite spécifique a davantage de chances de subir une spéciation après la colonisation d'un nouvel hôte qu'un parasite plus généraliste, plus tolérant aux changements d'hôte. Cette hypothèse sera étendue à la transmission par descendance. Elle implique que spécialisation et diversification sont liées, mais cela n'a été que peu étudié jusqu'a présent (Janzen, 1973 ; Futuyma & Moreno, 1988), même dans le cas des parasites (Price, 1980 ; Brooks & McLennan, 1991 ; Poulin, 1992).

Si cette hypothèse est exacte, on pourrait observer une augmentation de la spécificité avec le nombre de spéciations entre une espèce et la racine de la phylogénie (Figure I.1 ; voir Lanyon, 1992). En d'autres termes, plus les espèces montrent une spécificité stricte,

plus la diversification est importante. L'hypothèse nulle est l'absence de corrélation entre la diversification et la spécialisation. Ce cas de figure a cependant rarement été mis en évidence empiriquement (e.g., MacDonald & Brooks, 1989).

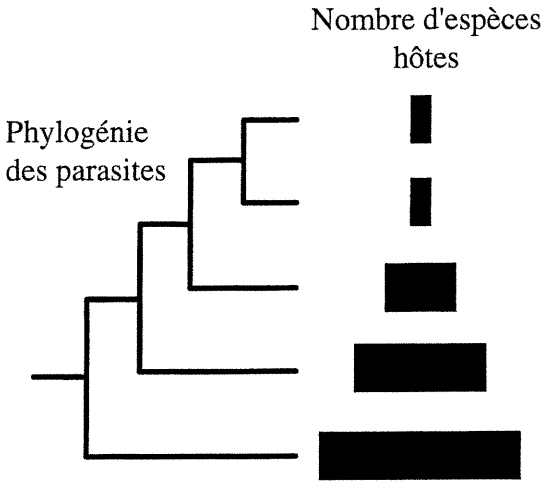


Figure I.1 : Lien hypothétique entre spécificité (nombre d'hôtes) et diversification taxonomique. Plus une espèce de parasite est dérivée, plus son nombre d'hôtes est faible.

Cette hypothèse d'une relation spécificité-diversification taxonomique sera étudiée dans ce travail, au niveau des grands groupes de parasites (classes et embranchements; **ARTICLE 1**), au niveau intergénérique (Diplectanidae; **ARTICLE 2**), et au niveau interspécifique (*Lamellodiscus*; **ARTICLE 6**). Cela répond aux **objectifs spécifiques a, b, et g**.

b. Phylogénie des parasites

La phylogénie des parasites peut également montrer une influence sur le profil observé de spécificité (Noble et al., 1989 ; Adamson & Caira, 1994). En d'autres termes, deux espèces proches de parasites peuvent avoir des degrés de spécificité semblables à cause des caractéristiques partagées qu'elles doivent à leur ancêtre commun. Cette hypothèse est liée à la précédente puisqu'elle fait intervenir les liens de parenté entre espèces, mais elle demeure différente et devra être traitée séparément.

On cherchera donc à savoir si la spécificité est contrainte par la phylogénie chez les *Lamellodiscus* (**ARTICLE 6**). Cela répond à l'**objectif spécifique g**.

c. Phylogénie des hôtes

Des hôtes apparentés représentent deux types d'habitats pouvant être supposés proches pour les parasites. On peut donc penser que des parasites exploitant ces espèces d'hôtes proches vont être soumis à des pressions évolutives relativement proches et pourraient ainsi montrer un même type de spécificité. Par conséquent, des différences de spécificité pourraient être au moins partiellement expliquées par des changements d'hôtes (captures).

Dans un cas idéal de coévolution par cospéciations successives, on observerait une parfaite congruence entre les phylogénies (règle de Farenholz), mais cela est rarement le cas dans les études effectuées en ce domaine (e.g., Brooks & Glen, 1982 ; Hafner & Nadler, 1988 ; Hafner et al., 1994), et on observe le plus souvent une histoire coévolutive faite de cospéciations et de captures.

Cette partie du travail, l'étude de la coévolution entre les Sparidae et les *Lamellodiscus*, représente une importante partie de la thèse. Elle suppose que l'on connaît la phylogénie des parasites et celle des hôtes. Il faut ensuite les comparer à l'aide de méthodes appropriées (**ARTICLES 3, 4 ET 5**). Cela répond aux **objectifs spécifiques d, e, et f**.

2. Influences écologiques (actuelles)

a. Taille de l'hôte

Nous retiendrons l'hypothèse, émise par Ward (1992), que les parasites ont tendance à se spécialiser sur des hôtes prédictibles. Sasal et al. (1999) ont suggéré que les monogènes se spécialisaient sur les hôtes les plus grands. Winemiller & Rose (1992) ont montré que de nombreux traits de vie étaient corrélés à la taille chez les poissons. On peut supposer que la ressource étant plus limitée pour les parasites spécialistes, ils deviennent plus dépendants de leur population hôte et ont intérêt à "choisir" des populations d'hôtes stables (Basset, 1992 ; Kithara & Fuji, 1994). Morand et al. (1996), Sorci et al. (1997) et Morand & Sorci

(1998) ont aussi montré que des parasites pouvaient développer des stratégies adaptatives en réaction à l'augmentation de la taille de l'hôte.

Ce lien entre la taille de l'hôte et la spécificité sera recherché chez les *Lamellodiscus* (**ARTICLE 6**). Cela répond à l'**objectif spécifique g**.

b. Abondance de l'hôte

Norton et Carpenter (1998) ont suggéré que la spécialisation pouvait être dépendante de l'abondance relative des hôtes potentiels. L'abondance des hôtes peut aussi être considérée comme un facteur de prédictibilité.

Cette variable est considérée dans la recherche des déterminants de la spécificité (**ARTICLE 6**). Cela répond à l'**objectif spécifique g**.

c. Grégarité

La grégarité des hôtes peut également jouer un rôle dans la prédictibilité de l'hôte et la détermination de la spécificité parasitaire. Des hôtes plus grégaires pourraient avoir tendance à "partager" plus facilement leur pool de parasites, ce qui favoriserait la spécificité. Rohde (1994) a souligné que la spécificité pour l'hôte était favorisée par le regroupement des individus d'une espèce ("monospecific schooling").

Cette variable est considérée comme un déterminant potentiel de la spécificité (**ARTICLE 6**). Cela répond à l'**objectif spécifique g**.

d. Nombre d'hôtes potentiels

Le nombre d'hôtes phylogénétiquement apparentés à l'hôte ou aux hôtes parasités pourrait être directement corrélé avec le nombre d'hôtes parasités, c'est-à-dire avec la spécificité. En effet, on peut penser que les hôtes proches partagent des caractéristiques permettant leur colonisation par une même espèce de parasite. Une telle corrélation a été observée par Poulin (1992) pour plusieurs genres de monogènes. Ce phénomène sera étudié ici.

Cette variable sera prise en compte dans la recherche des déterminants de la spécificité (**ARTICLE 6**). Cela répond à l'**objectif spécifique g**.

e. Morphologie des parasites

Morand et al. (2000) ont montré que certaines caractéristiques morphologiques des parasites pouvaient être liées à leur spécificité. La spécificité peut en effet être favorisée par certaines structures morphologiques (Noble et al., 1989). Un lien entre la taille des parasites et celle des hôtes sera recherché. Une telle corrélation pourrait être le fait d'une adaptation morphologique du parasite à l'hôte (e.g., Simkova et al., 2001). Un monogène parasitant un hôte de grande taille pourrait développer lui aussi une taille importante afin d'être mieux fixé pour résister à la pression de l'eau dans la cavité branchiale.

Le lien entre la taille des parasites et celle des hôtes, mécanisme potentiel du déterminisme de la spécificité ou adaptation causée par celle-ci, sera étudié (**ARTICLE 6**). Cela répond à l'**objectif spécifique g**.

D. Organisation de la thèse

Comme je l'ai mentionné plus haut, cette thèse a été réalisée et se présente sous forme de publications scientifiques, chacun de ces articles répondant à un ou plusieurs des objectifs spécifiques. Cela sera précisé au début de chaque article, dans la brève présentation qui le précède. Ce type de présentation implique que certaines informations peuvent être répétées dans un ou plusieurs articles ainsi que dans le texte de la thèse. Ma contribution à chaque publication est décrite dans le paragraphe de présentation.

Voici la liste des publications de la thèse. Celles-ci sont présentées en entier au Chapitre III.

- **ARTICLE 1** : SASAL Pierre, Yves DESDEVISES and Serge MORAND. 1998. Host-specificity and species diversity in fish parasites: phylogenetic conservatism? *Ecography* 21: 639-643.
- **ARTICLE 2** : DESDEVISES Yves, Serge MORAND, and Guy OLIVER. 2001. Linking Specialization to Diversification in the Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea). *Parasitology Research* 87(3): 223-230.
- **ARTICLE 3** : DESDEVISES Yves, Richard JOVELIN, Olivier JOUSSON and Serge MORAND. 2000. Comparison of ribosomal DNA sequences of

Lamellodiscus spp. (Monogenea, Diplectanidae) parasitizing *Pagellus* (Sparidae, Teleostei) in the North Mediterranean Sea: species divergence and coevolutionary interactions. *International Journal for Parasitology.* 30: 741-746.

- **ARTICLE 4** : DESDEVISES Yves. 2001. The phylogenetic position of *Furnestinia echeneis* (Monogenea, Diplectanidae) based on molecular data: a case of morphological adaptation? *International Journal for Parasitology* 31(2): 205-208.
- **ARTICLE 5** : DESDEVISES Yves, Serge MORAND, Olivier JOUSSON and Pierre LEGENDRE. Coevolution between *Lamellodiscus* (Monogenea) and Sparidae (Teleostei): the study of a complex host-parasite system. Soumis.
- **ARTICLE 6** : DESDEVISES Yves, Serge MORAND and Pierre LEGENDRE. Evolution and determinants of specificity in *Lamellodiscus* (Monogenea). Soumis.

II. MÉTHODOLOGIE

A. Espèces étudiées et zone d'échantillonnage

1. Espèces

Pour les raisons mentionnées en Introduction, nous avons choisi d'étudier un système monogène-poisson. Les monogènes (Plathelminthes) sont des parasites à cycle direct (Figure II.1) généralement très spécifiques (Noble et al., 1989 ; Schmidt & Roberts, 1989 ; Poulin, 1992), le plus souvent ectoparasites, et généralement non pathogènes (Schmidt & Roberts, 1989 ; Bakke et al., 1992). Le modèle choisi est l'association *Lamellodiscus*-Sparidae en Méditerranée. Ce complexe d'espèces présente une gamme d'associations hôte-parasite variée, avec des parasites très spécifiques ou plus généralistes (Euzet et al., 1993 ; Sasal et al., 1997 ; voir Tableau II.1), et des hôtes plus ou moins parasités. Il se prête donc très bien à l'étude envisagée.

a. Les parasites : *Lamellodiscus* spp.

Les monogènes du genre *Lamellodiscus* Johnston et Tiegs 1922 (voir Figure II.2) sont des ectoparasites branchiaux dont on connaît actuellement une quarantaine d'espèces (voir Oliver, 1987 , Roubal, 1994 ; Roubal et al., 1996), principalement en Méditerranée, en Australie et en Nouvelle-Zélande. Ils font partie de la classe des Monogenea (Carus, 1863) Bychowsky 1937, de la sous-classe des Monopisthocotylea Odhner 1912, de l'ordre des Dactylogyridea Bychowsky 1937, du sous-ordre des Diplectaninea Oliver 1967, de la super-famille des Diplectanoidea Bychowsky 1957, de la famille des Diplectanidae Oliver 1969 et de la sous-famille des Lamellodiscinae Oliver 1969. Comme presque tous les monogènes, ils sont hermaphrodites et ovipares. Ils possèdent un cycle direct, avec une larve ciliée issue de l'œuf, l'oncomiracidium, qui cherche activement un hôte convenable pour s'y fixer et se développer en adulte. Ils se nourrissent en ingérant l'épithélium des lamelles branchiales sur lesquelles ils vivent. Les caractéristiques anatomiques principales du genre *Lamellodiscus* sont : un hapteur comportant trois barres transversales, deux paires de hamuli, deux lamellodisques formés de lamelles paires sauf les lamelles extrêmes et 14 uncinuli; trois paires d'organes glandulaires céphaliques, des tâches oculaires généralement absentes chez l'adulte ; un appareil copulateur généralement constitué d'une pièce parfois complexe, avec ou sans pièce accessoire; un vagin le plus souvent sclérifié, situé sauf

exception dans la moitié gauche du corps; des œufs tétraédriques avec un long filament polaire; quatre paires (parfois cinq) de protonéphridies chez les oncomiracidia. Leur taille est généralement comprise entre 200 µm et 1100 µm. La taxonomie est basée sur la forme des pièces sclérifiées du hapteur et des organes copulateurs.

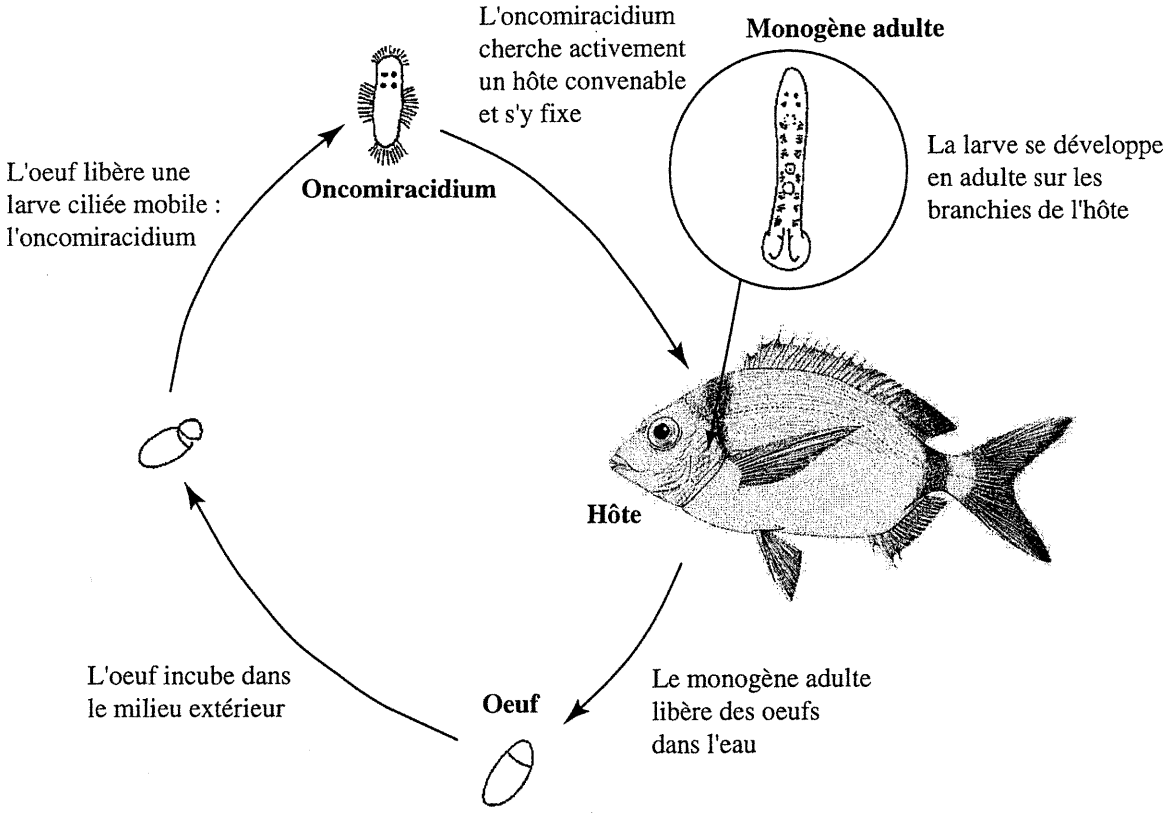


Figure II.1 : Cycle de développement typique des monogènes.

Ce genre a été choisi pour le nombre important d'espèces qu'il comporte en Méditerranée (20 espèces sont actuellement décrites dans la zone d'étude), la variation de spécificité qu'on peut y trouver (de 1 à 6 hôtes par espèce de parasite), et le fait qu'il ait été bien étudié (Euzet & Oliver, 1966, 1967 ; Oliver, 1968, 1973, 1974, 1987 ; San Filippo, 1978 ; Euzet, 1984 ; Euzet et al., 1993 ; Neifar, 1995 ; Kouider El Ouahed-Amine, 1998). L'identification de ces monogènes repose sur des critères morphologiques stables et bien établis (principalement les structures sclérifiées comme les éléments du hapteur ou les organes génitaux, ...) et leur taxonomie alpha est bien connue. Le profil de spécificité, c'est-

à-dire le nombre d'hôtes par espèce de parasite, a été étudié de manière intensive dans la région et peut être considéré comme bien connu.

Tableau II.1 : Liste des espèces de parasites étudiés et de leurs hôtes (*Furnestinia echeneis* est ajouté aux *Lamellodiscus*, car il fait partie du même genre sur la base de données moléculaires ; voir plus loin).

Lamellodiscus spp.	Sparidae (hôtes)	Nombre d'hôtes
F. echeneis	*Sparus aurata*	1
L. baeri	*Pagrus pagrus*	1
L. bidens	*Diplodus puntazzo*	1
L. coronatus	*Diplodus cervinus D. annularis, D. sargus*	3
L. drummondi	*Pagellus acarne*	1
L. elegans	*Diplodus annularis, D. sargus, D. vulgaris, Oblada melanura, Spondyliosoma cantharus*	5
L. ergensi	*Diplodus annularis, D. puntazzo, D. sargus, D. vulgaris*	4
L. erythrini	*Pagellus erythrinus*	1
L. fraternus	*Diplodus annularis, D. vulgaris*	2
L. furcosus	*Diplodus annularis, D. sargus*	2
L. gracilis	*Diplodus annularis, D. sargus, Oblada melanura*	3
L. hilii	*Diplodus puntazzo*	1
L. ignoratus	*Diplodus annularis, D. puntazzo, D. sargus, D. vulgaris, Lithognathus mormyrus, Sarpa salpa*	6
L. impervius	*Diplodus puntazzo*	1
L. knoepffleri	*Spondyliosoma cantharus, Spicara maena, Spicara smaris*	3
L. mirandus	*Diplodus sargus*	1
L. mormyri	*Lithognathus mormyrus*	1
L. parisi	*Sarpa salpa*	1
L. verberis	*Lithognathus mormyrus*	1
L. virgula	*Pagellus acarne, Pagellus bogaraveo*	2

b. Les hôtes : Sparidae

Les Sparidae sont une famille de Téléostéens marins fréquentant les eaux tropicales et tempérées en général à faible profondeur, comportant 119 espèces et 29 genres (Whitehead et al., 1986). Leur valeur culinaire en font des poissons d'intérêt commercial. Leur position systématique les situe dans l'ordre des Perciformes et le sous-ordre des Percoidés. Leur classification est principalement basée sur la dentition. Ces poissons font partie des rares poissons hétérodontes (ils possèdent des types de dents variés : canines, incisives, molaires), et possèdent des régimes alimentaires variés : herbivores (Saupes), carnivores

(Sars), conchyliphages (Dorades) et brouteurs d'invertébrés (Sparaillons). Les Sparidae comportent 16 espèces dans la région spécifiquement étudiée ici (Whitehead et al., 1986), dont 14 parasitées par des *Lamellodiscus* (Figure II.3). Des données biologiques et écologiques sont disponibles sur ces poissons (Whitehead et al., 1986 ; Froese & Pauly, 1995).

2. Échantillonnage

L'échantillonnage a été mené dans la partie ouest du golfe du Lion (voir Figure II.4), au nord-ouest de la Méditerranée, car de nombreuses données sont disponibles sur les hôtes comme sur les parasites étudiés dans cette région.

Les poissons ont été obtenus par pêche au chalut (avec l'aide des pêcheurs de l'Observatoire Océanologique de Banyuls-sur-Mer), par chasse sous-marine, ou directement auprès de pêcheurs professionnels de Port-Vendres (Pyrénées Orientales) ou de Sète (Hérault). Les poissons ont été soit directement disséqués pour en prélever les parasites, soit conservés à –20°C pour examen ultérieur. Les parasites prélevés des branchies sous la loupe binoculaire sont ensuite placés entre lame et lamelle, soit dans l'eau de mer (pour des échantillons très frais), soit dans l'alcool (pour des échantillons conservés plus longtemps). Après identification au microscope, les individus sont récupérés pour l'extraction d'ADN. Les données moléculaires sont utilisées pour inférer la phylogénie des espèces.

La spécificité hôte-parasite ainsi que les relations phylétiques entre les deux groupes seront étudiées au niveau géographique local. C'est à cette échelle que les études sont les plus exhaustive et que des tendances peuvent être bien visibles. La spécificité peut varier selon les régions (Thompson, 1994 ; Norton & Carpenter, 1998) et ce profil peut être différent à un niveau plus large. En outre, à une échelle plus grande, la spécificité mesurée peut correspondre à une moyenne artificielle n'ayant que peu de réalité biologique.

Il est important de noter que le profil de spécificité des *Lamellodiscus* du golfe du Lion est le même que sur les côtes tunisiennes et algériennes (Neifar, 1995 ; Kouider El Ouahed-Amine, 1998). Cela laisse supposer que ce profil est constant.

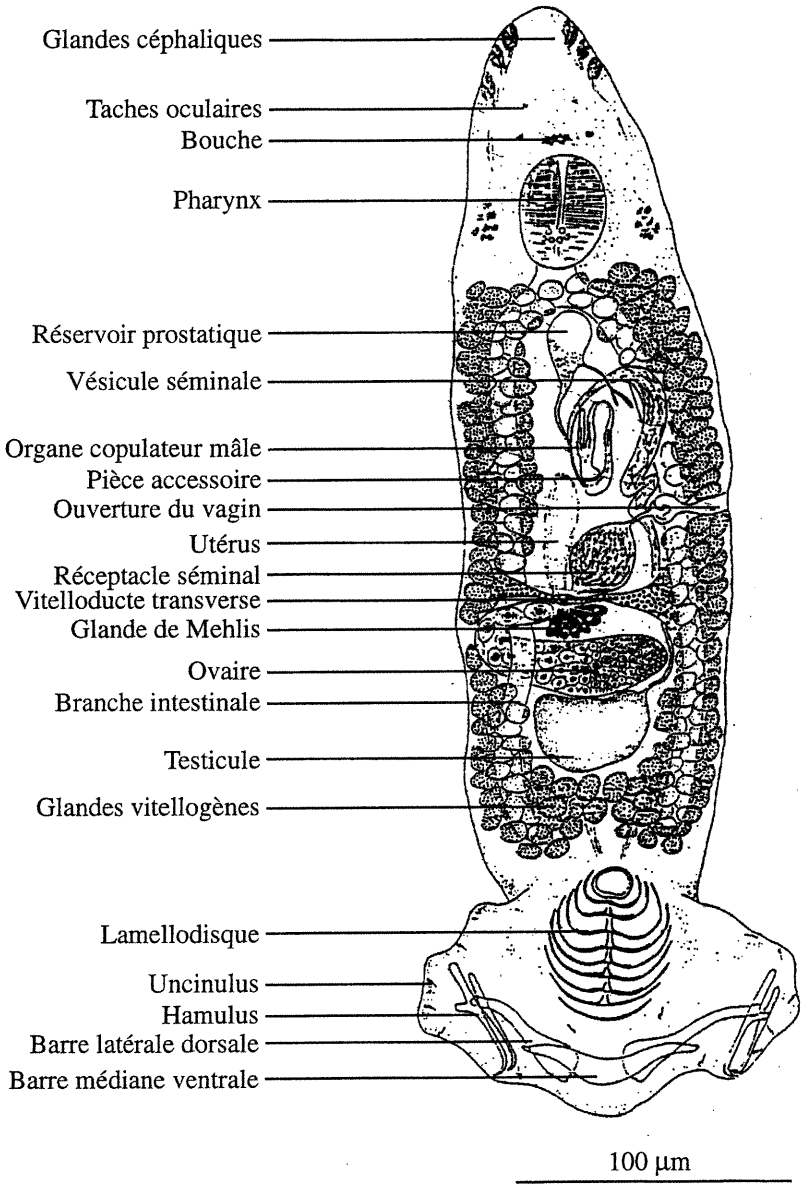


Figure II.2 : Une espèce-type de *Lamellodiscus* en détail (*L. ignoratus*).

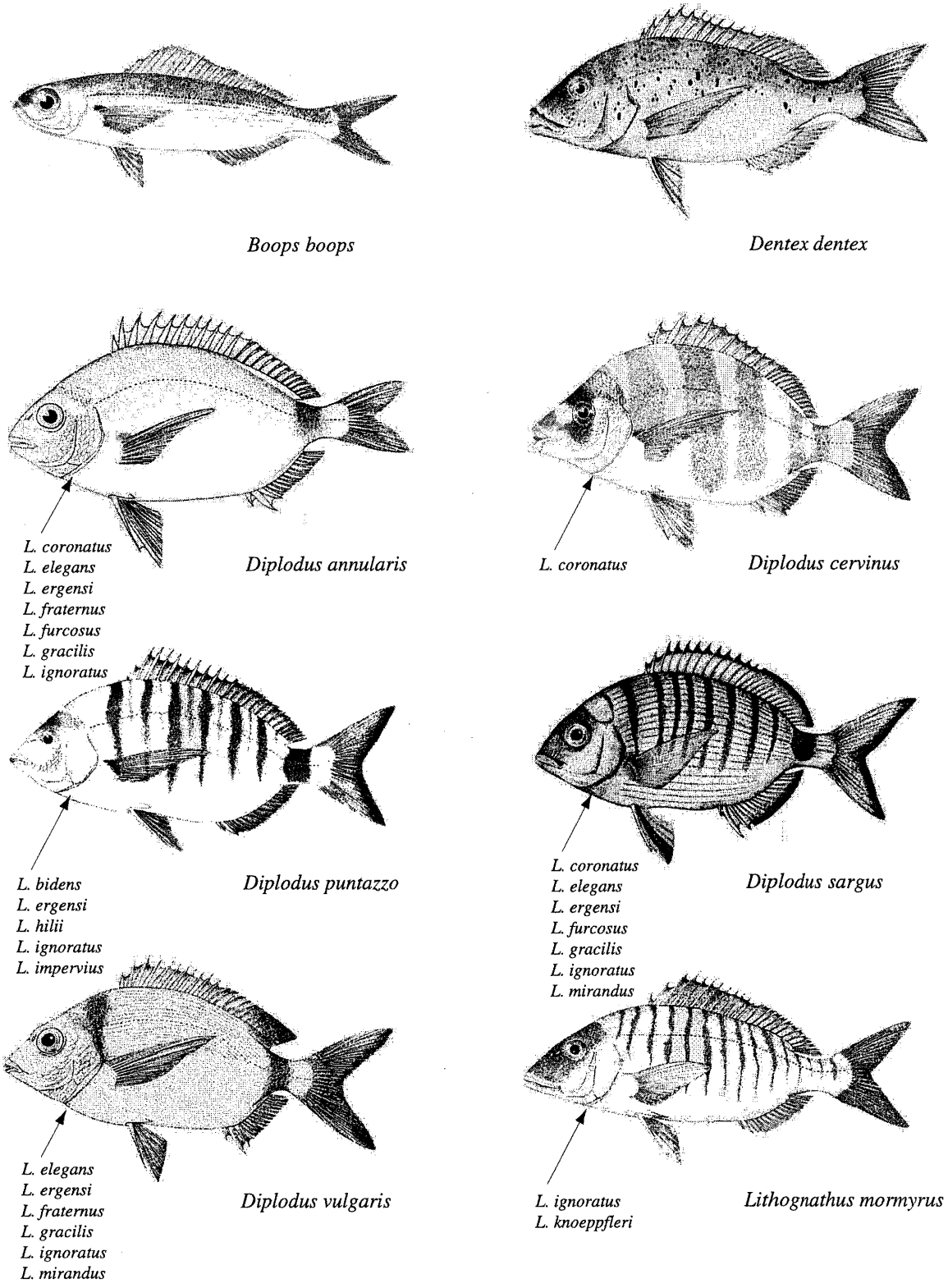
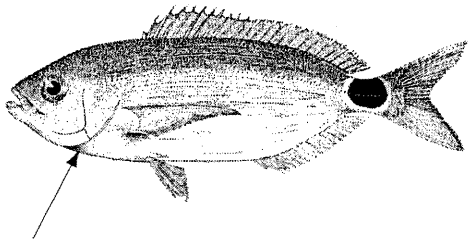
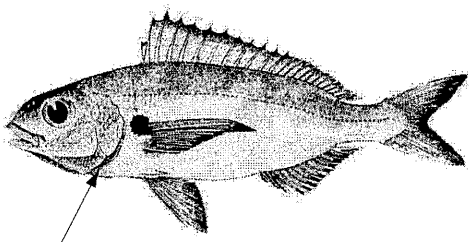


Figure II.3 : Espèces de Sparidae étudiées. Les espèces de *Lamellodiscus* qui s'y trouvent sont mentionnées.



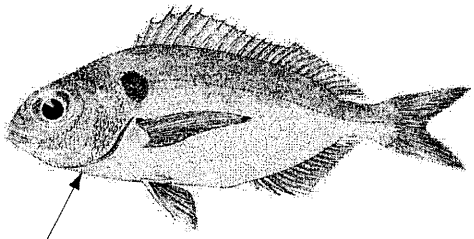
L. elegans
L. gracilis

Oblada melanura



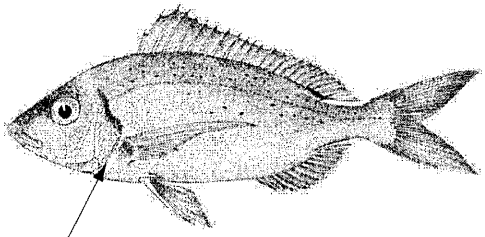
L. drummondi
L. virgula

Pagellus acarne



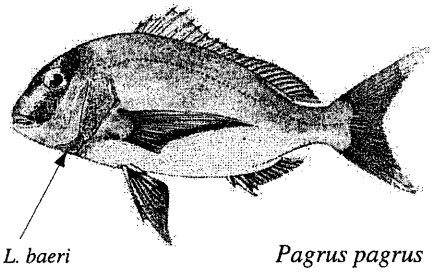
L. obeliae (L. virgula)

Pagellus bogaraveo



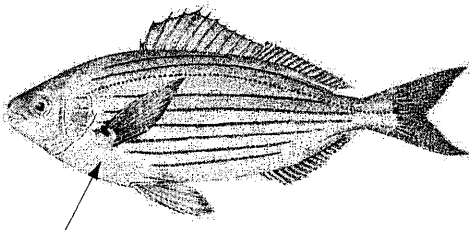
L. erythrini

Pagellus erythrinus



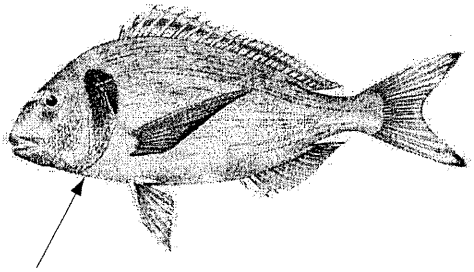
L. baeri

Pagrus pagrus



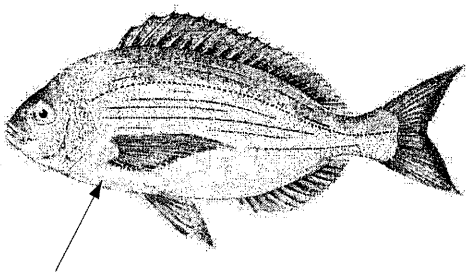
L. ignoratus
L. parisi

Sarpa salpa



Furnestinia echeneis

Sparus aurata



L. elegans
L. elegans

Spondyliosoma cantharus

Figure II.3 (suite)

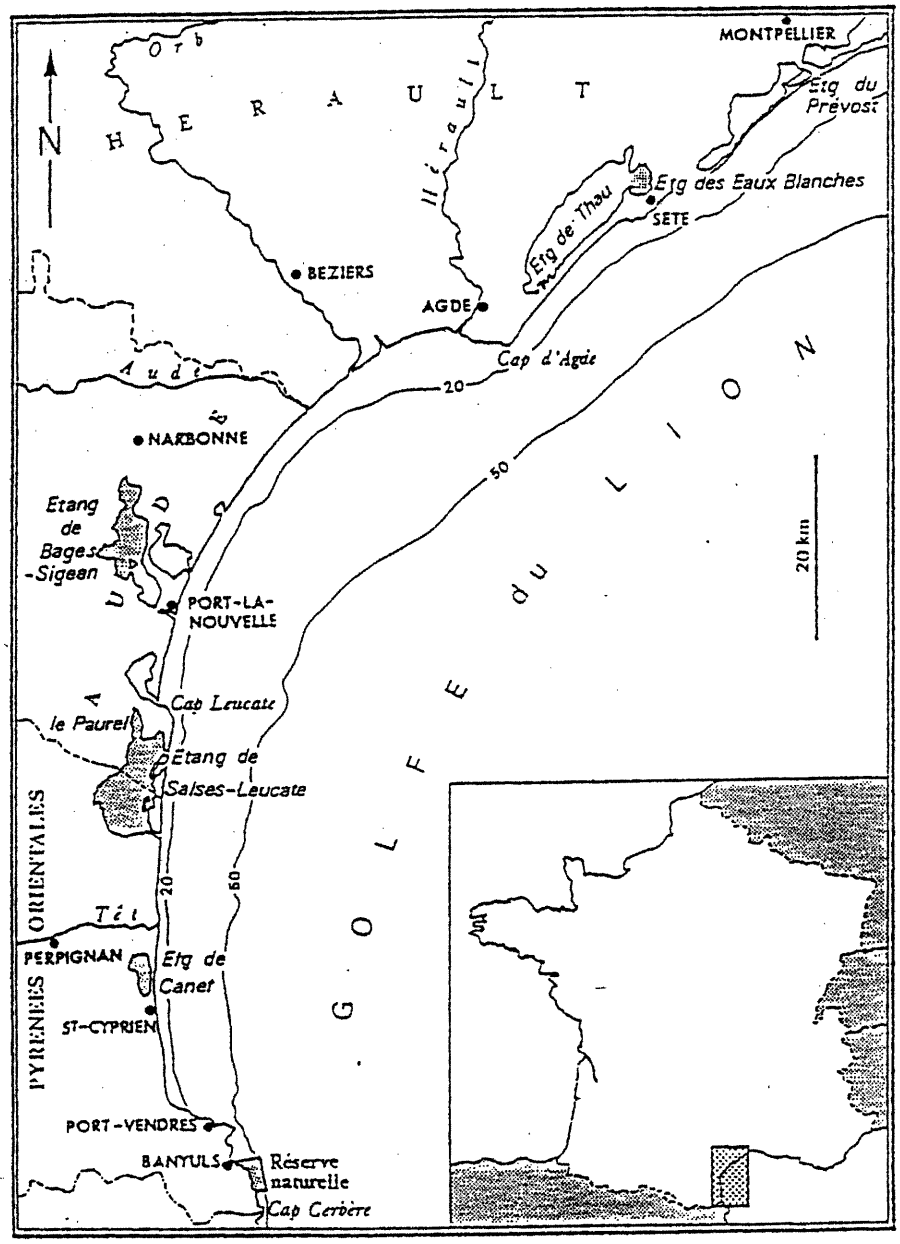


Figure II.4 : Zone d'étude : Golfe du Lion (Pyrénées-Orientales, France).

B. PHYLOGÉNIES

1. Méthodes

Le problème de la reconstruction phylogénétique a fait l'objet d'une littérature abondante (voir par exemple les revues de Felsenstein, 1988 ; Darlu & Tassy, 1993 ; Swofford et al., 1996 ; Page & Holmes, 1998). Plusieurs types de caractères peuvent être utilisés pour proposer une hypothèse des relations de parenté dans un groupe d'espèces : données morphologiques, biochimiques (nucléotides des séquences d'ADN, acides aminés correspondants, différents allèles enzymatiques, ...), écologiques, comportementaux. Les caractères les plus utilisés sont les attributs morphologiques des espèces ainsi que les séquences d'ADN de certaines parties du génome bien choisies. Il faut noter que dans ce dernier cas la phylogénie obtenue est celle des fragments d'ADN étudiés, qui n'est pas forcément celle des espèces qui les contiennent (voir Page & Charleston, 1998).

Il y a trois grandes familles de méthodes utilisées pour la reconstruction phylogénétique à partir de séquences d'ADN. Elles nécessitent toutes un bon alignement préalable des séquences :

- *Les méthodes de distance (phénétique)* : elles se basent sur un calcul de la distance globale réalisé pour chaque paire d'espèces. Les distances calculées à partir de données moléculaires, comme c'est le cas ici, peuvent faire intervenir des modèles d'évolution moléculaire tenant compte de différences *a priori* dans les fréquences de base et les taux de substitution des bases ; ce sont les même modèles que dans les méthodes de maximum de vraisemblance. La matrice de distance obtenue va servir à élaborer un arbre binaire au moyen de divers algorithmes. Les plus connus sont la méthode "neighbour-joining" (NJ) et le groupement selon l'association moyenne (UPGMA : unweighted pair group method using arithmetic averages). L'UPGMA produit des arbres ultramétriques supposant l'existence d'une horloge moléculaire (taux d'évolution constant), contrairement au NJ qui produit des arbres additifs.
- *Les méthodes de parcimonie* : ces méthodes se basent sur les caractères et non sur les distances globales entre paires d'espèces. Dans le cas de séquences d'ADN, les caractères sont les positions des bases alignées, et leurs états sont les différentes

bases (A, C, G et T). Les méthodes de parcimonie visent à reconstruire l'arbre postulant le moins de changements évolutifs possibles, ce qui revient à minimiser le nombre des homoplasies, donc à maximiser celui des homologies. Il est possible de pondérer différemment chaque type de substitution, par exemple de A vers G ou de T vers C : c'est la parcimonie généralisée ("generalized parsimony"). De la même façon, les sites peuvent se voir attribuer des pondérations différentes. Le problème est d'estimer les pondérations des substitutions ou des caractères.

- *Les méthodes de maximum de vraisemblance* : ces méthodes concernent également les caractères individuels. Elles sont basées sur un modèle d'évolution moléculaire tenant compte des fréquences des bases, des taux de substitution et éventuellement de l'hétérogénéité de ces taux entre les sites. Il existe plusieurs modèles plus ou moins complexes, en fonction du nombre de paramètres inférés. Le but de ces méthodes est de maximiser la vraisemblance des données observées en fonction du modèle (dont les paramètres sont éventuellement estimés pendant le processus), de la topologie de l'arbre et des données (les séquences). Pour sélectionner une topologie, on choisi celle qui maximise la vraisemblance des données observées.

Les trois types de méthodes seront utilisés dans cette thèse. Certains auteurs (Swofford et al., 1996 ; Page & Holmes, 1998) admettent qu'actuellement, on ne peut pas considérer qu'un type de méthode soit clairement "supérieur" aux autres. Les phylogénies seront validées à l'aide de méthodes de ré-échantillonnage comme le bootstrap (Efron, 1979 ; Felsenstein, 1985b ; voir Lapointe, 1998, pour une revue des méthodes de validation).

Dans ce travail, nous élaborerons des phylogénies pour les *Lamellodiscus* et leurs hôtes à l'aide de séquences d'ADN, et ce pour plusieurs raisons :

- Cela permet d'obtenir des arbres phylogénétiques possédant des longueurs de branches estimées, ce qui est utile dans l'analyse de la coévolution hôte-parasite basée sur des matrices de distances (voir plus loin).
- Par rapport à l'utilisation de caractères morphologiques, la reconstruction d'une phylogénie à l'aide de caractères moléculaires permet d'étudier l'évolution morphologique des espèces considérées sans risquer de tomber dans un

raisonnement circulaire du type : inférence de la phylogénie par les données morphologiques et discussion de l'évolution de ces mêmes caractères sur cette phylogénie. L'utilisation de données moléculaires permet donc de réaliser une étude morphologique indépendante de l'étude phylogénétique.

- Le nombre de caractères moléculaires (les positions des bases) obtenus peuvent être très nombreux, de l'ordre de plusieurs centaines ou plusieurs milliers, alors qu'il est difficile d'obtenir autant de caractères morphologiques, en particulier pour de nombreuses espèces proches comme c'est le cas ici dans le genre *Lamellodiscus*. Un nombre faible de caractère rend l'hypothèse phylogénétique obtenue (i.e., l'arbre) plus fragile et difficile à valider.
- Ces caractères permettent d'utiliser des modèles d'évolution moléculaire, ce qui autorise l'application des méthodes maximum de vraisemblance basées sur ces modèles.
- Les séquences d'ADN sont des données "absolues", ne dépendant pas du contexte particulier de l'étude réalisée. Elles sont directement comparables à ce qu'on peut trouver dans la littérature et dans les banques de données.

2. Phylogénie des parasites

Nous avons choisi d'utiliser la technique du clonage après amplification de l'ADN car étant donnée la très petite taille des parasites et la faible quantité d'ADN récupérée et, souvent, amplifiée, le clonage permet d'obtenir en grande quantité un ADN de très bonne qualité pour le séquençage. Par contre, cette méthode demande davantage de temps. Le séquençage proprement dit est réalisé à l'aide d'un séquenceur automatique.

Nous avons choisi une région de l'ADN comprenant une partie de l'ADN de la sous-unité ribosomique 18S (ce fragment sera simplement appelé 18S par la suite) et l'Internal Transcribed Spacer 1 (ITS1) entier (voir Figure II.5). Ce choix est fondé sur plusieurs raisons :

- Ce fragment d'ADN comprend une région à évolution rapide, l'ITS1, qui est intéressante pour étudier les relations entre espèces proches, ainsi qu'une région à évolution plus lente, le 18S, avec lequel on peut mettre en évidence les divergences

entre espèces éloignées. Ce choix est intéressant pour l'étude d'un groupe d'espèces dont on ne sait rien de l'âge ni du taux d'évolution, comme c'est le cas ici. On dispose actuellement de relativement peu de données sur l'ADN des monogènes (Gusev, 1995 ; Cunningham et al., 1995 ; Cunningham, 1997 ; Bentz et al., 2001 ; Sinnappah et al., 2001 ; Olson & Littlewood, sous presse). L'ADN 18S et l'ITS1 de l'ADN semblent être de bons marqueurs de l'évolution au niveau spécifique chez des monogènes gyrodactylides (Cunningham, 1997) et polystomatides (Sinnappah, 1998 ; Sinnappah et al. 2001), pour lesquelles des amorces bien conservées sont connues et disponibles. Nous avons utilisé les amorces L7 (5'- TGA TTT GTC TGG TTT ATT CCG AT -3') et H7 (5'- GCT GCG TTC TTC ATC GAT ACT CG -3') définies par Verneau et al. (1997).

- L'ADN ribosomique, et le 18S en particulier, est de loin le fragment d'ADN le plus étudié, ce qui permet de trouver facilement des séquences dans les banques de données sur Internet et donc de confirmer que l'on a bien de l'ADN de monogènes. Cela permet également de trouver facilement des extra-groupes.

Ce fragment a été séquencé pour au moins deux individus par espèce, et pour trois clones pour chaque individu, cela pour s'assurer de la validité des séquences.

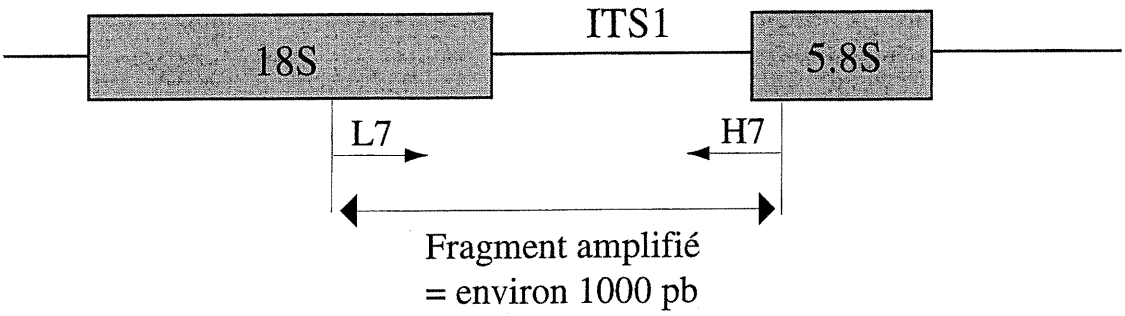


Figure II.5 : Région de l'ADN ribosomique amplifiée par le couple d'amorces L7-H7.

Nous utiliserons plusieurs extra-groupes : *Diplectanum aequans*, qui est un monogène de la même famille que les *Lamellodiscus*, les Diplectanidae; *Pseudomurraytrema ardens*, dont la séquence est disponible sur Internet dans GenBank, sera également utilisé car les Pseudomurraytrematidae, dont il fait partie, forment un

groupe-frère des Diplectanidae (Boeger & Kritsky, 1997); enfin, nous utiliserons aussi *Dactylogyrus minor* (Dactylogyridae) qui est plus éloigné des *Lamellodiscus*.

Pour la reconstruction phylogénétique, nous avons donc utilisé des méthodes de parcimonie, de maximum de vraisemblance et de distances. Pour la parcimonie, un algorithme "branch-and-bound" a été utilisé sans aucun schéma de pondération. En maximum de vraisemblance et en distance (NJ), un modèle HKY 85 a été employé ; l'hétérogénéité du taux de substitution a été estimée à l'aide une distribution gamma de paramètre α égal à 0.18, estimé à partir des arbres obtenus par parcimonie.

La phylogénie des Diplectanidae, nécessaire pour la recherche des déterminants de la spécificité dans ce groupe et pour définir des extra-groupes, a été élaborée à partir de caractères morphologiques définis à l'aide des descriptions des genres publiées dans la littérature. La matrice de caractères a été analysée à l'aide des méthodes de parcimonie avec l'algorithme "branch-and-bound" de PAUP* (Swofford, 2001). Le genre *Pseudomurraytrema* a été utilisé comme extra-groupe à cause de sa position de groupe-frère des Diplectanidae dans la phylogénie publiée par Boeger & Kritsky (1997).

3. Phylogénie des hôtes

Il est souhaitable d'obtenir la phylogénie des hôtes afin de pouvoir étudier les interactions coévolutives hôte-parasite ayant pu influencer le profil de spécificité, et également pour définir de façon plus fine la spécificité (voir plus loin). Hanel & Sturmbauer (2000) ont récemment proposé une phylogénie moléculaire des Sparidae de Méditerranée. Après avoir constaté que l'ADNr 18S ne fournissait pas assez de variabilité pour permettre d'inférer les relations phylogénétiques entre ces espèces, nous avons décidé de séquencer partiellement le gène mitochondrial du Cytochrome-b (appelé cyt-b par la suite), afin de confirmer et préciser leur résultat. Le séquençage proprement dit a été effectué par Olivier Jousson (Université de Genève). Ce fragment d'ADN a déjà été reconnu pour être un marqueur intéressant pour étudier l'évolution des Téléostéens (Cantatore et al., 1994 ; Song et al., 1998). Nous désirons comparer l'hypothèse phylogénétique obtenue par Hanel & Sturmbauer (2000) à l'aide des séquences d'ADNmt 16S, au résultat obtenu avec nos séquences de cyt-b. Ensuite, si les deux jeux de données ne

sont pas déclarés significativement différents par un test d'homogénéité (Partition Homogeneity Test ; Farris et al. 1994) et significativement semblables par un test de Mantel (1967), nous grouperons les deux jeux de séquences dans une analyse combinée ("total evidence", appelée 16S + cyt-b). Nous espérons ainsi augmenter la précision de l'arbre phylogénétique. Nous utiliserons *Dicentrarchus labrax* (Moronidae) comme extra-groupe dans l'analyse des séquences d'ADNmt cyt-b, et le même extra-groupe que Hanel & Sturmbauer (2000) pour l'ADNmt 16S, *Spicara maena* (Centracanthidae). Nous avons également utilisé les méthodes de parcimonie, de maximum de vraisemblance et de distances pour estimer la phylogénie des hôtes. Pour la parcimonie, un algorithme "branch-and-bound" a été utilisé sans aucun schéma de pondération. Un modèle HKY 85 a été utilisé pour les analyses en maximum de vraisemblance et NJ ; l'hétérogénéité du taux de substitution a été estimée à l'aide une distribution gamma de paramètre α de 0.17 estimé à partir de l'arbre le plus parcimonieux. En parcimonie, une pondération de 14 a été attribuée aux transitions à la première et à la troisième position des codons des séquences correspondant au cytochrome-b. Cette valeur est issue de l'estimation par maximum de vraisemblance du ratio transition/transversion (Ti/Tv), qui est sensiblement égal à la valeur maximale observée (13.4) pour les espèces les plus proches. L'algorithme heuristique de PAUP* a été utilisé pour la reconstruction phylogénétique.

C. Coévolution

Le terme "coévolution" est utilisé ici pour décrire les relations macroévolutives entre la phylogénie des parasites et celle de leurs hôtes. Il s'agit de mesurer à quel point ces deux arbres sont congruents. Quand ils sont parfaitement congruents, cela indique que la l'association hôte-parasite a subi uniquement des phénomènes de cospéciation, et ainsi que la coévolution est "parfaite". Cela correpond à la définition utilisée par Brooks (1979, 1985), Klassen & Beverley-Burton (1987), Brooks & McLennan (1991), et Klassen (1992). Cela ne doit pas être confondu avec un sens plus restrictif utilisé en génétique, et de manière générale dans un contexte microévolutif, où la coévolution est définie comme l'influence du génome des hôtes sur celui des parasites (Toft & Karter, 1990) et, d'une manière générale, comme l'apparition de modifications chez une espèce en réaction envers l'influence de l'autre (hypothèse de la Reine Rouge : Van Valen, 1973). En d'autres termes,

le sens utilisé est centré sur l'étude des événements de cladogénèse, alors que le second est davantage tourné vers l'anagénèse.

Il existe plusieurs méthodes pour étudier la coévolution entre les parasites et leurs hôtes. Parfois, quand l'association étudiée est suffisamment simple, l'observation visuelle des arbres peut suffire (e.g., Verneau et al., 1997). Mais dans la plupart des cas, une méthode analytique précise est préférable (voir revues dans Ronquist, 1995 ; Page & Holmes, 1998 ; Paterson & Banks, 2001).

La première méthode spécifiquement conçue pour l'étude de la coévolution dans les associations hôte-parasite est la "Brooks Parsimony Analysis" ou BPA (Brooks, 1981 ; Brooks and McLennan, 1991). Elle consiste en la reconstruction d'une phylogénie des hôtes en utilisant les parasites comme caractères, tout en prenant en compte les relations phylétiques entre ces derniers. L'arbre obtenu est comparé à l'arbre original des hôtes et les différences sont expliquées par des événements évolutifs comme la capture (voir par exemple Paterson et al., 1993 et Boeger & Kritsky, 1997). Cette méthode est essentiellement descriptive. Une autre méthode, l'analyse des composantes ("component analysis" : Nelson & Platnick, 1981) a été popularisée par Page (1993a,b,c). Elle est basée sur la comparaison des topologies des arbres. Cette méthode cherche les nœuds équivalents (conduisant aux mêmes espèces) dans les deux arbres et teste si la ressemblance obtenue est plus grande qu'une ressemblance due au hasard, mais elle ne permet pas la prise en compte des événements de capture. La méthode des arbres réconciliés ("reconciled trees"), également proposée par Page (1994a,b), pouvait incorporer de tels événements, mais sans les gérer correctement (Charleston, 1998). Le programme TreeMap permet de tester si le système hôte-parasite étudié est l'objet d'une "cospéciation significative", c'est-à-dire si le nombre d'événements de cospéciation estimé par TreeMap est supérieur à ce qu'on obtiendrait par hasard. Cela est réalisé en permutant aléatoirement l'arbre des parasites, celui des hôtes, ou les deux, et en estimant le nombre d'événements de cospéciation inférés pour chacune de ces associations aléatoires, qui représentent donc autant de réalisations de l'hypothèse nulle de non-congruence entre les arbres. TreeMap cherche ainsi à maximiser le nombre de cospéciations dans la reconstruction. Ronquist (1995) a proposé d'utiliser une méthode basée sur la parcimonie généralisée, dans laquelle les transitions entre états de caractères peuvent être pondérées. Cette méthode cherche également à obtenir une

congruence de l'arbre des parasites avec celui des hôtes en prenant en compte un coût différent pour les quatre types d'événements coévolutifs potentiels (Ronquist, 1995 ; Page and Charleston, 1998 ; Paterson and Banks, 2001) : cospéciation, duplication, disparition et capture (Figure II.6). La reconstruction optimale est celle qui minimise le coût global. Cela est réalisable à l'aide du programme TreeFitter de Ronquist (2001, disponible à l'adresse http://www.ebc.uu.se/systzoo/research/treefitter/treefitter.html) qui permet de tester statistiquement, par permutations, le coût global de la reconstruction ainsi que de chaque type d'événement. Un autre point intéressant de TreeFitter est qu'en faisant varier le coût de chaque événement, il permet de retrouver les conditions des méthodes BPA et TreeMap. Un problème est que la version actuelle (1.0) ne permet pas d'obtenir de sortie graphique des résultats, contrairement à TreeMap, ce qui rend difficile la proposition de scénarios évolutifs précis. Huenselbeck et al. (1997) ont proposé d'utiliser une méthode de maximum de vraisemblance pour tester l'hypothèse de cospéciation hôte-parasite. Cette méthode teste si les deux arbres sont significativement différents, en s'appuyant sur un modèle d'évolution moléculaire. Elle teste d'abord si les topologies sont les mêmes, puis si les longueurs de branches le sont, et enfin si les taux d'évolution sont les mêmes chez les hôtes et les parasites. Cette méthode est limitée à l'étude de phylogénies basées sur des séquences d'ADN et à des associations du type un hôte-un parasite. De plus, elle n'incorpore pas les événements de duplication et d'extinction. C'est aussi le cas d'une autre méthode proposée par Huenselbeck et al. (2000), basée sur le principe de l'inférence Bayesienne, qui permet en plus d'estimer des intervalles de confiance pour le nombre et la position des événements de capture.

Toutes les méthodes précédemment mentionnées fonctionnent de façon optimale avec des associations du type un hôte-un parasite, même si aucune limitation théorique ne s'oppose à des nombres différents, sauf pour les méthodes basées sur le maximum de vraisemblance et l'inférence Bayesienne. Si ces nombres augmentent, tout comme la taille des phylogénies, le problème peut devenir trop complexe pour permettre de trouver des solutions optimales. De plus, elles ont en commun de comparer les topologies des arbres évolutifs des hôtes et des parasites, ce qui permet de proposer des scénarios évolutifs pour l'association hôte-parasite concernée, mais qui suppose que ces arbres soient bien connus et, si possible, uniques. En effet, l'absence ou l'ajout d'une espèce à un des deux arbres peut

changer de façon plus ou moins importante la congruence entre les deux arbres, surtout si les nombres d'espèces considérés sont peu élevés. Cela suppose donc une bonne connaissance et un échantillonnage exhaustif des groupes étudiés. Mais il est impossible, en pratique, de savoir si une espèce actuellement éteinte prend place dans une des topologies, ce qui pourrait changer le scénario évolutif proposé (voir Brooks & McLennan, 1991).

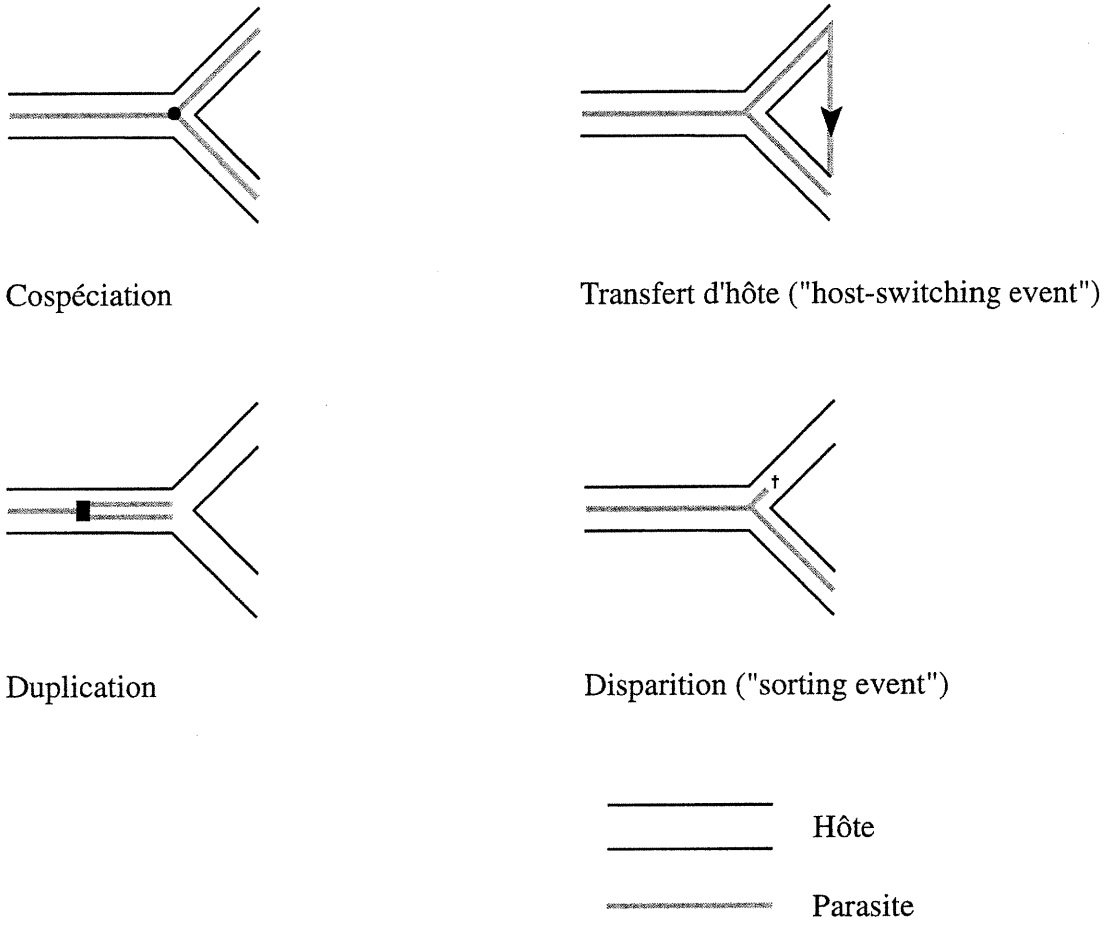


Figure II.6 : Les quatre événements coévolutifs.

Les distances phylogénétiques entre les espèces étudiées ne sont pas forcément bien estimées à partir des arbres évolutifs puisque ceux-ci sont eux-mêmes le résultat d'une estimation qui peut être plus ou moins heureuse. Les distances phylogénétiques peuvent éventuellement être directement estimées à partir des données brutes (séquences, caractères morphologiques, ...). Bien sûr, de telles distances sont des mesures moins précises des relations évolutives interspécifiques que celles qui seraient calculées à partir des arbres

phylogénétiques si ceux-ci étaient connus avec précision, mais elles ne dépendent pas des hypothèses nécessaires à la reconstruction d'un arbre (la moindre n'étant pas le choix de la méthode). De plus, si de multiples arbres sont générés par la reconstruction phylogénétique, par exemple plusieurs arbres également parcimonieux, il est fastidieux d'étudier toutes les associations possibles entre les arbres. Il peut être plus simple de travailler directement sur les distances brutes entre les espèces.

Une nouvelle méthode d'étude de la coévolution hôte-parasite, basée sur les distances phylogénétiques estimées entre les espèces, sera utilisée ici, en plus de TreeFitter et TreeMap. Cette méthode est mise en œuvre dans le programme ParaFit, et a été décrite par Legendre, Desdevises & Bazin (sous presse ; voir Annexe A). Le calcul utilise des matrices de distance brutes ou patristiques (i.e., calculées à partir des arbres phylogénétiques). Elle peut être utilisée avec toutes sortes de matrices de distance et avec n'importe quel nombre de parasites/hôtes par hôte/parasite. Elle est basée sur les statistiques du "quatrième coin" (Legendre et al., 1997 ; voir Legendre & Legendre, 1998, section 10.6), qui consistent à mesurer la congruence entre deux matrices incommensurables (ici des matrices de distance pouvant contenir des nombres différents d'objets, i.e. d'hôtes et de parasites), reliées par une troisième (ici une matrice décrivant les associations hôte-parasite individuelles, ou liens). Les matrices de distance phylogénétique sont transformées en un tableau rectangulaire par une analyse en coordonnée principale (Gower, 1966 ; voir Legendre & Legendre, 1998). La troisième matrice est une matrice binaire représentant les présences (1) ou absences (0) des parasites sur les hôtes. La méthode combine les trois matrices en une seule (le "quatrième coin"), d'où est extraite une mesure de la congruence entre les matrices de distance des hôtes et des parasites, contrainte par les associations hôte-parasite individuelles. Cette congruence est testée par une procédure permutationnelle, en permutant aléatoirement N fois la matrice de liens (les parasites sont attribués aléatoirement aux hôtes disponibles), ce qui génère N réalisations de l'hypothèse nulle de non-association entre les deux clades. La valeur correspondant à l'association observée est testée contre la distribution aléatoire générée par les permutations. ParaFit peut également tester l'influence de chaque lien hôte-parasite sur la congruence globale entre les matrices de distance, et ainsi trouver les espèces contribuant le plus à l'association évolutive entre les hôtes et leurs parasites. Ces espèces forment les associations coévolutives les plus probables.

Différentes méthodes vont donc être utilisées pour étudier la coévolution entre les *Lamellodiscus* et les Sparidae, une association complexe avec un nombre variable d'hôtes par parasite et de parasites par hôte. Nous tenterons ainsi d'estimer si l'histoire évolutive des parasites est dépendante de celles de leurs hôtes et donc de voir si la spécificité des parasites est contrainte par leurs liens historiques avec leurs hôtes, ou si au contraire elle semble plus dépendante des conditions écologiques actuelles.

D. ANALYSE COMPARATIVE

1. Déterminants de la spécificité

Dans cette étude, nous serons amenés à comparer certaines caractéristiques au sein d'un groupe d'espèces. Cependant, ces espèces sont liées par une histoire évolutive, et ne représentent donc pas des objets indépendants. Les corrélations recherchées entre les caractéristiques devront être établies à l'aide de méthodes spéciales, tenant compte des relations phylogénétiques entre les espèces : ce sont les méthodes comparatives (voir Brooks & McLennan, 1991 ; Harvey & Pagel, 1991). Il existe toute une gamme de méthodes comparatives, la plus connue étant celle des contrastes indépendants (Felsenstein, 1985a). Cette méthode consiste à estimer les différences (contrastes) entre les groupes-frères de la phylogénie, puis de réaliser les tests statistiques sur ces valeurs indépendantes. Les contrastes doivent être standardisés à travers l'arbre phylogénétique et la régression utilisée ensuite doit être forcée à l'origine (Garland et al., 1992). La méthode des contrastes indépendants est utilisable, entre autres, à l'aide du programme CAIC (Comparative Analysis using Independent Contrasts) de Purvis et Rambault (1995). Les contrastes peuvent ensuite être utilisés comme n'importe quelle variable quantitative, dans des analyses de régression simple ou multiple par exemple.

Dans la recherche des déterminants de la spécificité parasitaire, nous avons vu que plusieurs facteurs sont susceptibles d'influencer la spécificité parasitaire. De plus, ces facteurs peuvent être liés les uns aux autres. Il est donc important d'utiliser des méthodes qui permettent de prendre en compte toutes ces variables simultanément, soit des méthodes d'analyse multivariables (voir Legendre & Legendre, 1998).

Nous désirons étudier l'influence de toute une série de variables indépendantes (phylogénie, variables morphologiques et écologiques) sur une variable dépendante, la spécificité. Ceci requiert une approche de type régression multiple (Legendre & Legendre, 1998).

Il reste à définir maintenant la façon d'exprimer chaque variable :

- **Spécificité.** Elle est ici exprimée par le nombre d'hôtes. Il existe plusieurs façons d'exprimer la spécificité parasitaire. On peut prendre en compte la prévalence et l'intensité (Rohde, 1979, 1994) mais ces facteurs varient dans le temps et l'espace (Kennedy, 1975). Dans un contexte macro-évolutif, le nombre d'hôtes est mieux adapté. On peut définir comme spécialistes les parasites utilisant un seul hôte et comme généralistes les espèces en utilisant plusieurs (Euzet & Combes, 1980 ; Simkova et al., 2001). Cette distinction peut sembler simpliste, spécialement dans le cas des généralistes. En effet certaines espèces de parasites utilisent deux espèces d'hôtes très proches alors que d'autres utilisent de nombreuses espèces répandues à travers plusieurs genres. Il faut pouvoir rendre compte de cette différence. Il est donc important de prendre en compte la position phylogénétique des hôtes parasités. Ainsi, un indice composé de quatre classes de spécificité a été défini : 1- *specialistes* utilisant un seul hôte; 2- *spécialistes intermédiaires* utilisant deux hôtes proches phylogénétiquement; 3- *généralistes intermédiaires* utilisant des hôtes dans la même clade; 4- *généralistes* utilisant des hôtes appartenant à plusieurs clades. Il est donc nécessaire de posséder une phylogénie des hôtes et d'y définir des clades. Cet indice, comme le nombre d'hôtes, varie en sens inverse de la spécificité : plus il croît, plus la spécificité est faible. Cet indice est donc nommé INS, pour Indice de Non-Spécificité.

- **Variables morphologiques.** La taille du parasite, corrélée à celle du hapteur et à d'autres variables morphométriques, sera utilisée. Les mesures ont été prises directement sur les individus étudiés, ainsi que dans la littérature pour les espèces dont trop peu de représentants étaient disponibles.

- **Variables écologiques.** Ce sont essentiellement les variables liées à l'hôte : taille, abondance, grégarité. Ces variables sont tirées de la littérature (Whitehead et al.,

1986). La taille est une variable continue, l'abondance et la grégarité sont exprimées sous forme de classes.

- **Nombre d'hôtes potentiels.** Cette variable, utilisée par Poulin (1992), est le nombre d'hôtes présents dans le(les) clade(s) contenant le(s) hôte(s) parasité(s). On peut penser que ces hôtes sont plus compatibles avec le parasite considéré et donc plus susceptibles d'être parasités.

Afin d'étudier si la spécificité est liée à la phylogénie, il faut pouvoir exprimer celle-ci sous forme de variable. Une solution consiste à utiliser la matrice de distance patristique issue de l'arbre phylogénétique pour représenter la phylogénie dans l'analyse. En transformant la variable spécificité également en matrice de distance (en utilisant une distance euclidienne par exemple), il serait possible d'établir le lien statistique entre les deux matrices à l'aide du test de Mantel (1967). Cependant, Dutilleul et al. (2000) ont montré que la corrélation entre deux vecteurs était plus forte que la corrélation de Mantel entre deux matrices de distance issues de ces vecteurs. Legendre (2000) a montré, pour le même cas, que la puissance du test de la corrélation de Pearson était plus importante que celle du test de Mantel. Nous avons donc décidé de transformer plutôt la matrice de distance patristique en un tableau rectangulaire à l'aide d'une analyse en coordonnée principale (Gower, 1966). Les coordonnées principales issues de cette analyse sont des variables indépendantes dont la somme représente 100% de la variance phylogénétique (voir Figure II.7). Cette technique est efficace pour représenter l'inertie phylogénétique (Diniz-Filho et al., 1998). Les coordonnées principales sont ensuite simplement utilisées comme variables dans les analyses statistiques. Un lien significatif entre une ou plusieurs de ces variables et la spécificité, identifié par une régression multiple, indique la présence d'une corrélation entre la spécificité et la phylogénie.

Cette façon de quantifier l'inertie phylogénétique permet de partitionner la variation de la variable *spécificité* (INS) entre la variance phylogénétique et la variance due aux facteurs environnementaux. Comme la variation due à l'environnement et à la phylogénie peuvent ne pas être indépendants (Westoby et al., 1995), il est intéressant de pouvoir quantifier la portion de la variation de la variable dépendante qui due à l'effet conjugué de ces deux influences (Figure II.8). Une méthode a été proposée pour ce faire et elle sera

utilisée dans cette thèse. Pour une explication plus détaillée de cette méthode, voir Desdevises, Azouzi, Legendre & Morand (soumis, Annexe B).

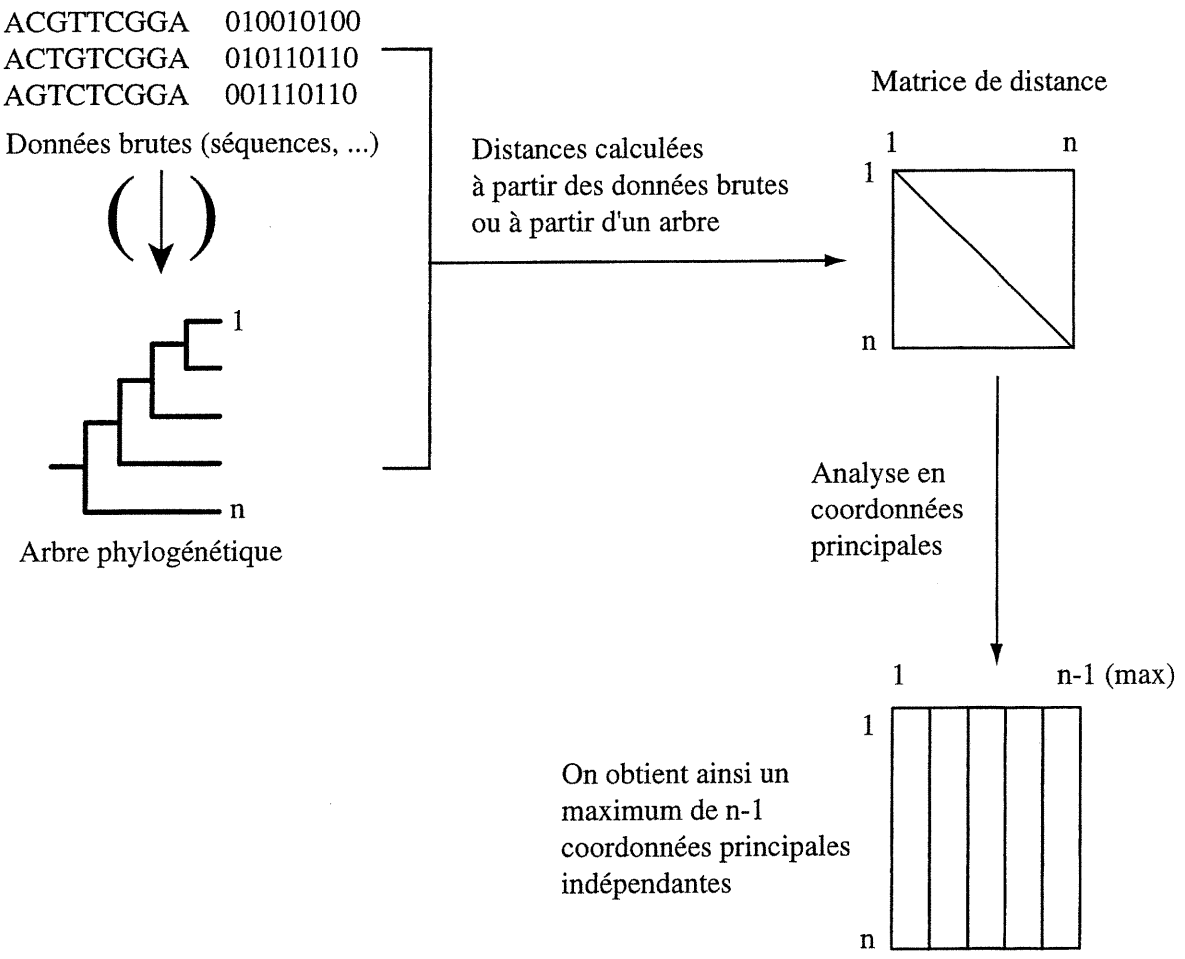


Figure II.7 : Transformation des relations phylogénétiques en coordonnées principales.

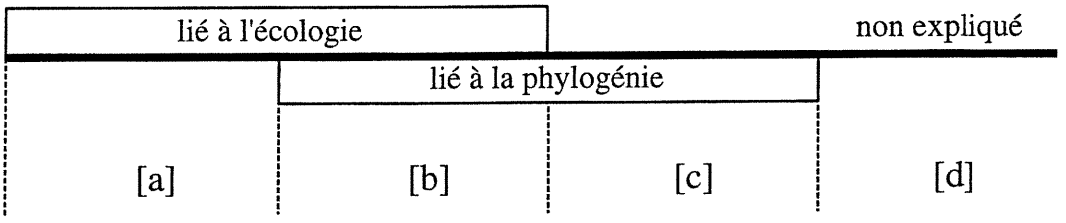


Figure II.8 : Partition de la variation d'une variable (trait épais) entre les effets écologiques et phylogénétiques.

2. Évolution de la spécificité

La technique de l'optimisation des caractères par parcimonie (Farris, 1970 ; Brooks & McLennan, 1991) sera utilisée afin de visualiser l'évolution de la spécificité sur l'arbre phylogénétique des parasites. L'indice INS sera cartographié sur cet arbre. Cela permettra de voir si la spécificité est une caractéristique dérivée, conduisant éventuellement à un cul-de-sac évolutif, comme cela a été proposé par plusieurs auteurs (Huxley, 1942 ; Simpson, 1953 ; voir Futuyma & Moreno, 1988 ; Thompson, 1994). Pour étudier cette même hypothèse, une régression de l'INS contre le nombre de nœuds séparant chaque espèce de la racine de l'arbre sera également utilisée. En effet, si la spécificité tend à être un caractère dérivé, cette relation devrait être significative, montrant une augmentation de la spécificité avec l'augmentation du nombre de nœuds.

3. Lien entre spécificité et diversification taxonomique

Ceci est une analyse comparative entre la spécificité, d'une part, et la diversification taxonomique des parasites d'autre part. Il est nécessaire d'utiliser la méthode comparative puisque l'effet de la phylogénie doit être contrôlé dans l'analyse. La diversification est représentée par le nombre d'espèces par clade. Cette variable ne peut donc être calculée pour les espèces individuelles, au niveau des feuilles de l'arbre, mais prend son sens au niveau des nœuds, c'est-à-dire des espèces ancestrales. Il s'agit de faire une comparaison de groupes-frères, ce que Barraclough et al. (1998) considèrent comme la meilleure approche pour étudier ce type de problème. Par ailleurs, la diversification ne peut pas être utilisée de la même façon que n'importe quelle autre variable dans une analyse comparative. En effet, la valeur d'une variable à un nœud est ordinairement la moyenne des valeurs aux nœuds descendants si on utilise des longueurs de branches égales et un modèle d'évolution brownien. Dans le cas de la diversification, cette valeur n'est pas la moyenne mais la somme des valeurs aux nœuds immédiatement issus du nœud pour lequel on la calcule. Le logiciel MacroCAIC écrit par P.-M Agapow (disponible à l'adresse http://www.bio.ic.ac.uk/evolve/software/macrocaic/) et dérivé du programme CAIC de Purvis et Rambault (1995), permet de calculer la corrélation entre une variable et la

diversité taxonomique. Les contrastes obtenus sont ensuite utilisés comme variables dans des régressions classiques. Pour chaque nœud de la phylogénie, la variable représentant la diversification taxonomique est le logarithme népérien (ln) du rapport entre le nombre d'espèces du clade dont la spécificité moyenne est la plus basse et le nombre d'espèces pour le clade de plus forte spécificité moyenne; ce rapport sera nommé CladeRatio. Quand ce rapport est plus petit que 1, le ln est négatif et le clade dont la spécificité moyenne est la plus faible contient davantage d'espèces, et inversement. L'analyse est réalisée pour chaque paire de groupes-frères à travers la phylogénie. Ce rapport est régressé contre le ln de la variable spécificité INS. Un lien entre la spécificité et la diversification taxonomique doit se matérialiser par une relation négative significative.

III. ARTICLES

A. DÉTERMINANTS DE LA SPÉCIFICITÉ AUX NIVEAUX SUPRA-SPÉCIFIQUES

Article 1

SASAL Pierre, Yves DESDEVISES and Serge MORAND. 1998. Host-specificity and species diversity in fish parasites: phylogenetic conservatism? *Ecography* 21: 639-643.

Objectif a

Ce travail est centré sur l'étude de la spécificité pour l'hôte dans les différents grands groupes de parasites (Monogènes, Digènes, Cestodes, Nématodes, Copépodes, Acanthocéphales). Nous montrons que le même type de spécificité semble conservé à l'intérieur des grands groupes de parasites étudiés, suggérant que des contraintes phylogénétiques à grande échelle sont impliquées dans son déterminisme. La localisation géographique et l'habitat ne semblent pas jouer un grand rôle quand au déterminisme de cette spécificité. Enfin, le lien entre diversité taxonomique et spécificité (nombre d'hôte) est mesuré.

Participation du thésard :

- Interprétation des résultats
- Discussion

Bien que je n'en sois pas le premier auteur, j'ai inclus cet article dans ma thèse parce que je pense que cela ajoute à la cohérence de l'ensemble. Ce travail offre une bonne introduction conceptuelle à la problématique constituant le cœur de ce travail et offre une vue du problème à une échelle taxonomique globale, qui sera resserrée ensuite. Ne pas inclure cette publication se traduirait par un manque car je dois m'y référer constamment.

HOST-SPECIALIZATION AND SPECIES DIVERSITY IN FISH PARASITES: PHYLOGENETIC CONSERVATISM?

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Abstract

The pattern of parasite species diversification and specialization, appreciated by host range, is investigated in fish parasites. We test whether host range is linked with phylogeny at a high taxonomic level, and if there is a relationship between host range and host species diversification. For this purpose we used two sets of data, one on macro-parasites of marine fishes of the Mediterranean Sea and the other on macro-parasites of marine and freshwater fishes of Canada. Similar patterns of host range among parasitic groups were found. Our findings suggest that habitat (marine *versus* freshwater) and geographic localisation (Canada *versus* Mediterranean region) play little role in determining the observed patterns of host range. We highlight the potential influence of phylogeny (high-taxonomic level) on the level host range in parasites. We find that parasites with free-swimming larval stages and with direct life cycles have a narrower range of host species than do parasites with indirect life cycle, even if we cannot control for phylogenetic effects because of the lack of variation of life cycles within each parasitic group. Finally, a positive relationship was found between the number of known hosts and parasite species diversity in the case of Mediterranean parasite species. The relationship between host range and species diversification should be related to the mechanism of cospeciation.

Introduction

Species diversification and species richness have been the topic of numerous studies (see Rosenzweig 1995) and many theoretical studies have tried to explain why some groups of organisms are much more diverse than others (Janzen 1973; May 1975; Stanley 1979; Cockburn 1991). Parasites are ideal candidates for testing evolutionary concepts because the resource environment can be more easily defined in space (individual hosts) and in time (host phylogeny) (Price 1980, 1982; Rohde 1982; Brooks and McLennan 1991, 1993a; Thompson 1994; Poulin 1997). Some authors have investigated the determinants of species diversification among parasitic organisms, referring to key innovations, i.e. the appearance of homoplasic character(s) which is (are) correlated with species diversification (Brooks and McLennan 1993b; but see also Rohde 1996). On the other hand, some studies have emphasized that specialization for a parasite, which is defined here as host range, may appear to be an important feature in species diversification (Brooks and McLennan 1991, 1993a; Poulin 1992).

There is a an apparent paradox between species diversification and specialization as emphasized by Brooks and McLennan (1993a) and one can hypothesize that the opportunities for host colonization and subsequent speciation are in inverse proportion to the degree of host specificity (or host range). That means, a more widely distributed parasite species, in term of host range, may be able to colonize more easily new host species. However, Brooks and McLennan (1993a) have argued that "if colonization of a new host leads to speciation and the establishment of a unique association, then the host must have acted as a strong directional selection force. The chances of this occurring should be higher for species with pronounced host specificity, because they are theoretically more sensitive to changes in the host component of their environments than their more tolerant, generalist relatives".

Our aim is to test whether host range is linked with (1) phylogeny at a high taxonomic level and (2) species diversification in host. For this purpose we used two sets of data, one on parasites of marine fishes of the Mediterranean Sea (Sasal, Morand and Guégan 1997) and the other on parasites of marine and freshwater fishes of Canada (Margolis and Arthur 1980).

Material and methods

Host range (Lymbery 1989; Rohde 1980) is defined as the number of species of hosts infected by a given species of parasites. Host range may be estimated from records of host occurrence. Rohde (1980) and Lymbery (1989) distinguish host specificity from host range by taking into account in the former "the prevalence and/or intensity of infection in each species of host". Unfortunately, all data on prevalence or intensity are not available, so host range will be used here as a measure of host specificity.

Data on hosts and parasites

Data for six groups of adult macro-parasites were collected: cestodes, nematodes, digeneans, monogeneans, copepods and acanthocephalans. Parasites can exhibit simple (or direct) life cycles or complex (or indirect) life cycles. In the case of a simple life cycle, parasites use a unique host. Complex life cycles involve two or more hosts. For the groups studied here, only monogeneans and copepods have a simple life cycle, all the other groups, cestodes, nematodes, digeneans and acanthocephalans, exhibit complex life cycles.

The factors responsible for interspecific variability in host-specificity were investigated within 225 species of metazoan parasite species found in Canadian freshwater fishes, 340 parasite species found in Canadian marine species (Margolis and Arthur 1980) and 155 species of metazoan parasites found in Mediterranean marine fishes (Sasal et al. 1997).

Statistical analysis

Mean host range (number of known hosts per parasite species) was compared with two two-way ANOVA, in which factors are parasite group and environment, and parasite group and geographic locality, to show the effect of each factor on the variable and their interaction.

Results

We found a positive relationship between the number of studies found in the litterature concerning one parasite species and the number of hosts recorded for this parasite species ($p<0.0001$), in the case of parasites of Canada. This relationship is quite

obvious since a largely distributed generalist parasite species (large host range) has a greater chance to be recorded in various studies. We found no relationship between the number of host species recorded by parasite species and the number of hosts studied ($p=0.37$) in the Mediterranean Sea. This result shows that host sample size has no influence on the estimation of host range, suggesting that it is not useful to control the data for host sampling effort.

Host range among parasitic groups

Host range according to parasitic groups was compared between freshwater fish parasites and marine fish parasites from Canada and the Mediterranean Sea (Fig. 1). The general pattern of host range depicted for Canadian fish parasites (marine and freshwater) was also found for Mediterranean fish parasites (Fig. 1). Host range differed significantly between parasitic groups (Tables 1 and 2). Nematodes and acanthocephalans had the largest mean host range whereas monogeneans and cestodes had lower host ranges. Similar mean numbers of known host species per parasite species were found for each group of parasites, in each geographic localisation.

There was a significant influence of parasitic groups on the values of host range (ANOVA, Table 1, Table 2).

There was no influence of host geographic localisation (freshwater Canada, marine Canada, Mediterranean Sea) on host range (ANOVA, Table 1).

There was also no influence of host environment (marine *versus* freshwater) on host range (ANOVA, Table 2).

Influence of parasite life cycle

The distribution of host range was aggregated: most parasite species were recorded from a few species of hosts (Fig. 2). The range of number of known hosts per parasite species is wider for parasites having indirect life cycles (1-50 host species) than for those having direct life cycle parasites (1-30 host species).

A significant effect of the type of parasite life cycle (direct *versus* indirect) was detected (Fig. 3; ANOVA, $F_{1,718}=38.12$, $p<0.0001$). Direct life cycle parasites have a significantly lower host range than indirect life cycle parasites. This result is found as a

general pattern for the global data, however it seems to be different when we considered only eucestoda and copepoda (see Fig. 1).

Host-range and parasite species richness

A negative relationship was found between the mean number of known hosts and the number of parasite species (R^2=0.26; b=-0.152; p=0.027) (Fig.4), which indicates that high host specificity for parasites (low mean number of infected hosts) is correlated with high species diversity.

A positive relationship was found between the number of known host species and the number of known parasite species in the case of Mediterranean fish (note that we add the case of parasitic isopodes) (R^2=0.85; b=1.492; p=0.0032) (Fig. 5).

Discussion

Several pitfalls have to be avoided in a study of host range. One important bias results in differential host sampling effort. Using data from well-known and intensively studied faunas (Canada and Mediterranean Sea) can prevent bias due to poorly or not studied hosts. Moreover, the lack of relationship between host range and host sampling effort in the Mediterranean Sea supports the view that our data set is suitable for this kind of analysis.

Poulin (1992) found a positive relationship between the number of potential host species and the number of known hosts among the 176 species of Canadian freshwater fish parasites included in his analysis. We found similar trends of host range between Canadian fish parasites (reanalyses of the same source of data as those of Poulin 1992) and Mediterranean fish parasites.

Our finding suggests that habitat (marine *versus* freshwater) and geographic localisation (Canada *versus* Mediterranean region) play little or no role in determining the observed pattern of host range.

Our finding also highlights the potential influence of phylogeny (high-taxonomic level) on the host range (Poulin 1992, 1997; Morand 1996) of parasites. Clearly, each group of parasite taken separatly reflect a similar host range in the different geographic localizations (that means in different environments); monogeneans on one extreme, high specialization (or low host range) and acanthocephalans on the other, low specialization (or

high host range). The view that parasites are more or less specific depending on the geographical localisation is thus strenghtened (see Rohde 1978). However, we did not test the influence of latitude on the relationship between host range and host diversification (see Rohde 1978; 1980).

As predicted by Noble et al. (1989), we found that parasites with free-swimming larval stages and with direct life cycles (monogeneans and copepodes) have a narrower range of host species than do parasites with indirect life cycle, which infect their definitive hosts through predator-prey relations. However, we cannot control for phylogenetic effects because of the lack of variation of life cycles within each parasite group (Morand 1996).

The determinants and patterns of host specialization among groups of parasites are not totally clarified by our findings. Poulin (1992) failed to find any relationship between host range and fish body size but, recently, Sasal and Morand (1998) argued that host range in the Monogenea is correlated with fish body size, large-bodied fish harbouring more specific parasites than small-bodied fish. The relationship between fish body size and host range of monogeneans was interpreted as a specialization on predictable hosts, as fish body size correlates with many life traits such as longevity or fecundity (Winemiller and Rose 1992). However, the pattern found for a certain group of parasites, here the Monogenea, is not necessary applicable to all parasite groups.

The relationship between parasite species diversity and host species diversity was explored by Price (1980, 1982). There are several examples indicating a significant relationship between the number of available host species and the number of parasites that exploit them (Strong 1977; see Price 1980; Rohde 1989; Poulin 1992). Poulin (1992) found, in the case of the Canadian freshwater fish parasites, that the number of known hosts is positively associated with the number of potential hosts, defined as the other species of the genus. A positive relationship was also found between the number of known Mediterranean hosts and the parasite species diversity, thus supporting this hypothesis.

The relationship between host range and species diversification could be related to the mechanism of cospeciation (Thompson 1994). There are strong arguments to support the view that cospeciation occurs more frequently with highly specific parasites than with poorly specific parasites (see Humphery-Smith 1989). Several examples on cospeciation

involve highly specific parasites: pocket gophers and their lice (Hafner et al.1994, Hafner and Page 1995) or oxyurid nematodes and primates (Brooks and Glen 1982).

The observed link between range and diversification raises the following question: is parasite diversification a consequence of parasite specialisation? Our study does not allow us to answer this question. For instance, much information is needed on the historical relationship between parasites and their hosts. As previously emphasized by Brooks and McLennan (1991), the phylogenies of parasites should be better known. These phylogenies would permit to test whether, within clades, diversification is related to specialization. Furthermore, it would allow to test if specialization arose prior to diversification, which supports the view of an adaptative role of specialization, or if diversification arose prior to specialization. The second case would imply that specialization is the product of another selective force.

References

Brooks, D. R. and Glen, D. R. 1982. Pinworms and primates: a case study in coevolution. Proc. Helminthol. Soc. Wash. - 49: 76-85.

Brooks, D. R. and McLennan, D. A. 1991. Phylogeny, Ecology, and Behavior. A Research Program in Comparative Biology. - The University of Chicago Press, London.

Brooks, D. R. and McLennan, D. A. 1993a. Parascript : Parasites and the Language of Evolution. - Smithsonian Institution Press, Washington.

Brooks, D. R., and McLennan, D. A. 1993b. Macroevolutionary patterns of morphological diversification among parasitic flatworms (Platyhelminthes: Cercomeria). - Evolution 47: 465-509.

Cockburn, A. 1991. An Introduction to Evolutionary Ecology. - Blackwell Scientific Publications, Oxford.

Hafner, M. S. and Page, R. D. M. 1995. Molecular phylogenies and host-parasite cospeciation. - Philos. Trans. R. Soc. Lond. Ser. B 349: 129-143.

Hafner, M. S., Sudman, P. D., Villablanca, F. X., Spradling, T. A., Demastes, J. W. and Nadler, S. A.. 1994. Disparate rates of molecular evolution in cospeciating hosts and their parasites. - Science 265: 1087-1090.

Humphery-Smith, I. 1989. The evolution of phylogenetic specificity among parasitic organisms. - Parasitol. Today 5: 385-387.

Janzen, D. H. 1973. Comments on host-specificity of tropical herbivores and its relevance to species richness. - In: Heywood, V. H. (ed.), Taxonomy and Ecology (ed.). Academic Press, New York, pp. 201-211.

Lymbery, A. J. 1989. Host specficity, Host range and Host preference. - Parasitol. Today 5: 298.

Margolis, L. and Arthur, J. R. 1980. Synopsis des parasites des poissons du Canada. - Ministères des Pêches et des Océans, Ottawa, Canada.

May, R. M. 1975. Patterns of species abundance and diversity. - In: Cody, M. L. and Diamond, J. M. (eds.). Ecology and Evolution of Community. Harvard University Press, Cambridge, MA, pp. 81-120.

Morand, S. 1996 Biodiversity of parasites in relation with their life cycle. - In: Hochberg, M., Clobert, J. and Barbault, R. (eds.). The Genesis and Maintenance of Biological Diversity, Oxford University Press, pp 243-260.

Noble, E. R., Noble, G. A., Schad, G. A. and MacInnes, A. J. 1989. Parasitology: The biology of Animal Parasites. - Lea and Febiger, Philadelphia.

Poulin, R. 1992. Determinants of host-specificity in parasites of freshwater fishes. - Int. J. Parasitol. 22: 753-758.

Poulin, R. 1997. Evolutionary Biology of Parasites. - Chapman and Hall, New York.

Price, P. W. 1980. Evolutionary Biology of Parasites.- Princeton University Press, Princeton.

Price, P. W. 1982. Communities of specialists: vacant niches in ecological and evolutionary times. - In: Strong, S. R., Simberloff, D., Abele, L. G. and Twistle, A. B. (eds.). Ecological communities: conceptual issues and the evidence. Princeton University Press, Princeton, New Jersey, pp. 510-523.

Rohde, K. 1978.Latidunal differences in host-specificity of marine Monogenea and Digenea. - Mar. Biol. 47: 125-134.

Rohde, K. 1980. Host specificity indices of parasites and their application. - Experientia 36: 1370-1371.

Rohde, K. 1982. Ecology of Marine Parasites. - University of Queensland Press, St Lucia, Queensland.

Rohde, K. 1989. Simple ecological systems, simple solutions to complex problems? - Evol. Theory 8: 305-350.

Rohde, K. 1996. Robust phylogenies and adaptive radiations: a critical examination of methods used to identify key innovations. - Am. Nat. 148: 481-500.

Rosenzweig, M. L. 1995. Species diversity in space and time. - Cambridge University Press, Cambridge.

Sasal, P., Morand S., and Guégan, J.-F. 1997. Parasite species richness for fish of the Mediterranean Sea. - Mar. Ecol. Prog. Ser. 149: 61-71.

Sasal, P. and Morand, S. 1998 Comparative analysis: a tool for studying Monogenean ecology and evolution. - Int. J. Parasitol. (in press).

Stanley, S. M. 1979. Macroevolution. Pattern and process. - Freeman and co, San Francisco.

Strong, D. R., Jr. 1977. Rolled-leaf hispine beetles (Chrysomelidae) and their Zingiberales host plants in Middle America. - Biotropica 9: 156-169.

Thompson, J. N. 1994. The coevolutionary process. - The University of Chicago Press, Chicago.

Winemiller, K. O. and Rose, K. A. 1992. Patterns of life history diversification in North American Fishes: Implications for population regulation. - Can. J. Fish. Aqua. Sci. 49: 2196-2218.

Figure Captions

Fig. 1 Host range (mean number of hosts ± sd) of fish parasites of Canada (freshwater and marine) and Mediterranean Sea (marine).

Fig. 2 Frequency distribution of numbers of parasite species among species of marine and freshwater fish parasites according to their life cycle: (A) simple life cycle (SLC) or (B) complex life cycle (CLC)

Fig. 3 Influence of parasite life cycle, simple life cycle (SLC) versus complex life cycle (CLC) on the host specificity (mean number of hosts in ln) (ANOVA, $F_{1,718}=38.12$, $p<0.0001$).

Fig. 4 Relationship between host range (mean number of host known for parasite, in ln) and the total number of known parasite species per group of parasite (in ln) ($R^2=0.26$; b=-0.152; p=0.027).

Fig. 5 Relationship between known host parasitized and known parasite species in the case of Mediterranean fish (note that we have added data on parasitic isopods) ($R^2=0.85$; b=1.492; p=0.0032).

Table 1. Effects of phylogenetic group and environment (marine or freshwater) detected by ANOVA on host range (in ln).

Effect	DF	F	p
parasite group	5	17.97	<0.0001
host environment	1	0.55	0.460
parasite group X host environment	5	2.64	0.022

Table 2. Effects of phylogenetic group and host geographic localisation (marine Canada, freshwater Canada, marine Mediterranean Sea) detected by ANOVA on host range (in ln).

Effect	DF	F	p
parasite group	5	14.60	<0.0001
host geographic localisation	2	2.40	0.092
group X host geographic localisation	10	2.06	0.025

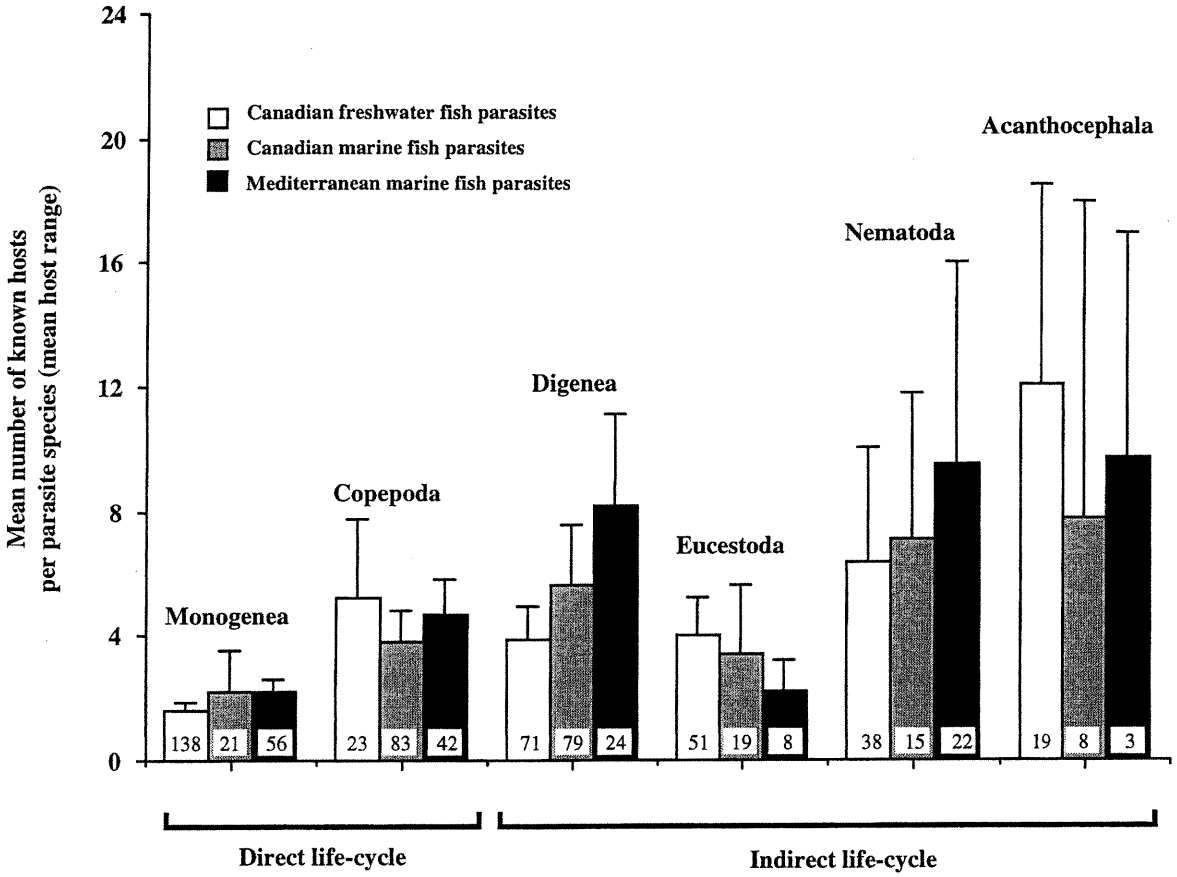

Fig. 1 Sasal et al.

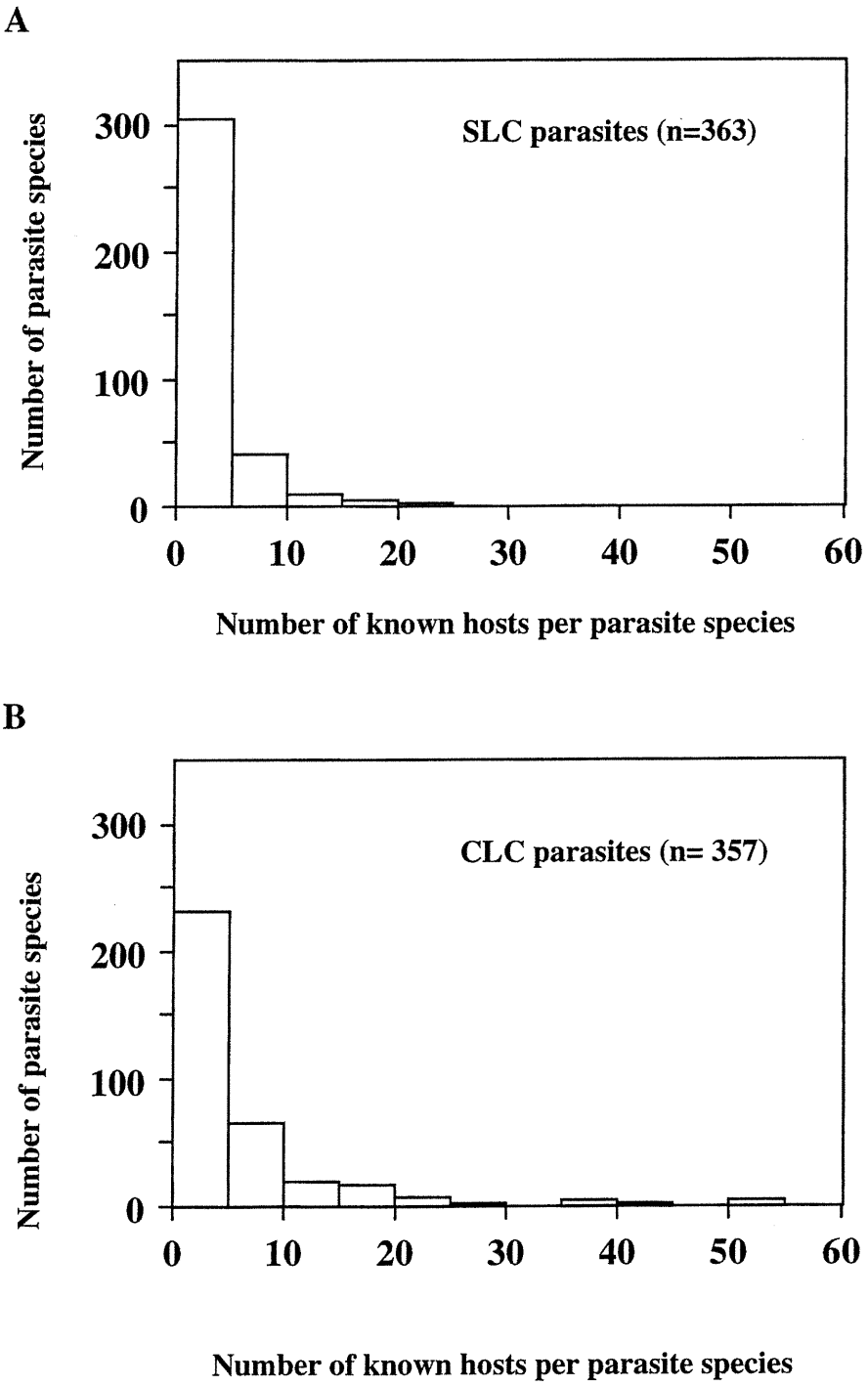

Fig. 2 Sasal et al.

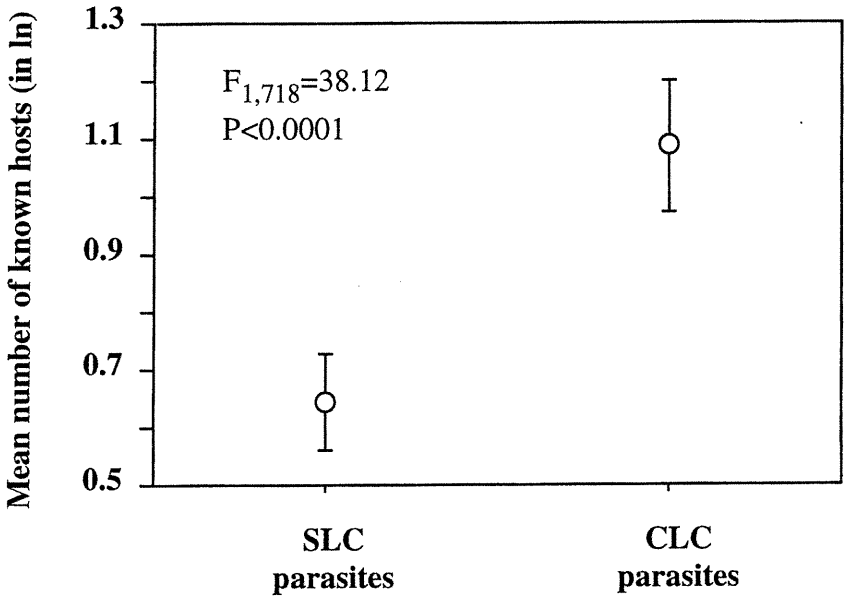

Fig 3 Sasal et al.

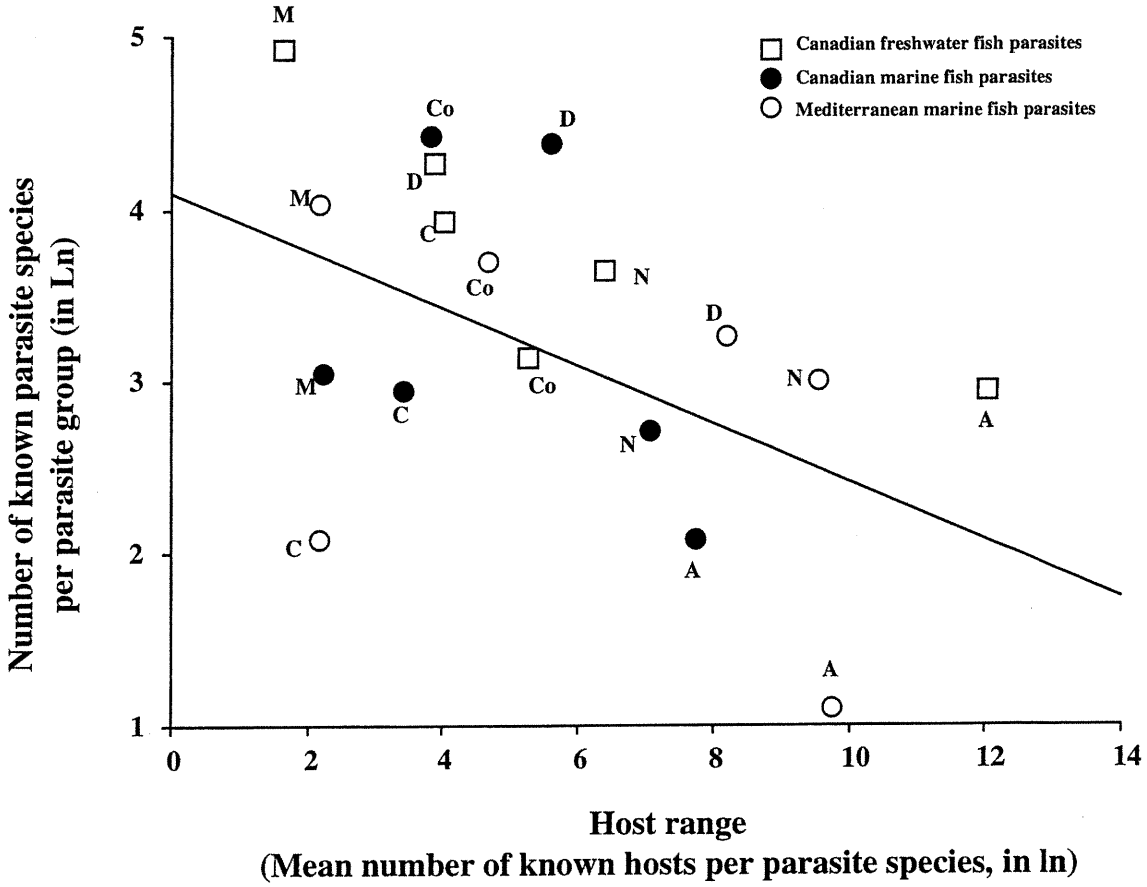

Fig 4 Sasal et al.

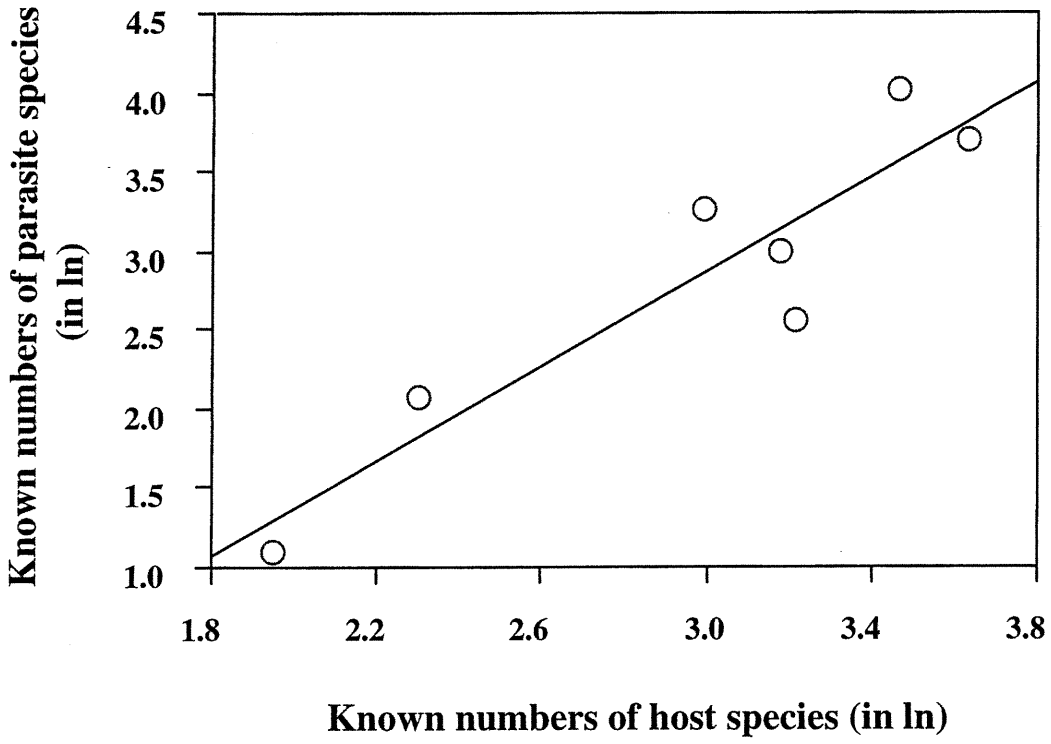

Fig 5 Sasal et al

Article 2

DESDEVISES Yves, Serge MORAND, and Guy OLIVER. 2001. Linking Specialization to Diversification in the Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea). *Parasitology Research* 87(3): 223-230.

Objectifs a et b

Dans cet article, nous cherchons à savoir si la spécificité est liée à la diversification taxonomique chez les Diplectanidae, la famille à laquelle appartient le genre *Lamellodiscus*. Cela est étudié dans un contexte phylogénétique à l'aide d'une méthode comparative appropriée. Pour cela, une phylogénie des Diplectanidae a été reconstruite à l'aide de caractères morphologiques. Cet arbre phylogénétique permet ensuite de définir des hors-groupes qui seront utilisés pour la phylogénie moléculaire des *Lamellodiscus*.

Participation du thésard :

- Recherche bibliographique
- Analyse phylogénétique
- Analyse comparative
- Rédaction

LINKING SPECIALISATION TO DIVERSIFICATION IN THE DIPLECTANIDAE BYCHOWSKY 1957 (MONOGENEA, PLATYHELMINTHES)

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Abstract

The hypothesis of a positive correlation between host-specificity and taxonomic diversification is tested in a family of fish ectoparasites, the Diplectanidae Bychowsky 1957 (Monogenea). A comparative analysis of correlation of species richness with host specificity was performed using an adapted independent contrasts method. In order to control for phylogenetic effects, a phylogenetic tree of the genera in the Diplectanidae using morphological characters was reconstructed. The current taxonomy is retrieved in this phylogenetic hypothesis, except for the Murraytrematoidinae subfamily, which appears to be paraphyletic. There is no significant correlation between host-specificity and taxonomic diversification in the Diplectanidae. The significance of this result is discussed, and different hypotheses which could have led to this observation are presented.

Introduction

Several studies have focused on the topic of taxonomic diversification, trying to explain what leads some groups of organisms to contain many different species while some harbor few (e.g. Slowinski and Guyer 1993; Rosenzweig 1995; Barraclough et al. 1998; Gittleman and Purvis 1998). Some authors have claimed that specialisation could be linked to taxonomic diversification (Janzen 1973; Futuyma and Moreno 1988) but, although there are many studies about specialisation (e.g. Fox and Morrow 1981; Futuyma and Moreno 1988; Thompson 1994), this hypothesis has not been intensively explored, especially in the field of parasitology (Price 1980; Brooks and McLennan 1991; Poulin 1997; De Meeûs et al. 1998).

Parasites are a subject of choice in evolutionary biology because their environment (i.e. host) is definable in space and time more easily than the external environment. Parasites are often described as an ideal biological model for ecological study (De Meeûs et al. 1998). Their ecological niches are easier to define than those of free-living animals. It is also possible to study the evolution of their environment in time, through a host phylogeny.

Brooks and McLennan (1991, 1993) have suggested that specialisation could be linked to diversification in parasitic organisms. They have suggested that the more a parasite is a specialist (i.e. restricted to few host species), the more it is subject to undergo speciation on a new host, because this parasite is, in theory, more sensitive (in an evolutionary way) to its environment than a generalist one, which is supposed to be more tolerant to its environment. This implies that the more a parasite is specialised on its host, the more it is susceptible to "produce" a variety of new species. We intend here to test this hypothesis.

Futuyma and Moreno (1988) have tried to test such an hypothesis with plant-feeding insects but failed because of the absence of a precise phylogeny. However, MacDonald and Brooks (1989) have observed an increase of specificity with taxonomic diversification in species of Telorchiidae Digenea. A phylogeny is indeed required to study the cause of specificity because it is supposed to be under a strong historical influence (see Adamson and Caira 1994).

When studying the macroevolution of a life-history trait, it is difficult to use an experimental design to test our hypothesis, but we can look for correlations between life-history traits by using a comparative method (Harvey and Pagel 1991; Brooks and McLennan 1991). To use such a comparative method, it is necessary to take into account the phylogeny of the taxa under study in order to avoid spurious correlation or lack of correlation (Felsenstein 1985; Harvey and Keymer 1991).

The study of taxa diversification leads to another problem: a poor-species taxa could be an artifact due to species extinction, or sampling errors. That is why great amplitude studies, where data are gathered along the literature, and where some species could be much more sampled and/or known than others, could be questionable. The study of a relatively small and well known group of organisms avoids some of those problems because sampling errors are less likely.

We will investigate here if specialisation is linked to taxonomic diversification in a monogenean family, the Diplectanidae Bychowsky 1957. Monogeneans are fish ectoparasites with a direct life cycle, and are known to be highly host-specific (e.g. Baer 1957; Noble et al. 1989; Sasal et al. 1998). The Diplectanidae are well known and for a long time (Wagener 1857; Parona and Perugia, 1889; Murray 1931; Yamaguti 1934, 1938, 1958; Price 1937; Palombi 1943; Sproston 1945; Ramalingam 1955; Bychowsky 1957; Laird 1958; Euzet and Audoin, 1959; Tripathi 1959; Paperna and Kohn 1964 ; Euzet and Oliver 1965, 1966a,b, 1967; Paperna 1965; Oliver 1968-1987; Thurston, Paperna 1969; Young 1969; Lambert and Maillard, 1974; Bychowsky and Nagibina 1976; Kumar and Agarwal 1978; Ogawa and Egusa 1978a,b; Paperna and Baudin Laurencin 1979 ; Maillard and Vala 1980; Vala et al. 1980; Venkatanarsaiah and Kultani 1980a,b; Beverley-Burton and Suriano 1981; Ergens 1981; Nasir 1983; Euzet 1984; Oliver and Paperna 1984; Kritsky and Thatcher 1984; Byrnes 1986; Roubal 1986; Rakotofiringa et al. 1987, Seng and Seng 1991; Euzet et al. 1993; Vidal-Martinez et al. 1997, 1998, Santos et al 2000). We made the most exhaustive possible survey of the literature in order to obtain the most precise known pattern of host specificity among the Diplectanidae. This family includes genera with various level of diversification and host specificity (Euzet et al. 1993; see Table 1), and is then appropriate for the study intended here.

Materials and Methods

Phylogeny

We used the classification scheme of Oliver (1987) and included the genus *Cycloplectanum* Oliver 1968 in the Diplectanidae, even if this is contested by Kritsky and Beverley-Burton (1986), who refuse the validity of this genus and replace it by *Pseudorhabdosynochus* Yamaguti 1958. This latter genus is mainly based on the genitalia morphology, while *Cycloplectanum* is essentially defined by the shape of the squamodisc. *Pseudorhabdosynochus* encompasses species from the genera *Cycloplectanum* and *Diplectanum*. We then replaced *Pseudorhabdosynochus* species in these genera on the basis of the shape of their squamodiscs.

The phylogeny of the genera in the Diplectanidae was reconstructed using morphological data found in the literature (Euzet and Oliver 1966a,b, 1967; Oliver 1968, 1969a, 1987 and references therein; Euzet 1984). Eighteen homologous series representing 49 character states were defined. These morphological data were coded using additive binary coding and linear coding methods (O'Grady and Deets 1987). A list of the homologous series of morphological characters used is given in Appendix 1, and the character matrix is shown in Appendix 2. We used a maximum parsimony method using the Branch-and-Bound algorithm implemented with PAUP* 4.0 (Swofford DL (1999) Laboratory of Molecular Systematic, Smithsonian Institution, Washington DC) to build a phylogenetic tree.

As we have relatively few morphological characters for the phylogenetic reconstruction, character resampling (i.e. bootstrap and jacknife) may be not very appropriate to validate the phylogeny, because a too small number of characters would be resampled at each iteration to build a fully-resolved phylogeny. Therefore, the probability to observe high support value would be low, even if all characters exhibit an important phylogenetic structure. Bootstrap proportions were calculated anyway and are given as indicative values, but should be taken with caution. We performed also a partition homogeneity test (PTP), which assesses tree length significance through a permutation procedure. For the bootstrap and the PTP test, a heuristic algorithm using the "closest"

addition sequence option and the "tree-bisection-reconnection" branch-swapping algorithm was used. All these analysis were performed with PAUP* 4.0.

We used the monogenean genus *Pseudomurraytrema* Bychowsky 1957 (Pseudomurraytrematidae) as outgroup, because of its status of close ancestor of the Diplectanidae (Boeger and Kritsky 1997).

Comparative analysis

We calculated the correlation of species richness with specificity through a comparative analysis using the independent contrasts method to control for phylogenetic effects (Felsenstein 1985; Martins and Garland 1991; Pagel 1992; Garland 1992). We analysed the data with the MacroCAIC program (Agapow et al. 2000), derived from CAIC (Comparative Analysis using Independent Contrasts; Purvis and Rambaut 1995). The independent contrasts method consists of estimating the differences (contrasts) between sister-taxa of the phylogeny, and implementing statistical tests with those independent values (see Felsenstein (1985) for more details). To be used, contrasts must be standardised across the phylogenetic tree (Felsenstein 1985), and the regression performed on the independent contrasts is forced through the origin (Garland et al. 1992). MacroCAIC allows to use species richness as a variable in a comparative analysis to estimate if traits (as here specificity) are associated with high speciation rate (clade species richness). Species richness cannot be used as other continuous variables with independent contrasts because, in that case, its estimated value at the nodes of the phylogeny is not the average of lower values but a sum of them. No contrasts were calculated for polytomies.

Specificity for a parasite species is defined here as the number of its (known) host species (as Poulin 1992). This is what Lymbery (1989) call "host range". As we work on genera, specificity in a genus is the mean specificity of all species in the genus. This variable will be called here MHR for Mean Host Range. Higher MHR means lower specificity, and vice-versa. The number of species in a genus and the number of host species by parasite species was found in the literature (Oliver 1987; Seng and Seng 1991; Dyer 1994; Dyer et al. 1995; Vidal-Martinez et al. 1997; Vidal-Martinez and Mandoza-Franco 1998; Bu et al. 1999; Santos et al. 2000). Note that *Lamellodiscus virgula* and *L.*

obeliae were considered as a single species on the basis of molecular evidence (Desdevises et al. 2000).

All variables were ln-transformed in order to obtain linearisation and normality of contrasts (Kolmogorov-Smirnov test, a = 5 %). Normality tests were performed with the program R 4.0 (Casgrain P and Legendre P (2000), freely available at the URL http://www.fas.umontreal.ca/BIOL /Casgrain/en/labo/R/v4/telecharger.html). Linear regressions were tested with a Pearson correlation coefficient.

The tested variable representing species richness is the natural log of the ratio CLS/CHS. CLS, for Clade with Low Specificity, is, for each node of the phylogeny, the number of species in the sister clade from whose the estimated value of specificity (at the node) is the lowest, compared to the value of specificity for the other sister clade, then called CHS (Clade with High Specificity). Then, when this ratio is smaller than one (negative ln), the clade with the lowest MHR contains more species. When the ratio is 1 (null ln), the number of species in each sister clade is the same, and when CLS/CHS is greater than one (positive ln), the clade with more species is has the lowest specificity (highest MHR). The analysis is then performed between sister clades at each node of the phylogeny.

The ln(CLS/CHS) are regressed against standardised contrasts for ln(MHR). If the tested hypothesis is true, we should observe an increase of diversification with specificity, then a negative relationship with MHR.

Results

Phylogeny

The parsimony analysis led to 64 most-parsimonious trees (MPT) (consistency index = 0.65). We used then character reweighting (Farris, 1988) based on the maximum value of the rescaled consistency index and obtained four MPT (CI = 0.81). We kept for our analysis the strict consensus of these four trees (Fig. 1), as its topology is also one of the four MPT. The four trees differs by the resolution of two polytomies: *Protolamellodiscus* and *Calydiscoides* are monophyletic in two MPT, and *Pseudolamellodiscus* is at the base of the

clade (*Lepidotrema*, *Heteroplectanum*, (*Diplectanum*, *Cycloplectanum*)) in two MPT. The PTP test (100 replicates) showed that tree length is highly significant ($p = 0.001$).

Comparative analysis

The regression equation forced through origin obtained after control for phylogeny is:

$\ln(CLS/CHS) = 9.392\ln(MHR)$; $r^2 = 0.103$, $p = 0.40$ (see graph on Fig. 2)

No significant relationship is found between specificity and diversification, i.e. clade with the higher species richness do not exhibit a lower host range per species. The same relationship was obtained with each of the three other MPT.

Note that the use of a standard comparative method (CAIC) leads to a significant positive relationship between MHR and taxonomic diversification ($p = 0.002$, Fig. 3). This emphasise the need to use a proper analytical method when dealing with clade species richness.

Discussion

Phylogeny of the Diplectanidae

The phylogenetic reconstruction presented here is supported by a high consistency index and a highly significant PTP test. It is also rather consistent with the actual taxonomy and a previously proposed phylogenetic hypothesis (Oliver 1987).

The Diplectanidae are composed of four subfamilies: the Murraytrematoidinae, the Rhabdosynochinae, the Lamellodiscinae and the Diplectaninae. The monophyly of the three latter subfamilies is supported by the phylogenetic hypothesis proposed here, but the Murraytrematoidinae appears to be paraphyletic.

We used *Cycloplectanum* Oliver 1968 in spite of its controversial status (Beverly-Burton and Suriano 1981; Kritsky and Beverley-Burton 1986). As previously mentioned, some authors place some species of this genus within the genus *Pseudorhabdosynochus* Yamaguti 1958. We have doubt on the validity of this classification for reasons developed elsewhere (Oliver 1984, 1986, 1987). Moreover, Oliver (1987) found that *Cycloplectanum* also has a specific microscopic morphology of the tegumentary scales. A publication by G. Oliver is currently in preparation to justify the validity of this genus.

Diversification and specialisation

Concerning the link between specialisation and diversification in the Diplectanidae, the observed results do not support the Brooks and McLennan's hypothesis (1991, 1993). We were expecting a high specificity in well-diversified groups and we could not find any statistically significant relationship. This could be attributed to the low power of the test, as only eight contrasts were calculated because of the presence of polytomies in the tree, but the same results were obtained when using the more resolved of the MPT obtained (ten contrasts). Moreover, the trend showed by the regression line (Fig. 2) is a positive relationship between MHR and diversification, which supports the inverse pattern as what would have been expected if taxonomic diversification was promoted by a high specificity. These findings then cannot confirm this hypothesis.

Several possibilities can be invoked to explain these results: 1) The hypothesis is true, but extrinsic factors have more influence on specificity than phylogeny, and therefore mask the influence of the intrinsic (phylogenetic) factors. A host phylogeny would be useful to understand the history of this host-parasite association and then to explain the possible influence of the ecological environment - i.e host - on the pattern of parasite specificity (see Page 1996). As an example, Poulin (1992) found a relationship between specificity and number of potential hosts, defined as hosts sharing common ancestry. 2) The hypothesis is true but the pattern of specificity is biased by a bad sampling - but this is unlikely for the reasons explained in the introduction - and/or extinction of species. All genera studied include old-described and well-known species (see Table 1), and it is likely that the number of known hosts increases with the time from which a parasite species is known. Then, to

control for a potential "sampling effect" affecting host specificity, it is necessary to control not for the number of studies (as previously seen in the literature, e.g. Poulin 1992), but for the date of parasite species description (i.e. the date since they are known). This is because a very specialized parasite species is likely to be seldom mentioned, because it parasitizes one (or few) host(s), and will therefore appear as less studied than more generalist species, which will be more often encountered in most cases. In other words, the more a parasite uses hosts, the more it is likely to be reported in the literature and an apparent bias will appear while controlling for the number of studies. But if we control for the date of species description, a true "sampling bias" on host specificity will lead to a positive correlation between the number of known hosts and the date of parasite species description, i.e. the more a species is "young", the less we know its hosts. There is no such significant correlation between the number of known hosts for a species and the time of species description ($p = 0.14$, calculated from Table 1) in our data. This allows one to think that the potential bias, if any, is small. 3) It is also possible, as stated by Futuyma and Moreno (1988), that genus is not the right phylogenetic level to test this hypothesis, because some genus are much more diverse than others and may reflect some artifacts of classification. In addition, the pattern of diversification may not be precisely seen at the genus level. The fact that species are often assigned to different genus through time by different authors, as seen in the Diplectanidae (see Oliver 1969a, 1987; Kritsky and Beverley-Burton 1986), shows that this division could sometimes be considered as fragile and somewhat artificial. This observation makes questionable all comparative studies made at the genus level. However, the present taxonomic state of the Diplectanidae family could now be considered as well known, and is supported by the present phylogeny. Other studies, as Brooks and McLennan (1988), have used such comparison of genera to study patterns of diversification. 4) The hypothesis is false. We could suggest an alternative to this hypothesis: generalist parasites face more diverse conditions than specialists and are therefore subject to different constraints. These parasites could encounter conditions leading to speciation more frequently than specialist parasites, confined to the same environmental conditions. Such a process could act simultaneously with the former and hide his effect, leading to the observation of an absence of change in taxonomic diversification with host specificity.

Therefore, host specificity would not be controlled by such phylogenetic influences but by other ecological and/or historical factors.

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References

Adamson ML, Caira JN (1994) Evolutionary factors influencing the nature of parasite specificity. Parasitology 109:S85-S95

Agapow P-M, etc. (2000) MacroCAIC: correlations of species richness revealed by comparative analysis. Bioinformatics: in press

Baer JG (1957) First Symposium on Host Specificity among Parasites of Vertebrates. Paul Attinger, Neuchatel

Barraclough TG, Vogler A, Harvey PH (1998) Revealing the factors that promote speciation. Phil Trans R Soc Lond B 353:241-249

Beverley-Burton M, Suriano DM (1981). A revision of *Cycloplectanum* Oliver, 1968 (Monogenea, Diplectanidae) and descriptions of *C. hongkongensis* n. sp. *C. lantauensis* n. sp. from *Epinephelus* spp. (Serranidae) in the South China Sea. Can J Zool 59(7):1276-1285

Boeger WA, Kritsky DC (1997) Coevolution of the Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. Int J Parasitol 27:1495-1511

Brooks DR, McLennan DA (1988) Comparative study of adaptive radiations with an example using parasitic flatworms (Platyhelminthes: Cercomeria). Am Nat 142(5):755-778

Brooks DR, McLennan DA (1991) Phylogeny, Ecology, and Behavior. A Research Program in Comparative Biology. The University of Chicago Press, London

Brooks DR, McLennan DA (1993) Parascript. Smithsonian series in Comparative Evolutionary Biology, Washington and London

Bu SSH, Leong TS, Wong SY, Woo YSN, Foo RWT (1999) Three diplectanid monogeneans from marine finfish (*Epinephelus* spp.) in the far east. J Helminthol 73(4):301-312

Bychowsky BE (1957) Monogenetic Trematodes. Their Systematic and Phylogeny. Moscow, Academy of Sciences

Bychowsky BE, Nagibina LF (1976) New species from the genus *Telegamatrix* Ramalingam, 1955 (Monogenoidea, Diplectanidae) (in Russian). Biol Mar 2:10-15

Bychowsky BE, Nagibina LF (1977) About the taxonomic position of some representants of the monopisthocotylean monogenean marine fish parasites (in Russian). Parazit Sb 27:7-17

Byrnes T (1986) Six species of *Lamellodiscus* (Monogenea: Diplectanidae) collected from australian bream (*Acanthopagrus* spp.). Proc Seto Mar Biol Lab 31:169-190

De Meeûs T, Michalakis Y, Renaud F (1998) Santa Rosalia revisited: or why are there so many kind of parasites in "The Garden of Earthly Delights"? Parasitol Today 14(1):10-13

Desdevises Y, Jovelin R, Jousson O, Morand S (2000) Comparison of ribosomal DNA sequences of *Lamellodiscus* spp. (Monogenea, Diplectanidae) parasitizing *Pagellus* (Sparidae, Teleostei) in the North Mediterranean Sea: species divergence and coevolutionary interactions. Int J Parasitol 30:741-746

Dyer WG, Williams EH, Bunkley-Williams L (1994) *Pseudorhabdosynochus monoaensis* n. sp. (Monogenea: Diplectanidae) on Rock Hind from Mona Island, Puerto Rico. J Aquat Anim Health 6:59-63

Dyer WG, Williams EH, Bunkley-Williams L (1995) *Pseudorhabdosynochus kritskyi* n. sp. (Monogenea: Diplectanidae) on Gag from the Gulf of Mexico. J Aquat Anim Health 7:337-340

Ergens R (1981) *Diplectanum lacustre* Thurston and Paperna, 1969 (Monogenea: Diplectanidae) from the egyptian *Lates niloticus*. Folia Parasitol 28(2):160

Euzet L (1984) Diplectanidae (Monogenea) parasites de poissons des Iles Kerkennah (Tunisie). Arch Inst Pasteur Tunis 61(4):463-474

Euzet L, Audouin J (1959) Sur un genre nouveau de Monogenoidea parasite de la dorade *Chrysophrys aurata* L. Rev Trav Inst Pêches Marit 23(3):317-322

Euzet L, Combes C, Caro A (1993) A checklist of Monogenea of mediterranean fish. Second International Symposium on Monogenea. Montpellier/Sète - 5-8 Juillet 1993

Euzet L, Oliver G (1965) Diplectanidae (Monogenea) des Téléostéens de la Méditerranée occidentale. II. Parasites d'*Epinephelus gigas* (Brünnich, 1768). Ann Par Hum Comp (Paris) 40(5):517-523

Euzet L, Oliver G (1966a) Diplectanidae (Monogenea) des Téléostéens de la Méditerranée occidentale. III. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Diplodus* (Sparidae). Ann Par Hum Comp (Paris) 41(6):573-598

Euzet L, Oliver G (1966b) Diplectanidae (Monogenea) de Téléostéens de la Méditerranée occidentale. IV. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Pagellus* Cuvier, 1829 (Sparidae). Ann Par Hum Comp (Paris) 42(4):407-425

Farris JS (1988) Hennig86, version 1.5. Distributed by the author, Port Jefferson Station, NY

Felsenstein J (1985) Phylogenies and the comparative method. Am Nat 125:1-15

Fox LR, Morrow PA (1981) Specialization: species property or local phenomenom? Science 211:887-893

Futuyma DJ, Moreno G (1988) The evolution of ecological specialization. Ann Rev Ecol Syst 19:207-233

Garland T (1992) Rate tests for phenotypic evolution using phylogenetically independant contrasts. Am Nat 140:509-519

Garland T Jr, Harvey PH, Ives RA (1992) Procedures for the analysis of comparative data using phylogenetically independent contrasts. Syst Biol 41(1):18-32

Gittleman JL, Purvis A (1998) Body size and species-richness in carnivores and primates. Proc R Soc Lond 265:113-119

Harvey PH, Keymer AE (1991) Comparing life histories using phylogenies. Phil Trans R Soc Lond B 332:31-39

Harvey PH, Pagel MD (1991) The comparative method in evolutionnary biology. Oxford University Press

Janzen DH (1973) Comments on host-specificity of tropical herbivores and its relevance to species richness. In: Heywood VH (ed) Taxonomy and Ecology. Academic Press, New York, p 201-211

Kritsky DC, Beverley-Burton M (1986) The status of *Pseudorhabdosynochus yamaguti* 1958 and *Cycloplectanum* Oliver 1968 (Monogenea: Diplectanidae). Proc Biol Soc Wash 99(1):17-20

Kritsky DC, Thatcher VE (1984) Neotropical Monogenea. 6. Five new species of *Diplectanum* (Diplectanidae) from freshwater Teleosts *Plagioscion* spp. (Scieanidae), in Brazil. Proc Biol Soc Wash 97(2):425-439

Kumar R, Agarwal GP (1978). On a new species of the genus *Lobotrema* Tripathi, 1959 (Monogenea: Dactylogyridae, Bychowsky, 1933) from a freshwater fish, *Sciaena coitre* (Ham.) at Varanasi, India. Indian J Helminth 30(2):90-95

Laird M (1958) Parasites of South Pacific Fishes II. *Diplectanum melanesiensis* n. sp., a monogenetic trematode from Fiji and the New hebrides. Can J Zool 36(2):167-173

Lambert A, Maillard C (1974) Parasitisme branchial simultané par deux espèces de *Diplectanum* Diesing, 1858 (Monogenea, Monopisthocotylea) chez *Dicentrarchus labrax* (L., 1758) (Téleostéens). C R Hebd Séanc Acad Sci Paros 279, Sér. D(16):1345-1347

Le Brun N, Renaud F, Berrebi P, Lambert A (1992) Hybrid zones and host-parasite relationships: effect on the evolution of parasitic specificity. Evolution 46(1):56-61

Lymbery, A. J. 1989. Host specificity, host range and host preference. Parasitol Today 5(9): 298.

MacDonald CA, Brooks DR (1989) Revision and phylogenetic analysis of the North American species of *Telorchis* Luehe, 1899 (Cercomeria: Trematoda: Digenea: Telorchiidae). Can J Zool 67:2301-2320.

Maillard C, Vala J-C (1980) Two new species of Monogenea of the genus *Pseudodiplectanum* Tripathi, 1955, branchial parasites of *Galeoides decadactylus*, a teleostean from the coasts of Gabon. Syst Parasitol 1(2):111-116

Martins EP, Garland T (1991) Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. Evolution 45:534-557

Murray FV (1931) Gill trematodes from some Australian fishes. Parasitology 23:492-506

Nasir P (1983) Occurrence and significance of the monogenean *Cycloplectanum americanum* (Price, 1937) Oliver, 1968, on a freshwater host. J Parasitol 69(5):957-962

Noble ER, Noble GA, Schad GA, MacInnes AJ (1989) Parasitology. The Biology of Animal Parasites. 6th Edition. Lea & Febiger, Philadelphia

O'Grady RT, Deets GB (1987) Coding multistate characters, with special reference to the use of parasites as characters of their hosts. Syst Zool 36(3):268-279

Ogawa K, Egusa S (1978a) Five species of monogeneans obtained from the Japanese Black Sea Bream, *Acanthopagrus schlegeli* (Bleeker) (Teleostei: Sparidae). Proceedings of the Fourth International Congress of Parasitology, 19-26 August 1978, Warsawa, Section B: 27

Ogawa K, Egusa S (1978b) Three species of *Lamellodiscus* (Monogenea: Diplectanidae) from the gills of the Japanese Black Sea Bream, *Acanthopagrus schlegeli* (Bleeker). Bull Jap Soc Scient Fish 44(6):607-612

Oliver G (1968) Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. I. Diplectaninae Monticelli 1903. Vie Milieu 19(1-A):95-138

Oliver G (1969a) Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. II. Lamellodiscinae nov. sub. fam. Vie Milieu 20(1-A):43-72

Oliver G (1969b) Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. III. Biologie. Vie Milieu 20(2-A):397-420

Oliver G (1973) *Lamellodiscus obeliae* n. sp. une nouvelle espèce de Diplectanidae (Monogenea, Monopisthocotylea) parasite de *Pagellus centrodontus* (Delaroche, 1809) (Pisces, Sparidae). Z Parasitenk 41:103-108

Oliver G (1974) Nouveaux aspects du parasitisme des Diplectanidae Bychowsky 1957 (Monogenea, Monopisthocotylea) chez les Téléostéens Perciformes des côtes de France. C R Acad Sc Paris 279:803-805

Oliver G (1980a) Les Diplectanidae Bychowsky 1957 (Monogenea, Monopisthocotylea) parasites des Sciaenidae (Pisces, Perciformes) du Golfe de Gascogne. Bull Mus Nat Hist Nat Paris, 4ème Sér., 2, Sect A (3):669-689

Oliver G (1980b) Description de *Pseudodiplectanum kearni* n. sp., (Monogenea, Diplectanidae), des côtes européennes, parasite d'un poisson pleuronectiforme. Bull Mus Nat Hist Nat Paris, 4ème Sér., 2, Sect A (3):691-695

Oliver G (1984a) Description de deux nouvelles espèces du genre *Cycloplectanum* Oliver 1968 (Monogenea, Monopisthocotylea, Diplectanidae). Ann Par Hum Comp 59(1):31-39

Oliver G (1984b) Quelques espèces du genre *Calydiscoides* Young (Monogenea, Monopisthocotylea, Diplectanidae), parasites de Perciformes du Récif de la Grande Barrière (Australie). Zool Scr 13(3):189-193

Oliver G (1986) *Cycloplectanum riouxi* n. sp., une nouvelle espèce de Diplectanidae (Monogenea, Monopisthocotylea) parasite d'*Epinephelus gaza* (Linnaeus, 1758) (Pisces, Serranidae). Syst Parasitol 8:317-322

Oliver G (1987) Les Diplectanidae Bychowsky 1957 (Monogenea, Monopisthocotylea, Dactylogyridea). Systématique. Biologie. Ontogénie. Écologie. Essai de phylogénèse. Thèse d'état. Université des Sciences et Techniques du Languedoc

Oliver G, Paperna I (1984) Diplectanidae Bychowsky 1957 (Monogenea, Monopisthocotylea) parasites de Perciformes de Méditerranée orientale, de la Mer Rouge et de l'Océan Indien. Bull Mus Nat Hist Nat Paris, 4ème Sér., 6, Sect A (1):49-65

Page RDM (1996) Temporal congruence revisited: comparison of mitochondrial DNA sequence divergence in cospeciating pocket gophers and their chewing lice. Syst Biol 45(2):151-167

Pagel MD (1992) A method for the analysis of the comparative data. J Theor Biol 156:431-442

Palombi A (1943) Notizie Elmintologiche. V. *Diplectanum* (*Dactylogyrus*) *echeneis* (Wag.) Par. et Per. = *Lamellodiscus ignoratus* n. sp. Diagnosi della specie e suoi opistatori. Annuario Mus Zool Napoli (N. S.) 7(16):1-5

Paperna I (1965) Contribution to the knowledge of the Red Sea. No 32. Monogenetic Trematodes from the gills of Red Sea fishes. Bull Sea Fish Res Stn Israël 39 :17-26

Paperna I, Baudin Laurencin F (1979). Parasitic infection of sea bass, *Dicentrarchus labrax*, and gilt head sea bream, *Sparus aurata*, in mariculture facilities in France. Aquaculture 16(2) :173-175

Paperna I, Kohn A (1964) Report on Monogenetic Trematodes collected from eastern Mediterranean. Revta Bras Biol 24(3) :243-248

Parona C, Perugia A (1889) Di alcuni trematodi ectoparasiti di pesci marini. Nota preventiva. Ann Mus Civ Stor Nat Genova, ser. 2a(7):740-474

Poulin R (1992) Determinants of host specificity in parasites of freshwater fishes. Int J Parasitol 22(6):753-58

Poulin R (1997) Parasites faunas of freshwater fish: the relationship between richness and the specificity of parasites. Int J Parasitol 27(9):1091-1098

Price EW (1937) North American Monogenetic Trematodes. I. The superfamily Gyrodactyloidea. (continued). J Wash Acad Sci 27(4):146-164

Price PW (1980) Evolutionary Biology of Parasites. Princeton University Press, Princeton

Purvis A, Rambaut A (1995) Comparative analysis by independent contrasts (CAIC): and Apple Macintosh application for analysis comparative data. Comput Appl Biosci 11:247-251

Rakotofiringa S, Oliver G, Lambert A (1987) *Heteroplectanum* n. gen., un nouveau genre de Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea) parasite de Téléostéens marins de Madagascar. Bull Mus Nat Hist Nat Paris, 4ème Sér., 9, Sect A (1):145-157

Ramalingam K (1955) A remarkable organism, *Telegamatrix pellona* gen. And sp. nov. (Monogenea: Diplectaninae) parasitic in an Indian herring. Proc Indian Acad Sci, Sect B, 42(5):209-218

Roubal F (1986) Studies on monogeneans and copepods parasitizing the gills of a sparid (*Acanthopagrus australis* (Günther)) in northern New South Wales. Can J Zool 64(4):841-849.

Rozensweig ML (1995) Species diversity in space and time. Cambridge University Press, Cambridge

Santos CP, Buchmann K, Gibson DI (2000) *Pseudorhabdosynochus* spp. (Monogenea : Diplectanidae) from the gills of *Epinephelus* spp. in brazilian waters. Syst Parasitol 45(2):145-153

Sasal P, Desdevises Y, Morand S (1998) Host-specificity and species diversity in fish parasites: phylogenetic conservatism? Ecography 21:639-643

Seng LK, Seng, LT (1991) A redescription of *Pseudorhabdosynochus latesi* (Tripathi 1955) and description of *Diplectanum penangi* n. sp. (Monogenea: Diplectanidae) from *Lates calcarifer* cultured in floating cages in Malaysia and Thailand. J Bioscience 2(1-2):77-84

Slowinski JB, Guyer C (1993) Testing whether certain traits have caused amplified diversification: an improved method based on a model of random speciation and extinction. Am Nat 142(6):1019-1024

Sproston NG (1945) A synopsis of the Monogenetic Trematodes. Trans Zool Soc Lond 25(4):185-600

Thompson JN (1994) The coevolutionary process. The University of Chicago Press, Chicago and London

Thurston JP, Paperna I (1969) *Diplectanum lacustris* sp. nov. (Dactylogyroidea, Diplectanidae), a monogenetic Trematode from the gills of the Nile Perch. Proc Helminth Soc Wash 36(2):214-218

Tripathi YR (1959) Monogenetic Trematodes from fishes of India. Indian J Helminth 9(1-2):1-149

Vala JC, Lopez Roman R, Boudaoud K (1980) *Pseudiplectanum kearnei* n. sp. (Monogenea, Diplectanidae) parasite branchial de Solea vulgaris Quensel, 1806 en Méditérranée. Revta Iber Parasit 40(1):21-26

Venkatanarsaiah J, Kultani T (1980a) New monogenetic trematodes of the genus *Calydiscoides* Young, 1969 from the gills of *Nemipterus japonicus*. India. Proc Indian Acad Parasit 1(1):20-22

Venkatanarsaiah J, Kultani T (1980b) On a new monogenetic trematode, *Lamellodiscus caballeroi* n. sp., from the gills of *Crenidens indicus* at Machilipatnam Coast, Andhra Pradesh, India. Proc Indian Acad Parasit 1(1):23-26

Vidal-Martinez VM, Aguirre-Macedo L, Mendoza-Franco, EF (1997) *Pseudorhabdosynochus yucatanensis* sp. n. (Monogenea, Diplectanidae) from the gills of the red grouper *Epinephelus morio* (pisces, serranidae) of the yucatan peninsula, mexico. Folia Parasitol 44(4):274-278

Vidal-Martinez VM, Mendoza-Franco EF (1998) *Pseudorhabdosynochus capurroi* sp. n. (Monogenea, Diplectanidae) from the gills of *Mycteroperca bonaci* (Pisces, Serranidae) of the Yucatan peninsula, Mexico. Folia Parasitol 45(3):221-224

Wagener G (1857) Helminthologische Bemerkungen aus einem Sandsschreibe an C. Th. V. Siebold Zeit Wiss Zool 9:73-90

Yamaguti S (1934) Studies of the helminth fauna of Japan. Part 2. Trematodes of fishes. I. Jap J Zool 5(3) :249-541

Yamaguti S (1938) Studies of the helminth fauna of Japan. Part 25. Trematodes of fishes. V. Jap J Zool 8(1) :15-74

Yamaguti S (1958) Studies of the helminth fauna of Japan. Part 53. Trematodes of fishes. XII. Pub Seto Mar Biol Lab 7(1) :53-88

Young PC (1969) Some monogenoideans of the family Diplectanidae Bychowsky 1957, from Australian Teleost fishes. J Helminthol 43(1-2):223-254

Table 1 : Number of parasites species per genus, mean number of host species per parasite species in each genus, host range per genus, mean date of species description per genus and range of date of species description per genus

Parasite genus	Number of species	Mean number of host per species	Host range	Mean date of species description	Range of dates of species description
Murraytrematoides	5	1.20	1-2	1963	1955-1976
Murraytrema	2	1	1	1954	1931-1977
Lobotrema	7	1.57	1-5	1974	1959-1984
Rhabdosynochus	1	1	1	1941	1941
Protolamellodiscus	3	1.67	1-3	1968	1953-1987
Calydiscoides	7	1.29	1-3	1968	1953-1984
Lamellodiscus	35	1.97	1-7	1968	1922-1987
Telegamatrix	4	1	1	1971	1955-1976
Furnestinia	1	1	1	1959	1959
Monoplectanum	1	1	1	1969	1969
Latericaecum	1	1	1	1969	1969
Lepidotrema	6	1	1	1924	1922-1931
Diplectanum	65	1.28	1-3	1966	1857-2000
Pseudodiplectanum	5	1.20	1-2	1974	1957-1980
Heteroplectanum	9	1.22	1-2	1978	1968-1987
Pseudolamellodiscus	4	1.25	1-2	1968	1953-1979
Cycloplectanum	19	1.26	1-4	1974	1937-1999

Legends

Fig. 1: Phylogenetic relationships between the genus in the Diplectanidae reconstructed by parsimony: strict consensus tree. *Pseudomurraytrema* is used as outgroup. Numbers are Bootstrap values (1000 replicates)

Fig. 2: Linear regression of the diversification ratio between sister clades (Clade with Low Specificity/Clade with High Specificity) on independent contrasts of mean host range per genus (MHR) between sister clades (all values are ln-transformed)

Fig. 3: Linear regression of independent contrasts of number of species per genus on independent contrasts of mean host range per genus (all values are ln-transformed)

Appendix 1

List of homologous series used for the phylogenetic reconstruction of the Diplectanidae (Monogenea).

1. *Hooks*. (0) Equal. (1) Dissymetrical.

2. *Ventral bar*. (0) Thick. (1) Thin with curved ends.

3. *Transversal bars*. (0) Three. (1) Two. Bychowsky and Nagibina (1977) consider that there is a loss of a transversal bar allowing the passage from three to two.

4. *Transversal bars*. (0) Connected. (1) Separated.

5. *Sexual appendice*. (0) Absent. (1) Present.

6. *Echinodiscs*. (0) Present. (1) Absent. Echinodiscs are primitive structures also present in the Rhamnocercidae.

7. *Number of accessory adhesive organs*. (0) None. (1) One. (2) Two.

8. *Opisthohaptoral tegumentary scales*. (000000) Unmodified. (100000) Bonded (placodisc). (011000) Lamellodisc. (010101) Primitive squamodisc. (010110) Evolved squamodisc. The squamodisc of the "primitive" type has the same structure of the first stage of squamodisc development (see Oliver 1987).

9. *Shape of accessory adhesive organ*. (0) Full. (1) Split.

10. *Anterior lamellae of accessory adhesive organs*. (0) Opened. (1) One lamellae closed. (2) More than one lamellae closed.

11. *Accessory piece*. (0) Absent. (1) Present.

12. *Cephalic glands*. (0) Numerous. (1) Three or four.

13. *Vas deferens*. (0) Looping left caecum. (1) Do not looping left caecum.

14. *Eyespots*. (0) Eyespot or pigmentary grains present. (1) Eyespots present or absent. (2) Eyespots always present.

15. *Number of marginal hooks in adult*. (0) 14. (1) 12.

16. *Lateral expansions on caecum*. (0) Absent. (1) Present.

17. *Caeca*. (0) Confluent. (1) Nonconfluent.

18. *Shape of testis*. (0) Irregular. (1) Regular.

Appendix 2

Character matrix used in reconstruction of the phylogeny of the Diplectanidae. Polymorphic character states (either 0 or 1 in the genus) are noted P. Unapplicable character states are coded 9.

Homologous serie	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Pseudomurraytrema	0	0	0	0	0	0	0	000000	9	9	1	1	1	2	0	0	0	0
Murraytrematoides	0	0	0	0	0	0	0	000000	9	9	P	0	P	1	0	0	0	1
Murraytrema	0	0	0	1	0	0	0	000000	9	9	P	1	P	0	0	0	1	0
Lobotrema	0	9	1	1	0	0	0	000000	9	9	P	1	1	0	0	0	1	0
Rhabdosynochus	1	1	0	0	0	0	0	100000	9	9	1	1	0	2	0	0	1	1
Protolamellodiscus	0	0	0	0	0	0	2	011000	0	2	1	1	0	2	0	0	1	0
Calydiscoides	0	0	0	0	0	0	2	011000	0	2	P	1	0	2	0	0	1	0
Lamellodiscus	0	0	0	0	0	0	2	011000	1	1	1	1	P	1	0	0	1	0
Telegamatrix	0	0	0	0	1	0	2	011000	1	1	1	1	9	2	0	0	1	0
Furnestinia	0	0	0	0	0	0	1	011000	1	1	1	1	0	2	0	0	1	0
Monoplectanum	0	0	0	0	0	0	1	010101	0	0	1	1	0	2	0	0	1	0
Latericaecum	0	0	0	0	0	0	2	010101	0	0	0	0	0	2	1	1	1	1
Lepidotrema	0	0	0	0	0	1	2	010110	0	0	0	1	0	2	0	0	1	0
Diplectanum	0	0	0	0	0	0	2	010110	0	0	P	1	0	0	0	0	1	1
Pseudodiplectanum	1	1	0	0	0	0	2	010110	0	0	1	1	0	1	0	0	1	0
Heteroplectanum	0	0	0	0	0	0	2	010110	0	0	0	1	0	2	0	0	1	0
Pseudolamellodiscus	0	1	0	0	0	0	2	010110	0	0	0	0	0	2	0	0	1	0
Cycloplectanum	0	0	0	0	0	0	2	010110	0	1	0	1	0	2	0	0	1	1

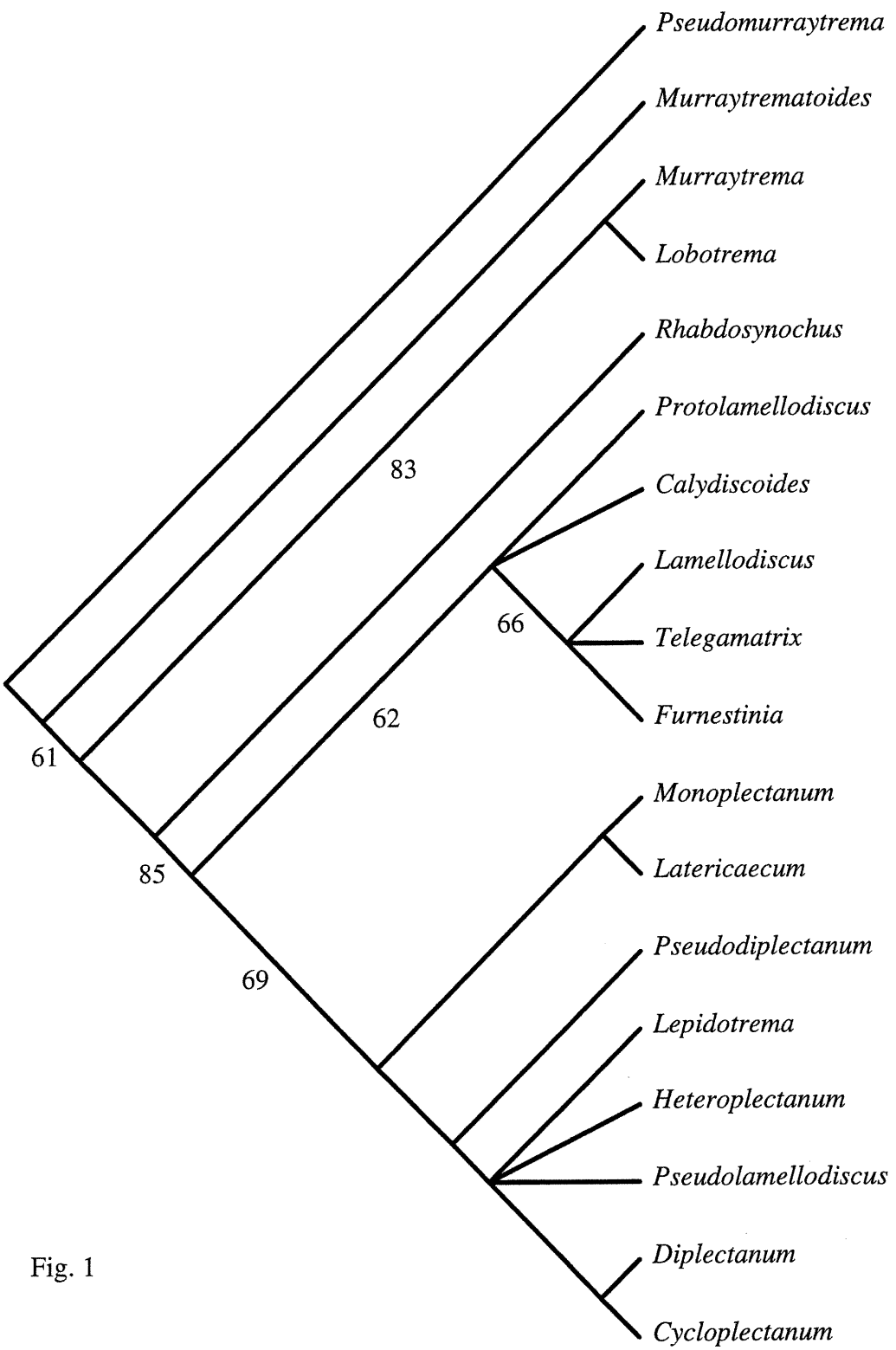
Pseudomurraytrema
Murraytrematoides
Murraytrema
Lobotrema
Rhabdosynochus
Protolamellodiscus
Calydiscoides
Lamellodiscus
Telegamatrix
Furnestinia
Monoplectanum
Latericaecum
Pseudodiplectanum
Lepidotrema
Heteroplectanum
Pseudolamellodiscus
Diplectanum
Cycloplectanum
83
66
62
61
85
69

Fig. 1

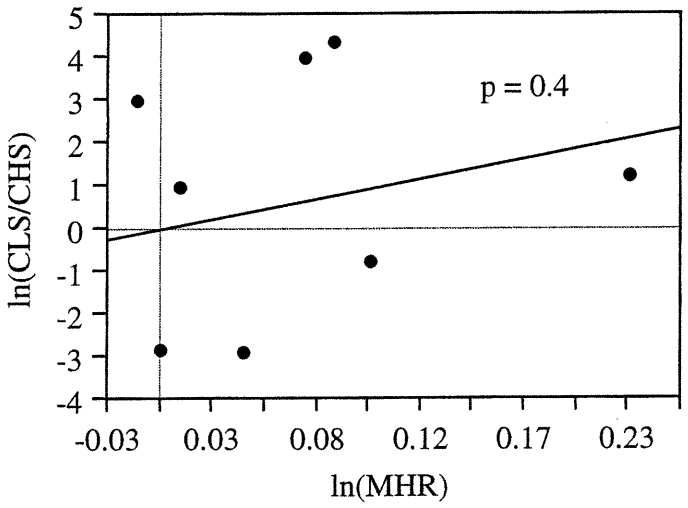

Fig. 2

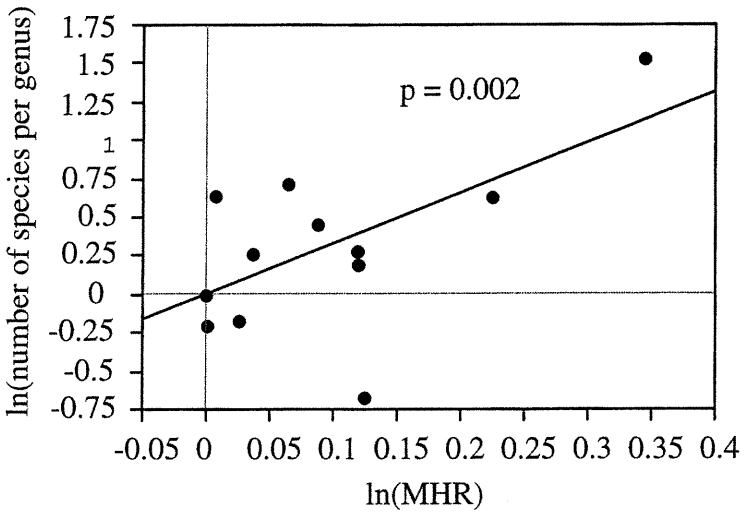
ln(number of species per genus)
1.75
1.5
1.25
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
p = 0.002
-0.05 0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4
ln(MHR)

Fig. 3

B. PHYLOGÉNIE DES *LAMELLODISCUS*: ÉTUDE PRÉLIMINAIRE, CHOIX DES MARQUEURS ET PRÉCISIONS DE CERTAINES INCERTITUDES TAXONOMIQUES

Article 3

DESDEVISES Yves, Richard JOVELIN, Olivier JOUSSON and Serge MORAND. 2000. Comparison of ribosomal DNA sequences of *Lamellodiscus* spp. (Monogenea, Diplectanidae) parasitizing *Pagellus* (Sparidae, Teleostei) in the North Mediterranean Sea: species divergence and coevolutionary interactions. *International Journal for Parasitology.* 30: 741-746.

Objectifs c et d

Ce travail est le premier basé sur le séquençage de l'ADN des *Lamellodiscus* et des Sparidae. C'est un travail préliminaire sur la coévolution entre les deux groupes, qui se focalise sur une partie seulement des espèces appartenant aux deux complexes d'espèces. Les résultats laissent entendre que des incongruences existent entre les deux phylogénies, et donc que des événements de capture existent probablement entre les parasites et leurs hôtes. Un résultat important est que deux espèces précédemment décrites, *Lamellodiscus virgula* et *L. obeliae*, s'avèrent être une seule espèce sur la base de la divergence de leurs séquences d'ADN (ITS1). Nous avons également montré que l'ADN ribosomal 18S semble être un bon marqueur pour inférer la phylogénie des *Lamellodiscus*. Ces informations seront pris en compte dans la reconstruction de la phylogénie globale de tous les *Lamellodiscus* de Méditerranée.

Participation du thésard :

- Recherche bibliographique
- Séquençage de l'ADN des parasites
- Analyse phylogénétique
- Rédaction

COMPARISON OF RIBOSOMAL DNA SEQUENCES OF *LAMELLODISCUS* SPP. (MONOGENEA, DIPLECTANIDAE) PARASITISING *PAGELLUS* (SPARIDAE, TELEOSTEI) IN THE NORTH MEDITERRANEAN SEA: SPECIES DIVERGENCE AND COEVOLUTIONARY INTERACTIONS*

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* Note: Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession numbers AJ276439-AJ276447 and AJ276879-AJ276881.

Abstract

We sequenced DNA fragments from four monogenean species of the genus *Lamellodiscus* and their three fish host species from the genus *Pagellus* in the North Mediterranean Sea, in order to estimate the molecular divergence and the coevolutionary interactions in this association. By comparing the ITS1 sequences of the parasites, we assessed their level of interspecific differences and tested the phylogenetic status of *Lamellodiscus virgula* and *Lamellodiscus obeliae,* formerly described as two different species. Moreover, we wanted to know if closely related parasites used closely related hosts, to investigate the coevolutionary interactions in this complex. Phylogenetic relationships among *Lamellodiscus* species were estimated with partial 18S ribosomal DNA sequences while mitochondrial cytochrome-b DNA sequences were used for their fish hosts. The ITS1 sequences appear to be highly variable among *Lamellodiscus* species, except *L. virgula* and *L. obeliae*, suggesting an old divergence time or a rapid molecular evolution within this genus. This fish-parasite association seems to exhibit coevolutionary interactions. *L. virgula* and *L. obeliae* are proposed to be a single species on the basis of their almost identical ITS1 sequences.

Key words: Species status, 18S rDNA, ITS1, cytochrome-b DNA, *Lamellodiscus*, *Pagellus*, Coevolution

Introduction

Monogeneans are commonly recognised as highly host-specific [1-2]. This group of parasites is characterized by an important taxonomic diversification [3], with about 3000 described species [4, 5]. Species description and identification of monogeneans are almost only made through morphological studies. However, morphological identification are sometimes based on very small differences, and the host is considered as a criterion for new species identification in some cases [6]. Moreover, molecular [7] and developmental studies [8] have shown that morphologically different parasite species can be a single species. It has also been demonstrated that a single species of monogenean (*Gyrodactylus salaris*) can show morphological variations because of extrinsic factors such as host species or temperature [9-11]. The taxonomic status of some species of monogeneans could therefore be questioned and we addressed this idea through the study of monogeneans from the genus *Lamellodiscus* Johnston and Tiegs, 1922.

The genus *Lamellodiscus* is currently composed of 36 described species [12]. These species are generally highly host specific. They parasitise fish from the Sparidae family around the world [12, 13]. Among Sparidae, the genus *Pagellus* is composed of three sympatric species in the North Mediterranean Sea [14]: *Pagellus erythrinus* (Linnaeus, 1758), *Pagellus acarne* (Risso, 1826) and *Pagellus bogaraveo* (Brünnich, 1768). These three *Pagellus* species are parasitised by four *Lamellodiscus* species [15, 16], following a specific pattern: *P. erythrinus* is parasitized by *Lamellodiscus erythrini* [15], *P. acarne* harbors *Lamellodiscus drummondi* and *Lamellodiscus virgula* [15], and *P. bogaraveo* is the exclusive host of *Lamellodiscus obeliae* [17]. In comparison to *L. erythrini*, *L. virgula*, *L. obeliae* and *L. drummondi* share a morphological homogeneity [15, 17], by the shape of their copulatory organ, their haptor type and their absence of eyespots. *Lamellodiscus virgula* and *L. obeliae* are even closer based on the morphology of their copulatory organ, but they use different hosts. Thus, at the morphological level, these four species present an array of similarities, with *L. obeliae* and *L. virgula* being the closest, *L. drummondi* slightly different from these two species, and *L. erythrini* much more different. We need to ask, then, if the morphological difference among species is reflected by their molecular divergence, and if the closest species are really different at the molecular level (i.e. distinct

species). The amount of interspecific differences can be assessed with DNA sequence comparison, in particular by using the internal transcribed spacers (ITS) [18].

The ITS lies in the ribosomal DNA cluster between the 18S rRNA and 28S rRNA coding regions. The ITS is divided into two (ITS1 and ITS2), separated by the gene coding for 5.8S rRNA. The ribosomal coding regions are relatively slow-evolving and highly conserved, while the ITS are known to show a lot of variability [18, 19] because of their faster evolving rate. The ITS have already been used for diagnostic purposes at the species level among the Digenea [20] and the Nematoda [10]. Evolutionary relationships of monogeneans have also been depicted through rDNA analysis [21-23], and among-species differences in ITS have been assessed for monogeneans [24].

ITS1 sequences were used here to assess the level of differences between the *Lamellodiscus* parasitising *Pagellus*, and especially to test if *L. virgula* and *L. obeliae* are effectively distinct species. As coevolutionary interactions could have shaped the specificity pattern of these parasites, we also wanted to assess the phylogenetic relationships of both parasites and hosts to see if closely related parasites use closely related hosts, i.e. if distance among parasites is related to distance among hosts. Indeed, as monogeneans are highly host-specific, they have been suggested to show tight coevolutionary interactions with their hosts [2]. This has been shown at the family level [25], where cospeciation events are widespread, but remains to be investigated at a finer scale (genus or species level) where coevolution studies are scarce [26]. The phylogenetic relationships within the genus *Lamellodiscus* are unknown and DNA sequences were also used to assess the phylogeny of the group. As there is no information on the molecular divergence between these species, we used partial 18S rRNA sequence of the 3' terminal end. It has been shown that sibling species can be very different at the molecular level [27], thus ITS1 may not be suitable to assess the phylogenetic relationships among these species. *Pagellus* phylogeny is also poorly known [28] and the evolutionary relationships among the three species studied here were estimated from cytochrome b sequences since this mitochondrial gene has already been used to infer fish phylogenetic relationships at the order [29] and family level [30].

Material and methods

Host

Pagellus spp. were sampled in the Golfe du Lion near Banyuls-sur-Mer (France) for *P. acarne* and *P. bogaraveo* and in Corsica (Scandola Natural Reserve, France) for *P. erythrinus*.

Mitochondrial DNA was extracted from fresh specimens of *Pagellus* in guanidine lysis buffer, precipitated with isopropanol and dissolved in distilled water [18]. The following reagents were used in each amplification: 1.25 unit TAQ polymerase, 5 ml 10X PCR buffer, 1 ml of each dNTP at 10 mM, 10 pM of each primer, 1 ml template DNA, made up to 40 ml with water. PCR amplifications were performed in a total volume of 50 ml with an amplification profile consisting of 40 cycles of 30 s at 94°C, 30 s at 50°C and 2 min at 72°C, followed by 5 min at 72°C for final extension. The cytochrome b gene of the mitochondrial DNA was amplified using specific primers. The primers were designed in adjacent regions coding for the transfer RNA Glutamate (cbtd2: 5'- AAT GAY WTG AAA AAC CAC CGT TG -3') and Tryptophane (cbtr2: 5'- CGG MTT ACA AGR CCG RYG CT -3') using the complete mitochondrial genome sequences available for *Gadus morhua* (EMBL Accession Number NC002081), *Salmo salar* (NC001960) and *Cyprinus carpio* (NC001606). Amplified PCR products were purified using High Pure PCR Purification Kit (Roche Diagnostics) and sequenced directly using the ABI 377 DNA sequencer (Perkin-Elmer). The amplification primers were also used for DNA sequencing.

Parasites

Lamellodiscus were sampled from fish gills under a dissecting microscope and identified through the observation of haptor and copulatory organ morphology under an optical microscope with a 400 X magnification. *Diplectanum aequans* (Wagener, 1857), used as outgroup in the phylogenetic analysis, was sampled and identified from alcohol-preserved gills of *Dicentrachus labrax* (Linnaeus, 1758) from Atlantic Ocean. Parasites were kept in 95% alcohol before DNA extraction.

After identification, DNA was extracted from one parasite individual per species with BioRad Chelex 100™ [18]. Partial 18S rDNA sequence and the entire ITS1 region were amplified using the L7 (5'- TGA TTT GTC TGG TTT ATT CCG AT –3') and H7 primers (5'- GCT GCG TTC TTC ATC GAT ACT CG -3') which anneal to the 18S rRNA and 5.8S rRNA respectively [27]. PCR was carried out in a final volume of 25 µl with the following steps: 4 min at 95°C, following by 35 cycles of 1 min at 45°C, 1 min 30 at 72°C, 1 min at 94°C, and a final elongation of 10 min at 72°C. Electrophoresis was then performed on a 1% agarose gel stained with ethidium bromide for DNA visualisation under UV light. The band of interest was excised and the DNA purified using the Concert™ Rapid Gel Extraction System (Life Technologies). The DNA fragment was then cloned in PGEM®-T Vector (Promega) and transfected in JM109 competent cells (Promega). Positive colonies were visually screened and purified with the QIAprep Spin Miniprep Kit (QIAgen). Sequencing was done with an automated sequencer by Genome Express S.A. (Grenoble).

Three clones were sequenced when possible and inter-clone variability was always negligible (less than 0.5 %).

Phylogenetic reconstructions

Sequence alignment was first performed with ClustalW [31] and improved by eye with Se-Al (v1d1, A. Rambaut, University of Oxford, 1995) and analyses were carried out on all substitutions.

The phylogenetic tree was reconstructed using neighbour-joining analysis with PAUP* 4.0 [32]. Distances were estimated using the Kimura-2-parameter model, correcting for transition bias. The outgroup taxa are *Dentex dentex* (Sparidae, EMBL Accession Number AF143197) for the fish tree, and *Diplectanum aequans* (Diplectanidae) for the parasite tree. Phylogenetic reconstructions (phenograms) were validated with a bootstrap procedure [33].

Results

Sequences

Total length of *Pagellus* cytochrome b sequences are 1106 bp each.

For *Lamellodiscus* DNA sequences, the length of the 18S rRNA fragments were comprised between 534 bp and 537 bp, while the length of the ITS1 fragments were between 370 and 469 bp (Table 1). The ITS1 sequences are highly variable, and impossible to align unambiguously, except those between *L. virgula* and *L. obeliae*, which differ by only one base insertion. Therefore, we used the 18S rDNA sequence to perform the phylogenetic analysis. The 18S rDNA differences among species lies between 0% (*L. virgula* - *L. obeliae*) and 6.54% (*L. drummondi* - *L. erythrini*). Among-species differences for the ITS1 and 18S rDNA are shown in Table 1. The morphological differences among species mentioned earlier are reflected in their molecular divergence: *L. virgula* and *L. obeliae* are the closest species (0% difference for 18S), *L. drummondi* is slightly different (3.55%), and *L. erythrini* is much more divergent (> 5%).

Phylogenetic reconstructions

The fish tree is shown in Fig. 1a. This tree groups *P. acarne* and *P. bogaraveo*, which have indeed the most similar morphology and share a protandrous hermaphrodism (*P. erythrinus* is protogynous) [14].

The parasite tree (Fig. 1b) is built from the 18S rDNA sequence only because of the high between-species variability of the ITS1 fragment.

Discussion

Although the copulatory organ morphologies of *L. virgula* and *L. obeliae* are slightly different [12], these species present almost no variation for the whole DNA fragments sequenced here (ITS1 and 18S rDNA). This is especially noteworthy when this is compared with the otherwise highly variable ITS1. To assess the inter and intra-specific difference of ITS in monogeneans, Cunningham [24] compared the ITS region of three *Gyrodactylus* species by RFLP and showed several nucleotide differences among these species. Harris et

al. [34] reported an important difference in ITS1 length between *Gyrodactylus turnbulli* (475 bp) and *Gyrodactylus salaris* (625 bp), which is of the same order as the variation in ITS1 size we observed among *Lamellodiscus* species. Concerning digeneans, Jousson et al. [35] studied the ITS from Mesometridae and measured interspecific differences ranging from 6.6% to 19.1% for the ITS1, and from 3.4% to 15.1% for the ITS2. For another digenean family, the Opecoelidae, Jousson et al. [36] studied the ITS and measured interspecific differences ranging from 2% to 28%. Adlard et al. [20] sequenced the ITS2 from Fasciolidae species, and measured a 2.8% divergence between *Fasciola hepatica* and *Fasciola gigantica* sequences. The amount of intraspecific variation in *F. hepatica* was 0.4% (one substitution in 263 nucleotides compared). Several studies have assessed the ITS variations in nematodes. Newton et al. [7] reported ITS2 differences ranging from 1.7% to 5.0% between *Cooperia* species, and 3.9% to 24,7% between *Nematodirus* species [37]. Chilton et al. [38] assessed differences ranging from 25.0% to 28.3% among ITS2 sequences from three morphologically similar *Hypodontus* species. Stevenson et al. [39] measured a difference of 1.3% between the ITS2 of two *Haemonchus* species. Hoste et al. [40] defined a range of 1.3% - 7.6% differences in ITS2 to distinguish *Trichostrongylus* species. Contrary to our ITS1 monogenean sequences, it was possible to align the ITS sequences for all these digenean and nematode species. In the present study, given that the ITS1 sequences among *Lamellodiscus* species are so different that they cannot be aligned between species and that the differences between *L. obeliae* and *L. virgula* species are small (0.27%: one nucleotide in 370 bp), we propose that *L. virgula* and *L. obeliae* are a single species.

From this, two not mutually exclusive hypotheses can be suggested: 1) these two species are presently undergoing a speciation event and the morphological differentiation precedes the molecular one, or 2) as these two morphological types are exclusively and respectively found on two different sympatric hosts, this morphological differentiation could be host-induced. Downes [8] found that parasitic mites of the mussel can exhibit a different morphological development depending on the colonised host, and this has been suggested for the monogenean *Gyrodactylus salaris* by Mo [10]. If this host-induced variation is the case for at least some species of monogeneans, the assumption of their high host specificity should be considered with caution.

As previously mentioned, *Lamellodiscus* species parasitising *Pagellus*, except *L. erythrini*, are very similar at the morphological level. In addition, they are different from other existing *Lamellodiscus* species [12, 13, 15]. For example, they exhibit the same type of copulatory organ, and they have no eyespot, a feature shared by only five *Lamellodiscus* species (*L. drummondi*, *L. mormyri*, *L. obeliae*, *L. virgula* and *L. verberis*), which parasitise only two host genera, *Pagellus* and *Lithognathus* (formerly *Pagellus* [14]). This strongly suggests a coevolutionary pattern. *Pagellus erythrinus* and its specific parasite *L. erythrini* are phylogenetically isolated from the clade formed by *P. acarne* and *P. bogaraveo* on the one hand and *L. drummondi*, *L. virgula* and *L. obeliae* on the other (see Fig. 1). We cannot speculate on co-speciation events between fishes from the genus *Pagellus* and their *Lamellodiscus* parasites as we do not know if these two clades are monophyletic [41]. However, if *L. virgula* and *L. obeliae* are considered as a single species, the presence of this species on two different hosts could be explained by a host-switching event. Thus, this association does not show a clear co-speciation pattern (as suggested by Farenholz's rule [42]. Three of the four *Lamellodiscus* species studied show a high molecular variability among their ITS1 sequences. This could be due to either a rapid molecular evolution or an old divergence time among the species. This latter hypothesis suggests that the speciation events could be ancient.

The fact that *L. obeliae* and *L. virgula* probably are the same species in spite of morphological differences suggests that the number of species in *Lamellodiscus*, and by extension in other monogeneans, may be overestimated. This could be a result of the concept of very strict host specificity for monogeneans, which could have led to the erection of new species on different hosts, thus reinforcing this conception.

Acknowledgments

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References

[1] Baer JG, editor. First Symposium on Host Specificity among Parasites of Vertebrates. Paul Attinger, Neuchatel, 1957.

[2] Noble ER, Noble GA, Schad GA, MacInnes AJ. Parasitology. The Biology of Animal Parasites. 6th Edition. Lea & Febiger, Philadelphia, 1989.

[3] Sasal P, Desdevises Y, Morand S. Host-specialization and species diversity in fish parasites: phylogenetic conservatism? Ecography 1998; 21:639-643.

[4] Brooks DR, McLennan DA. Parascript. Smithsonian series in Comparative Evolutionary Biology, Washington and London, 1993.

[5] Rohde K. Robust phylogenies and adaptive radiations: a critical examination of methods used to identify key innovations. Am Nat 1996; 148: 481-500.

[6] Euzet L, Combes C. Les problèmes de l'espèce chez les animaux parasites. In: Les problèmes de l'espèce dans le règne animal. Tome III. In: Boquet C, Génermont J, Lamotte M, editors. Mém Soc Zool Fr 1980; 40: 239-285.

[7] Newton LA, Chilton NB, Beveridge I, Gasser RB. Genetic evidence indicating that *Cooperia surnabada* and *Cooperia oncophora* are one species. Int J Parasitol 1998; 28: 331-336.

[8] Downes BJ. Host-induced morphology in mites: implication for host-parasite coevolution. Syst Zool 1990; 39: 162-168.

[9] Mo TA. Seasonal variations of opisthohaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Bastnfjorselva, Norway. Syst Parasitol 1991; 19: 231-240.

[10] Mo TA. Variations of opisthohaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss*

(Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in South-eastern Norway. Syst Parasitol 1991; 20: 1-9.

[11] Mo TA. Variations of opisthohaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. Syst Parasitol 1991; 20: 11-19.

[12] Oliver G. Les Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea, Dactylogyridea). Systématique. Biologie. Ontogénie. Écologie. Essai de phylogénèse. Thèse d'état. Université des Sciences et Techniques du Languedoc, 1987.

[13] Oliver G. Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. II. Lamellodiscinae nov. sub. fam. Vie Milieu 1968; 19: 95-138.

[14] Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E. Checklist of the fishes of the North-eastern Atlantic and of the Mediterranean. Paris, UNESCO, Vol. III, 1986.

[15] Euzet L, Oliver G. Diplectanidae (Monogenea) de Téléostéens de la Méditerranée occidentale. IV. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Pagellus* Cuvier, 1829 (Sparidae). Ann Par Comp (Paris) 1967; 42: 407-425.

[16] Euzet L, Combes C, Caro A. A checklist of Monogenea of Mediterranean fish. Second International Symposium on Monogenea. Montpellier/Sète - 5-8 July 1993.

[17] Oliver G. *Lamellodiscus obeliae* n. sp. une nouvelle espèce de Diplectanidae (Monogenea, Monopisthocotylea) parasite de *Pagellus centrodontus* (Delaroche, 1809) (Pisces, Sparidae). Z Parasitenk 1973; 41: 103-108.

[18] Hillis DM, Moritz C, Mable BK: editors. Molecular Systematics. Second Edition. Sinauer Associates. Sunderland, USA, 1996.

[19] Hillis DM, Dixon MT. Ribosomal DNA: molecular evolution and phylogenetic inference. Quaterly Rev Biol 1991; 66: 411-453.

[20] Adlard RD, Barker SC, Blair D, Cribb TH. Comparison of the second internal transcribed spacer (ribosomal DNA) from populations and species of Fasciolidae (Digenea). Int J Parasitol 1993; 23: 423-425.

[21] Baverstock PB, Fielke R, Johnson AM, Bray RA, Beveridge I. Conflicting phylogenetic hypotheses for the parasitic platyhelminths tested by partial sequencing of 18S ribosomal RNA. Int J Parasitol 1991; 21: 329-339.

[22] Cunningham CO, McGillivray DM, MacKenzie K. Phylogenetic analysis of *Gyrodactylus salaris* Malmberg, 1957 based on the small subunit (18S) ribosomal gene. Mol Biochem Parasitol 1995; 71: 139-142.

[23] Littlewood DTJ, Rohde K, Clough KA. Parasite speciation within or between host species? Phylogenetic evidence from site-specific polystome monogeneans. Int J Parasitol 1997; 27: 1289-1297.

[24] Cunningham CO. Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. J Parasitol 1997; 83: 215-219.

[25] Boeger WA, Kritsky DC. Coevolution of the monogenoidea (platyhelminthes) based on a revised hypothesis of parasite phylogeny. Int J Parasitol 1997; 27: 1495-1511.

[26] Klassen GJ, Beverley-Burton M. Phylogenetic relationships of *Ligictaluridus* spp. (Monogenea: Ancyrocephalidae) and their Ictalurid (Siluriformes) hosts: an hypothesis. Proc Helminthol Soc Wash 1987; 54: 84-90.

[27] Verneau O, Renaud F, Catzeflis F. Evolutionary relationships of sibling tapeworm species (Cestoda) parasitizing teleost fishes. Mol Biol Evol 1997; 14: 630-636.

[28] Basaglia F. Interspecific gene differences and phylogeny of the Sparidae family (Perciformes, Teleostei), estimated from electrophoretic data on enzymatic tissue expression. Comp Biochem Physiol 1991; 99 :495-508.

[29] Cantatore P, Roberti M, Pesole G et al. Evolutionary analysis of cytochrome b sequences in some perciformes: evidence for a slower rate of evolution than in mammals. J Mol Evol 1994; 39: 589-597.

[30] Song CB, Near TJ, Page LM. Phylogenetic relationships among percid fishes as inferred from mitochondrial cytochrome b sequence data. Mol Phyl Evol 1998; 10: 343-353.

[31] Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucl Acids Res 1994; 22: 4673-4680.

[32] Swofford, DL. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, 1999.

[33] Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985; 39: 793-791.

[34] Harris PD, Cable J, Tinsley RC, Lazarus CM. Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. J Parasitol 1999; 85: 188-191.

[35] Jousson O, Bartoli P, Zaninetti L, Pawlowski J. Use of the ITS rDNA for elucidation of some life-cycles of Mesometridae (Trematoda, Digenea). Int J Parasitol 1998; 28: 1403-1411.

[36] Jousson O, Bartoli P, Pawlowski J. Molecular identification of developmental stages in Opecoelidae (Digenea). Int J Parasitol 1999; 29: 1853-1858.

[37] Newton LA, Chilton NB, Beveridge I, Gasser RB. Differences in the second internal transcribed spacer of four species of *Nematodirus* (Nematoda: Molineidae). Int J Parasitol 1998; 28: 337-341.

[38] Chilton NB, Gasser RB, Beveridge, I. Differences in a ribosomal DNA sequence of Morphologically indistiguishable species within the *Hypodontus macropi* complex (Nematoda: Strongyloidea). Int J Parasitol 1995; 25: 647-651.

[39] Stevenson LA, Chilton NB, Gasser RB. Differentiation of *Haemonchus placei* from *H. contortus* (Nematoda: Trichstrongylidae) by the ribosomal DNA second internal transcribed spacer. Int J Parasitol 1995; 25: 483-488.

[40] Hoste H, Chilton NB, Gasser RB, Beveridge I. Differences in the second internal transcribed spacer (ribosomal DNA) between five species of *Trichostrongylus* species (Nematoda: Trochostrongylidae). Int J Parasitol 1995; 25: 75-80.

[41] Page RDM. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics 1994; 10: 155-173.

[42] Fahrenholz H. Ectoparasiten und Abstammungslehre. Zool Anz 1913; 41: 371-74.

Legends to Figures:

Figure 1: (a) Phylogenetic tree of North Mediterranean *Pagellus* species obtained by a neighbour-joining analysis with a Kimura-2-parameter model; (b) Phylogenetic tree of *Lamellodiscus* from North Mediterranean *Pagellus* species obtained by a neighbour-joining analysis with a Kimura-2-parameter model. Thin lines depict host-parasite associations, where *L. virgula* and *L. obeliae* are considered as a single species. Numbers are Bootstrap values (1000 replicates).

Table 1

ITS1 lengths and species - species distances (in % differences) for the ITS1 (first value) and the18S rDNA (second value) sequences of the four *Lamellodiscus* species used in this study.

Species (ITS1 length in bp)	*L. drummondi*	*L. virgula*	*L. obeliae*
L. erythrini (397)	NA[1]/6.54	NA/5.42	NA/5.42
L. drummondi (469)	0	NA/3.55	NA/3.55
L. virgula (370)	-	0	0.27/0
L. obeliae (371)	-	-	0

[1] Non-alignable

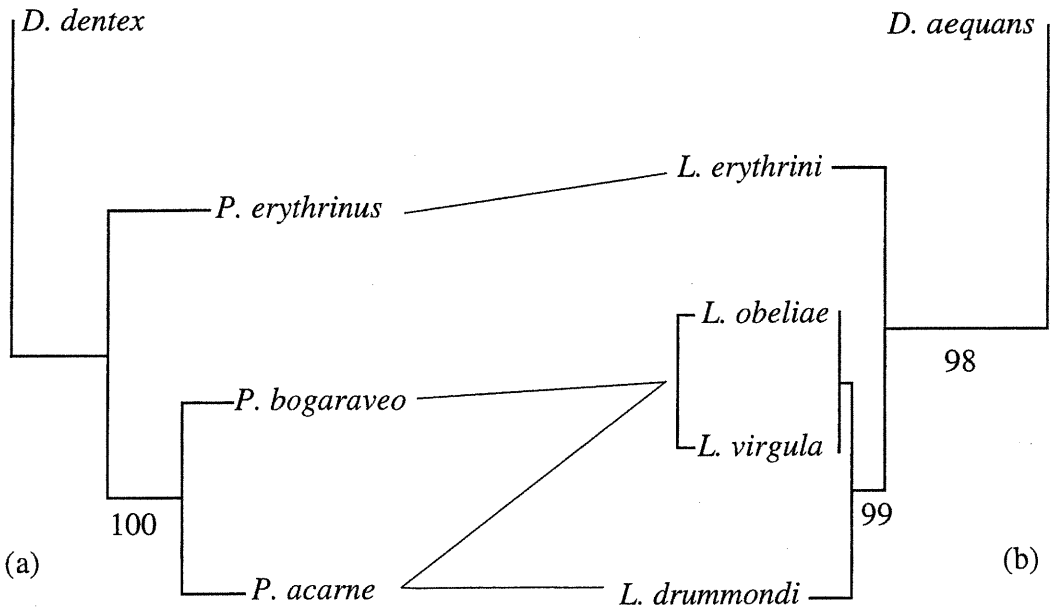
D. dentex
D. aequans
L. erythrini
P. erythrinus
L. obeliae
98
P. bogaraveo
L. virgula
99
100
(a)
(b)
P. acarne
L. drummondi

Fig. 1

Article 4

DESDEVISES Yves. 2001. The phylogenetic position of *Furnestinia echeneis* (Monogenea, Diplectanidae) based on molecular data: a case of morphological adaptation? *International Journal for Parasitology* 31(2): 205-208.

Objectifs c et d

Dans ce travail, je suggère, sur la base de séquences d'ADN ribosomal 18S partiel, que le genre *Furnestinia*, contenant une seule espèce, *F. echeneis*, parasite de *Sparus aurata* (Sparidae), devrait être inclus dans le genre *Lamellodiscus*, car une reconstruction de la phylogénie de plusieurs espèces de *Lamellodiscus* et de *F. echeneis* indique que cette espèce est clairement nichée dans l'arbre évolutif des *Lamellodiscus*. En outre, la morphologie de *F. echeneis* semble compatible avec l'arbre obtenu. L'hypertrophie de son unique lamellodisque est proposée comme une adaptation morphologique à l'accrochage sur l'hôte.

Participation du thésard :

- Tout

RESEARCH NOTE

THE PHYLOGENETIC POSITION OF *FURNESTINIA ECHENEIS* (MONOGENEA, DIPLECTANIDAE) BASED ON MOLECULAR DATA: A CASE OF MORPHOLOGICAL ADAPTATION?*

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Key Words: 18S rDNA, Monogenea, *Furnestinia echeneis*, *Lamellodiscus*, phylogeny, adaptation

* Note: Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession numbers AF294952-AF294957 and AJ276439-AJ276442

Abstract

The genus *Furnestinia* (Diplectanidae) contains only one species, *Furnestinia echeneis*. However, its close morphological similarity with the genus *Lamellodiscus* (Diplectanidae) raises doubt concerning its placement in a separate genus. These two genera differ only by their number of lamellodiscs: one for *Furnestinia*, two for *Lamellodiscus*. Here, the taxonomic position of *F. echeneis* is investigated via a phylogenetic reconstruction based on partial 18S rDNA for *F. echeneis* and several *Lamellodiscus* species. *Furnestinia echeneis* appears to be clearly nested into the *Lamellodiscus* genus, these two genera should then be synonymised. The hypertrophy of its unique lamellodisc is hypothesised to be a morphological adaptation for attachment to the host.

The taxonomic status of the monogenean *Furnestinia echeneis* (Diplectanidae), a fish gill ectoparasite, has been debated since it was first described as belonging to the genus *Dactylogyrus* by Wagener (1857). In 1889 it was transferred to the genus *Diplectanum* Diesing, 1858 by Parona and Perugia (1859). Later it was assigned to the genus *Lamellodiscus* Johnston and Tiegs, 1922 by Palombi (1943). Euzet and Audoin (1959) contains a more detailed account of the changes in classification. Euzet and Audoin (1959) created the genus *Furnestinia* for only this species and placed it in the subfamily Diplectaninae Monticelli, 1903, now considered as part of the family Diplectanidae Monticelli, 1903 (Oliver, 1968; Oliver G., 1987. Les Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea, Dactylogyridea). Systématique. Biologie. Ontogénie. Écologie. Essai de phylogénèse. Thèse d'état. Université des Sciences et Techniques du Languedoc; Euzet L., Combes C., Caro A., 1993. A checklist of Monogenea of mediterranean fish. Second International Symposium on Monogenea. Montpellier/Sète - 5-8 July, 1993). Oliver (1969) placed the genus *Furnestinia* in the subfamily Lamellodiscinae Oliver, 1969. This genus was based on a specific morphological feature: the presence of a single adhesive organ on the opistohaptor. Typical diplectanids possess two adhesive organs, except a few genera among the 17 comprising the entire family: *Murraytrematoides* Yamaguti, 1958, *Murraytrema* Price, 1937, *Lobotrema* Tripathi, 1957 and *Rhabdosynochus* Mizelle and Blatz, 1941 which have no squamodisc (opisthaptoral adhesive organ of the Diplectaninae), and *Monoplectanum* Young, 1969, which possesses one (Oliver, 1968, 1969; Oliver, 1987, Thesis; Euzet et al., 1993, Second Int. Symp. on Monogenea). Among the Diplectanidae, the only morphological difference between *Furnestinia* and *Lamellodiscus* is the number of adhesive organs. These structures are of the same type in these two genera. They are composed of two vertical rows of lamellae, and are therefore called lamellodiscs, but the unique lamellodisc of *Furnestinia* is strongly developed compared with the same structure in *Lamellodiscus*. Moreover, the oncomiracidium in these two genera possesses an excretory system with four pairs of protonephridia (Oliver, 1987, Thesis), versus five in the Diplectaninae. The genus *Lamellodiscus* contains 36 described species throughout the world (Oliver, 1987, Thesis), mainly in the Mediterranean Sea (Euzet et al., 1993, Second Int. Symp. on Monogenea) and Australia (Oliver, 1987, Thesis; Young, 1969), which parasitise almost exclusively teleost fishes from the family

Sparidae. The majority of the 20 known *Lamellodiscus* species inhabit the Mediterranean Sea (Oliver, 1987, Thesis). *Furnestinia echeneis* is found only on the sparid fish *Sparus aurata* Linnaeus, 1758, in the Mediterranean Sea. Considering the important morphological similarity between these two genera, it is unclear if the genus *Furnestinia* with only one species should be considered as a *Lamellodiscus* species.

The objective of this study was to investigate whether *Furnestinia* is a distinct group from *Lamellodiscus* or if it belongs to this latter genus, by reconstructing a phylogeny comprising the unique *Furnestinia* species, *F. echeneis*, and several *Lamellodiscus* species. To solve this problem, I used data independent of morphology to study the phylogenetic relationships between *F. echeneis* and *Lamellodiscus* species, thus avoiding circular reasoning of using morphology to build a phylogeny which will be used to discuss morphology.

The 3' terminal portion of the 18S rDNA was used to estimate the phylogenetic relationships among these species, as it has been an appropriate phylogenetic marker in monogeneans (e.g. Cunningham et al., 1995), and can accurately be used to resolve evolutionary relationships among *Lamellodiscus* species (Desdevises et al., 2000).

Several *Lamellodiscus* species were included in the phylogenetic tree: *Lamellodiscus drummondi* Euzet and Oliver, 1967, *Lamellodiscus virgula* Euzet and Oliver, 1967, *Lamellodiscus erythrini* Euzet and Oliver, 1967, *Lamellodiscus mormyri* Euzet and Oliver, 1967, *Lamellodiscus verberis* Euzet and Oliver, 1967, *Lamellodiscus elegans* Bychowsky, 1957 and *Lamellodiscus ignoratus* Palombi, 1943. The first three species were previously sequenced for this DNA fragment (Desdevises et al., 2000), the others are new data. *Lamellodiscus drummondi* and *L. virgula* are found on *Pagellus acarne* (Risso, 1826), *L. erythrini* parasitizes *Pagellus erythrinus* (Linnaeus, 1758), *L. mormyri* and *L. verberis* parasitise *Lithognathus mormyrus* (Linnaeus, 1758), while *L. elegans* and *L. ignoratus* are generalist monogeneans, parasitising *Diplodus annularis* (Linnaeus, 1758), *Diplodus sargus* (Linnaeus, 1758), *Diplodus vulgaris* (E. Geoffroy Saint-Hilaire, 1817), *Oblada melanura* (Linnaeus, 1758) and *Spondyliosoma cantharus* (Linnaeus, 1758) for the former, and *Diplodus annularis*, *Diplodus puntazzo* (Cetti, 1777), *Diplodus sargus*, *Diplodus*

vulgaris, *L. mormyrus*, and *Sarpa salpa* (Linnaeus, 1758) for the latter (Euzet and Oliver, 1966a,b; Oliver, 1969, 1974; Oliver, 1987, Thesis; Euzet et al., 1993, Second Int. Symp. on Monogenea). *Lamellodiscus elegans* was sampled from *D. sargus*, and *L. ignoratus* from *S. salpa*.

Fish hosts were all sampled in the Mediterranean sea in the Golfe du Lion near Banyuls-sur-Mer (France). Parasites were sampled from host gills under a dissecting microscope and identified through the observation of opisthaptor and copulatory organ morphology with an optical microscope at 400 X magnification. *Diplectanum aequans* (Wagener, 1857) and *Dactylogyrus minor* (Wagener, 1857), which were used as outgroups, were sampled respectively from *Dicentrarchus labrax* (Linnaeus, 1758) from the Atlantic Ocean, and *Alburnus alburnus* (Linnaeus, 1758) from the Morava River (Czech Republic). All parasites were fixed and stored in 95% ethanol before DNA extraction.

DNA amplification, cloning and sequencing followed the protocol in Desdevises et al. (2000), except that sequencing was performed on an automated sequencer (Applied Biosystems 373A DNA Sequencer). Primers used for DNA amplification were L7 (5'- TGA TTT GTC TGG TTT ATT CCG AT -3') and H7 (5'- GCT GCG TTC TTC ATC GAT ACT CG -3'), which anneal to the 18S rRNA and 5.8S rRNA, respectively (Verneau et al., 1997). The DNA fragment amplified with these primers comprises the 3' terminal end of the 18S rDNA, the entire internal transcribed spacer 1 (ITS1) and a very small fragment of 5.8S rDNA. It was shown elsewhere (Desdevises et al., 2000) that ITS1 was too variable to resolve phylogenetic relationships among *Lamellodiscus* species, but it was used to amplify ITS1 at the same time as partial 18S rDNA because the amplification of the sole partial 18S rDNA fragment through the use of a reverse primer at the 3' end of the 18S rDNA appeared not to be reproducible, probably sometimes amplifying pseudogenes. This did not happen with the L7-H7 generated fragment, where variations in amplified sequences were negligible if any. These DNA fragments were independently sequenced from two individuals per species. Three clones were sequenced for each individual, inter-individual and inter-clone variability were always negligible (less than 0.5 %), and consensus sequences were used.

Sequence alignment was first performed with ClustalX (Thompson et al., 1997) and improved by eye with Se-Al (v1d1, A. Rambaut, University of Oxford, 1995). After alignment, ambiguous regions (i.e. containing gaps) were removed. Analyses were carried out on all substitutions. Saturation level was assessed by plotting the proportion of differences for transitions (in ordinate) versus transversions (in abscissa) between pairs of species (Fig. 1). The relationship is almost linear, revealing the absence of saturation in the data and allowing the use of the whole alignment for phylogenetic reconstruction. The phylogenetic tree was reconstructed with PAUP* 4.0 (Swofford D.L., 2000. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4b4a. Sinauer Associates, Sunderland, Massachusetts). A neighbour-joining (NJ) analysis was performed with a minimum evolution objective criterion. Distances were estimated using the Kimura-2-parameter model, correcting for transition bias. A maximum parsimony method was also used, with an exhaustive search for the most-parsimonious tree, as permitted by the relatively low number number of taxa. Phylogenetic reconstructions were validated with a bootstrap procedure for the NJ analysis, and a partition tail probability test, which assesses tree length significance through a permutation procedure, for the maximum parsimony analysis.

Total length of the partial 18S rDNA fragments sequenced was between 524 and 526 bp. After alignment and removal of ambiguous regions, 520 bp remained for phylogenetic reconstruction (alignments are available from the author). The parsimony analysis led to four most-parsimonious trees (number of parsimony-informative characters = 56) of length 194 (partition tail probability test with 100 replicates, $p = 0.01$) and a consistency index (CI) of 0.76. After character reweighting by the maximum values of rescaled consistency indices, one most-parsimonious tree was obtained (CI = 0.94), which is the same as the tree obtained by the NJ analysis. This tree is shown in Fig. 2. *Furnestinia echeneis* appeared to be clearly nested in the clade formed by *Lamellodiscus* species, as shown by high bootstrap support values. The tree exhibits two main clades: one containing *L. virgula*, *L. elegans*, *L. drummondi*, *L. verberis*, *L. mormyri*, and *F. echeneis*, supported by a bootstrap proportion of 83%, and another clade containing *L. erythrini* and *L. ignoratus*, with a bootstrap value of 97%. Phylogenetic relationships are not well supported inside the first clade, except for the (*L. verberis*, *L. mormyri*) clade (bootstrap value of 78%).

Given the phylogenetic tree shown in Fig. 2, it seems clear that *Furnestinia echeneis* belongs to the genus *Lamellodiscus*. There are two types of lamellodiscs among *Lamellodiscus* spp., namely a "split type", where the lamellae are deeply curved and disposed in two well separated vertical rows, and a "non-split type", where the lamellae are less curved, slightly linked two by two across the two vertical rows they form, which are therefore not clearly split. *Lamellodiscus drummondi*, *L. virgula*, *L. mormyri*, *L. verberis* and *L. elegans* belongs to the "split type", while *L. ignoratus* and *L. erythrini* are of the "non-split type" (Euzet and Oliver, 1966a,b; Oliver, 1969; Oliver, 1987, Thesis). These two groups form clearly separated clades in the phylogenetic tree. The unique lamellodisc of *F. echeneis* is of the "split type" and this species is associated in the tree with *Lamellodiscus* spp. possessing the same type of lamellodisc. Moreover, *F. echeneis* seems to be closely related to *L. verberis* and *L. mormyri*, with which it shares the same type of copulatory organ (Euzet and Audouin, 1959; Oliver, 1969; Euzet and Oliver, 1966b). Among these species, the male genital organ is composed of two pieces articulated together.

From this, it can be proposed that *Furnestinia echeneis* is a *Lamellodiscus* with only one lamellodisc. These two genera should then be synonymised. Compared with the relative size of the lamellodisc in *Lamellodiscus* (the ratio lamellodisc height/parasite length vary between 0.12 and 0.15 among the *Lamellodiscus* studied), this organ is clearly more developed in *F. echeneis* (lamellodisc height/parasite length = 0.25). The surface represented by the lamellodisc of *F. echeneis* is at least four times greater than the surface of a lamellodisc belonging to any *Lamellodiscus* spp. Therefore, the total surface represented by lamellodiscs is less important in *Lamellodiscus*. This observation suggests that the loss of a lamellodisc in *F. echeneis* (or its ancestor) was followed by a hypertrophy of the remaining organ, highlighting the possible important role of this structure in the attachment of the parasite to its host. This hypertrophy could then be considered as a morphological adaptation appearing in *F. echeneis*. The fact that *F. echeneis* is the only known species in the *Lamellodiscus* genus harbouring this peculiarity suggests that this haptor morphology is recent in the history of the genus.

It has been suggested that the taxonomy of the Diplectanidae, mainly based on the opisthaptoral adhesive organ structure (Oliver, 1987, Thesis), may be not appropriate, as this structure seems to be subject to morphological adaptation (Mo, 1991). For example, the taxonomic status of the genus *Monoplectanum* should be re-examined. Other characters, such as copulatory organ morphology should also play an important role in the taxonomy of this family (Kritsky and Beverley-Burton, 1986). More generally, this study suggests that taxonomy based on single or very few morphological characters, as strong as they may seem, may not be a reliable procedure.

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References

Cunningham C.O., McGillivray D.M., MacKenzie K., 1995. Phylogenetic analysis of *Gyrodactylus salaris* Malmberg, 1957 based on the small subunit (18S) ribosomal gene. Mol. Biochem. Parasitol. 71, 139-142.

Desdevises Y., Jovelin R., Jousson O., Morand S., 2000. Comparison of ribosomal DNA sequences of *Lamellodiscus* spp. (Monogenea, Diplectanidae) parasitizing *Pagellus* (Sparidae, Teleostei) in the North Mediterranean Sea: species divergence and coevolutionary interactions. Int. J. Parasitol. 30(6), 741-746.

Euzet L., Audouin J., 1959. Sur un genre nouveau de Monogenoidea parasite de la dorade *Chrysophrys aurata* L. Rev. Trav. Inst. Pêches Marit. 23(3), 317-322.

Euzet L., Oliver G., 1966a. Diplectanidae (Monogenea) des Téléostéens de la Méditerranée occidentale. III. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Diplodus* (Sparidae). Ann. Par. Hum. Comp. (Paris) 41(6), 573-598.

Euzet L., Oliver G., 1966b. Diplectanidae (Monogenea) de Téléostéens de la Méditerranée occidentale. IV. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Pagellus* Cuvier, 1829 (Sparidae). Ann. Par. Hum. Comp. (Paris) 42(4), 407-425.

Kritsky D.C., Beverley-Burton M., 1986. The status of *Pseudorhabdosynochus yamaguti* 1958 and *Cycloplectanum* Oliver 1968 (Monogenea: Diplectanidae). Proc. Biol. Soc. Wash. 99(1), 17-20.

Mo T.A., 1991. Variations of opisthohaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in South-eastern Norway. Syst. Parasitol. 20, 1-9.

Oliver G., 1968. Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. I. Diplectaninae Monticelli 1903. Vie Milieu 19(1-A), 95-138.

Oliver G., 1969. Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. II. Lamellodiscinae nov. sub. fam. Vie Milieu 20(1-A), 43-72.

Oliver G., 1974. Nouveaux aspects du parasitisme des Diplectanidae Bychowsky 1957 (Monogenea, Monopisthocotylea) chez les Téléostéens Perciformes des côtes de France. C. R. Acad. Sc. Paris 279, 803-805.

Palombi A., 1943. Notizie Elmintologiche. V. *Diplectanum* (*Dactylogyrus*) *echeneis* (Wag.) Par. et Per. = *Lamellodiscus ignoratus* n. sp. Diagnosi della specie e suoi opistatori. Annuario Mus. Zool. Napoli (N. S.) 7(16), 1-5.

Parona C., Perugia A., 1889. Di alcuni trematodi ectoparasiti di pesci marini. Nota preventiva. Ann. Mus. Civ. Stor. Nat. Genova, ser. 2a. 7, 740-474.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876-4882.

Verneau O., Renaud F., Catzeflis F., 1997. Evolutionary relationships of sibling tapeworm species (Cestoda) parasitizing teleost fishes. Mol. Biol. Evol. 14, 630-636.

Wagener G., 1857. Helminthologische Bemerkungen aus einem Sandsschreibe an C. Th. V. Siebold Zeit. Wiss. Zool. 9, 73-90.

Young P.C., 1969. Some monogenoideans of the family Diplectanidae Bychowsky 1957, from Australian Teleost fishes. J. Helminthol. 43(1-2), 223-254.

Legends to Figures

Fig. 1: Saturation plot of distances estimated from transitions (Ti) versus distances estimated from transversions (Tv). Distances are calculated from the aligned sequences of 18S rDNA and corrected by the Kimura-2-parameter model.

Fig. 2: Phylogenetic reconstruction of several *Lamellodiscus* species and *Furnestinia echeneis* obtained by a neighbour-joining analysis and an exhaustive search for the most-parsimonious tree (consistency index = 0.95 after character reweighting) based on partial 18S rDNA sequences. Numbers are boostrap values (1000 replicates). *Dactylogyrus minor* and *Diplectanum aequans* are used as outgroups.

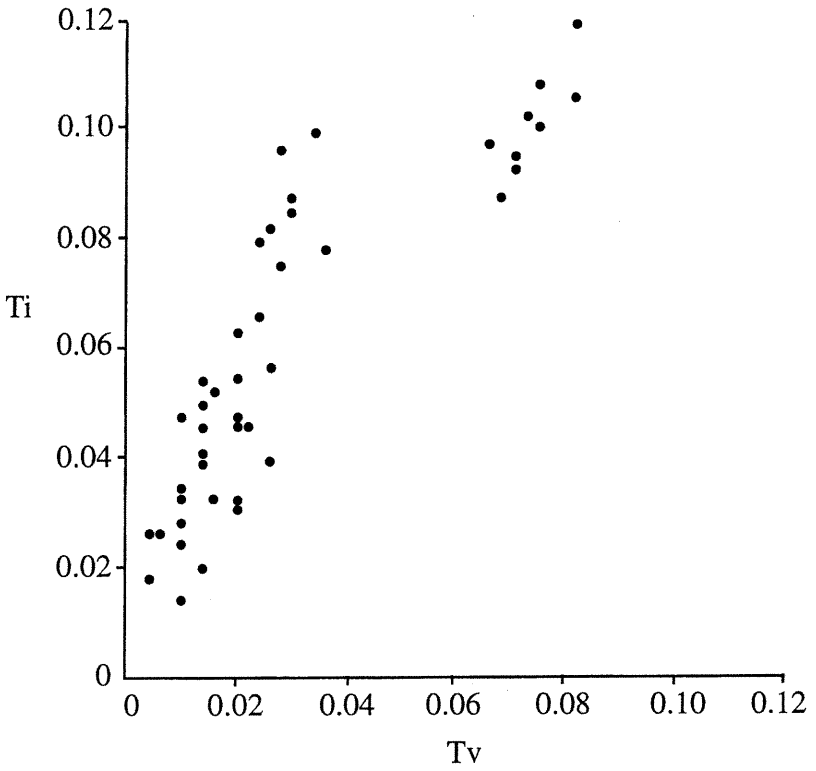

Figure 1

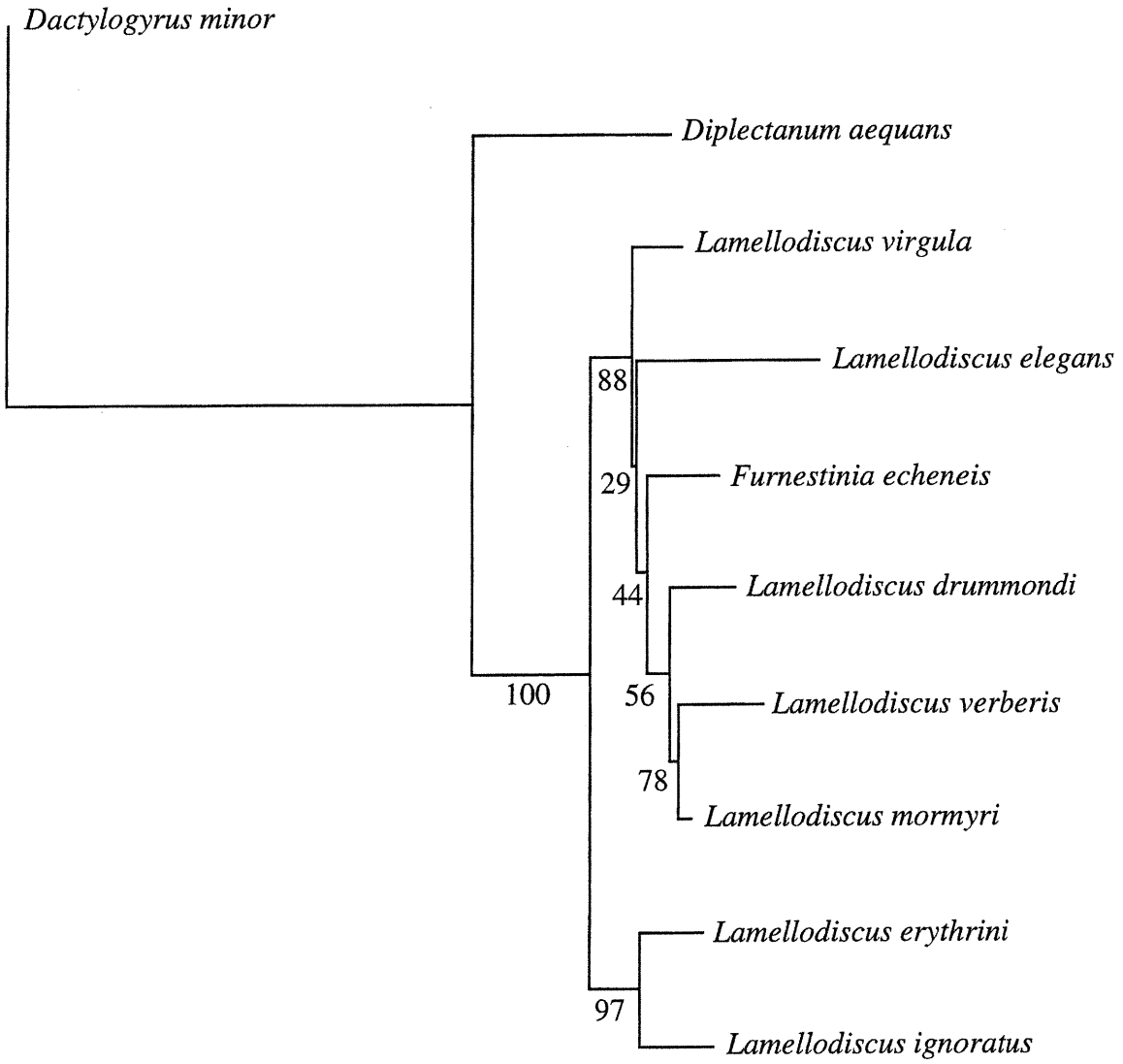
Dactylogyrus minor
Diplectanum aequans
Lamellodiscus virgula
Lamellodiscus elegans
88
Furnestinia echeneis
29
Lamellodiscus drummondi
44
100
56
Lamellodiscus verberis
78
Lamellodiscus mormyri
Lamellodiscus erythrini
97
Lamellodiscus ignoratus

Figure 2

C. Coévolution *Lamellodiscus*-Sparidae

Article 5

DESDEVISES Yves, Serge MORAND, Olivier JOUSSON and Pierre LEGENDRE. Coevolution between *Lamellodiscus* (Monogenea) and Sparidae (Teleostei): the study of a complex host-parasite system. Soumis.

Objectifs d, e, et f

Cet article est centré sur la coévolution entre les Sparidae du nord de la Méditerranée et leurs parasites monogènes *Lamellodiscus*. Des phylogénies moléculaires complètes de ces deux groupes sont proposées. La phylogénie obtenue pour les *Lamellodiscus* est compatible avec leur morphologie, alors que celle des Sparidae, si elle contredit la taxonomie actuelle, est compatible avec une hypothèse précédemment publiée, basée sur le séquençage d'ADN mitochonrial 16S. Une nouvelle méthode d'étude de la coévolution, ParaFit, est utilisée ici, en plus de méthodes existantes, TreeMap et TreeFitter. Toutes les méthodes semblent indiquer une absence de coévolution par cospéciation entre les *Lamellodiscus* et leurs hôtes. Les monogènes étudiés ici semblent suivre une stratégie opportuniste quant au choix de leurs hôtes, probablement basée sur les caractéristiques écologiques de leurs hôtes. Ainsi, la spécificité chez les *Lamellodiscus* ne semble pas conduire à la cospéciation, et l'absence de cospéciation est compatible avec une forte spécificité chez ces monogènes, suggérant des possibilités d'adaptation rapides.

Participation du thésard :

- Recherche bibliographique
- Séquençage de l'ADN des parasites
- Analyse phylogénétique
- Rédaction

COEVOLUTION BETWEEN *LAMELLODISCUS* (MONOGENEA: DIPLECTANIDAE) AND SPARIDAE (TELEOSTEI): THE STUDY OF A COMPLEX HOST-PARASITE SYSTEM

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LRH: YVES DESDEVISES ET AL.

RRH: COEVOLUTION *LAMELLODISCUS*-SPARIDAE

ABSTRACT

Host-parasite coevolution was studied between Sparidae (Teleostei) fishes and their parasites of the genus *Lamellodiscus* (Monogenea, Diplectanidae), in the Northwestern Mediterranean Sea. Molecular phylogenies were reconstructed for both groups. The phylogenetic tree of the Sparidae was obtained from previously published 16S mtDNA sequences associated with new Cyt-b mtDNA sequences via a "total evidence" procedure. The phylogeny of *Lamellodiscus* species was reconstructed from 18S rDNA sequences that we obtained. Host-parasite coevolution was studied through different methods: TreeFitter, TreeMap, and a new method, ParaFit. If the cost of a host switch is not assumed to be high for parasites, all methods agree on the absence of widespread cospeciation processes in this host-parasite system. Host-parasite associations were interpreted to be due more to ecological factors than to coevolutionary processes. Host specificity appeared not to be related to host-parasite coevolution.

Key Words: Coevolution, Monogenean, Fish, *Lamellodiscus*, Sparidae, 18S rDNA, cyt-b mtDNA

Host-parasite coevolution has been the subject of numerous studies, and this for a long time (e.g., Kellogg 1913; Bychowsky 1961; Brooks 1979, 1981; Brooks and Glen 1982; Cressey et al. 1983; Hafner and Nadler 1988; Klassen and Beverly-Burton 1988; Page 1993c, 1994a; Paterson et al. 1993; Hafner et al. 1994; Boeger and Kritsky 1997; Desdevises et al. 2000; Paterson and Banks 2001); see Klassen (1992) for a historical perspective. From this important body of work, we now recognized that "Farenholz' rule" (that "the parasite phylogeny mirrors the host phylogeny") does not seem to be generally true in host-parasite associations (see Paterson and Banks 2001). It was suggested early (Kellogg 1913) that host switching could be a component of host-parasite coevolution, even if priority was given to evolution through cospeciation (Bychowsky 1961; see Klassen 1992). The term "coevolution" is used here to describe the extent to which the host and parasite phylogenetic trees are congruent. When the trees are perfectly congruent, coevolution is the equivalent of cospeciation. This corresponds to the definition of Brooks (1979, 1988), Klassen and Beverley-Burton (1987), Brooks and McLennan (1991), and Klassen (1992), and refers to the macroevolutionary context. This should not be confused with a more restrictive meaning, used in genetic and microevolutionary studies, which defines coevolution as the influence of the host genome on the parasite genome, and vice versa (Toft and Karter 1990).

Most studies where the host and parasite phylogenies were found to be congruent, with parasites found in similar positions on the tree as their hosts, involve very particular groups in which biological characteristics made host-switching events highly improbable (see Barker 1994). This is the case of the well-known association between pocket gophers and their chewing lice (Hafner and Nadler 1988; Hafner at al. 1994), and that of swiftlets and their parasitic lice (Page et al. 1998). In a review about coevolution of lice and their hosts, Barker (1994, but see Page et al. 1996) pointed out that every time a possibility of host switching was encountered (i.e., contact between hosts), it effectively took place (see Hafner and Nadler 1988). Cospeciation of parasites with their hosts mainly happened when the hosts were allopatric to one another.

Monogenean-fish associations were seldom studied in this context (Klassen and Beverly-Burton 1987, 1988; Guégan and Agnèse 1991; Boeger and Kritsky 1989, 1997;

Desdevises et al. 2000). The high host specificity encountered in monogeneans, in terms of the number of hosts parasitized (Baer 1957; Llewellyn 1957; Kennedy 1975; Rohde 1979, 1982; Noble et al. 1989; Sasal et al. 1998), allows one to suppose close interactions with their hosts, and therefore anticipate a high level of coevolution via cospeciation (Noble et al. 1989; Kearn 1994). This kind of host-parasite complex is characterized by high dispersion of the mobile larval stage (oncomiracidium) of the parasites, which finds its host through chemical cues (Kearn 1967, 1988). Chemical interaction could be supposed to be a determinant of host specificity, and therefore would support a coevolution hypothesis. Moreover, the direct life cycle of monogeneans avoids the influence of an intermediate host in the phylogenetic relationship of the final host with its parasite. However, other authors have argued that the capacity of dispersion of the larval stage in monogeneans suggests important possibilities for new host colonization (Brooks and McLennan 1991). Moreover, adults of some monogenean species are supposed to be able to survive for a short time period outside the host (see Bakke et al. 1992 on *Gyrodactylus*), thus increasing the possibility of dispersion. It has been suggested that monogeneans are transmitted via host contact. It has also been suggested that no or little competition exists among ectoparasitic monogeneans (Rhode 1979, 1994; Simkova et al. 2000). So, potentially competing species would not be an obstacle to host switching in monogeneans, contrarily to lice for example (see Barker 1994). Studies on the coevolution of monogeneans with their hosts apparently supports the existence of host-switching events: Boeger and Kristky (1997) have suggested that several dispersion events took place in the early history of monogeneans; Klassen and Beverley-Burton (1987, 1988) suggested that *Ligictaluridus* ancyrocephalid monogeneans have not strictly cospeciated with their fish hosts; Desdevises et al. (2000) suggested that the *Lamellodiscus* species associated with *Pagellus* (Sparidae) have not cospeciated with their hosts; and Sinnappah at al. (2001) proposed the existence of dispersion events in the history of Polystomatid monogeneans.

The precise reconstruction of a hypothetical coevolutionary scenario between hosts and their parasites is not always straightforward. Page (1993b, 1994b), and Page and Charleston (1998) have shown that sometimes, and at least in theory, no switching event is necessary to reconcile the host and parasite trees. A host-parasite association whose phylogenetic trees are not congruent can closely coevolve via duplication, cospeciation and

lineage sorting, without any host-switching events. Likewise, the absence of congruence may not always be equated with a lack of historical association between the two components. When the host and parasite phylogenetic trees are similar or almost similar, with parasites found in similar positions on the tree as their hosts, the use of a special analytical method to study coevolution among them may not be necessary. In that case, the putative colonization events can be inferred by visual examination of the trees (e.g., Verneau et al. 1997). However, if the pattern becomes more complicated, a rigorous method should be used to choose among a number of potential scenarios. Several methods have been developed to study host-parasite coevolution. These are Brooks' Parsimony Analysis (BPA: Brooks 1981, Brooks and McLennan 1991), Component analysis (Component: Page 1993a), trees reconciliation (TreeMap: Page 1994b), event-based methods (TreeFitter: Ronquist 1995, 1997; Jungles: Charleston 1998), the maximum-likelihood method (Huelsenbeck et al. 1997), and a method based on Bayesian inference (Huelsenbeck et al. 2000). These methods search for an optimal evolutionary scenario of the association between a set of hosts and their parasites. To achieve that, each method has a specific way of using cospeciation, duplication, sorting, and switching events. For more explanation on the terminology, see Page (1994b), Ronquist (1997), Charleston (1998), Page and Charleston (1998), and Paterson and Banks (2001).

We will here investigate coevolutionary interactions in a Mediterranean fish-monogenean association which has been known for a long time and is well-studied (Euzet and Oliver 1966, 1967; Oliver 1968, 1973, 1974, 1987; Euzet 1984; Euzet et al. 1993). Therefore, the known pattern of host specificity may be considered to be close to reality. The inventory of fish parasites in the Mediterranean Sea is considered to be one of the most exhaustive in the world (Caro et al. 1997). The host-parasite association studied here is formed by the monogenan gill ectoparasites of the genus *Lamellodiscus* (Johnston and Tiegs 1922), which are parasites of teleost fishes of the Sparidae family (Oliver 1987; Euzet et al. 1993). The pattern of host-parasite relationships is described in Table 1. There are 21 described *Lamellodiscus* species and 16 Sparidae in the study area. This association is characterized by a high number of parasite and host species living in sympatry (Whitehead et al. 1986). All potential hosts are then always "available" to the parasites. The observation of a tight cospeciation pattern, in this case, would be the sole result of the

influence of the hosts on the parasite evolutionary history, because no ecological or geographic barrier could be invoked to explain parasite speciation. Several of the above-mentioned methods will be used here to study coevolution, in addition to a new method which makes use of the host and parasite phylogenetic distance matrices as well as a matrix describing the host-parasite association links (Legendre et al. submitted).

The objectives of this study are to reconstruct molecular phylogenies for the hosts (Sparidae) and their monogenean parasites (*Lamellodiscus*), to assess the extent of coevolution in this association, and to propose an interpretation of the observed pattern.

MATERIAL AND METHODS

Sampling

Sparid fish were caught in several locations in the northwestern Mediterranean Sea: in the Golfe du Lion near Banyuls-sur-Mer (France), near Marseilles (France), and in Corsica (Scandola Natural Reserve, France). *Lamellodiscus* were dislodged from the gills of the fish under a dissecting microscope and identified using the morphology of the haptor and copulatory organ observed under an optical microscope with 400x magnification. Parasites were stored in 95% alcohol before DNA extraction.

Phylogenies

For the host and parasite phylogenetic analyses, saturation levels in the DNA sequences were estimated by plotting distances (% differences) calculated only for transversions against distances calculated only from transitions. Phylogenetic analyses were carried out using the maximum parsimony (MP) and maximum likelihood (ML) methods. Patterns of base changes (transitions and transversions) and transitions/transversions ratios (Ti/Tv) were estimated by ML from the most parsimonious trees previously obtained, and used in the ML phylogenetic reconstruction. The α parameter of the Γ distribution (accounting for substitution rate heterogeneity) was also estimated from the most-parsimonious tree (MPT), as its estimation is considered to be consistent if reasonable tree topologies are used (Yang 1994, 1995, 1996; Yang et al. 1994; Sullivan et al. 1996). All

phylogenetic analyses were performed with PAUP* 4.0d8 (Swofford 2001). MP trees were validated with a bootstrap procedure using 1000 replicates.

Hosts

Phylogenetic relationships among Sparid fish species have long been controversial. Their present classification only relies upon morphological characters, particularly the types of fin rays and dentition (Whitehead et al. 1986). There are presently four recognized subfamilies: the Sparinae, Denticinae, Boopsinae and Pagellinae (Fiedler 1991; Smith and Smith 1986). These subfamilies are differentiated by their dentition and trophic specialization. However, no clear phylogeny has been proposed until recently (Hanel and Sturmbauer 2000). Previous attempts were unsatisfactory (Cataudella et al. 1980; Basaglia 1991; Reina et al. 1994; Garridos-Ramos et al. 1995, 1998, 1999). Hanel and Sturmbauer (2000) reconstructed a phylogenetic tree of Sparid fishes based on 16S mtDNA, for 24 species from the Atlantic Ocean and the Mediterranean Sea (among which the 16 Mediterranean species under study here). Their tree showed considerable differences with current taxonomy, but was not fully resolved. To infer the phylogenetic relationships among Sparids and perform an independent external validation of their dataset, we partially sequenced the cytochrome-b mtDNA. DNA extraction and sequencing followed the same protocol as in Desdevises et al. (2000). Some of these sequences were used in Jousson et al. (2000). A phylogenetic tree was estimated from these data, with *Dicentrachus labrax* (Moronidae) as the outgroup. Then we added the 16S mtDNA sequence data from Hanel and Sturmbauer (2000) to our data table and performed a partition homogeneity test (PHT: Farris et al. 1994) on the pooled dataset (16S + cyt-b mtDNA), as well as a Mantel test (Mantel 1967) between pairwise species distances computed for each dataset, to assess if it can be safely used in a "total evidence" approach (see Lapointe 1998). Since the homogeneity (or concordance) of the two datasets was not rejected (PHT: 100 heuristic searches, $P = 0.18$; Mantel test: 999 permutations, $P = 0.001$), this analysis was carried out. As outgroup, we merged the sequences from the two outgroups used in the previous separate analyses, *Dicentrachus labrax* and *Spicara maena* (Centracanthidae) used in Hanel and Sturmbauer (2000). In doing so, we were hoping to increase the resolution of the phylogenetic tree.

Parasites

It has been suggested that 18S rDNA was a reliable marker to infer phylogenetic relationships among *Lamellodiscus* species (Desdevises et al. 2000; Desdevises 2001). The same protocol as in Desdevises et al. (2000) was used here for extraction, amplification, cloning and sequencing of the partial 18S rDNA. These DNA fragments were independently sequenced from two individuals per species. Three clones were sequenced for each individual. Inter-individual and inter-clone variability were always negligible (less than 0.5 %), so that consensus sequences were used. With the primers used (L7 and H7, designed by Verneau et al. 1997), the internal transcribed spacer 1 (ITS1) is amplified and sequenced with the partial 18S rDNA. It has been shown that ITS1 is highly variable and not useful to infer evolutionary relationships among *Lamellodiscus*, but it can be used to identify species, which can exhibit different morphologies (Desdevises et al. 2000). The amplification of the sole partial rDNA 18S fragment, with L7 and an internal primer, sometimes led to nonreproducible results, perhaps because pseudogenes were present. *Furnestinia echeneis* (Euzet and Audoin 1959), the parasite of *Sparus aurata*, was added to the 20 *Lamellodiscus* species under study, after it was suggested that it should be included in the genus *Lamellodiscus* on the basis of 18S rDNA (Desdevises 2001). Based on the molecular data, *Lamellodiscus obeliae*, which had been described as a specific parasite of *Pagellus bogaraveo* by Oliver (1973), has been considered to be the same species as *L. virgula* (Desdevises et al. 2000) which is a parasite of *P. acarne*. In the present study, these two species are considered to be *L. virgula* which then parasitizes *P. acarne* and *P. bogaraveo*. Sequence alignment was done with ClustalX (Thompson et al. 1997) and visually checked. Gaps were treated as missing data.

Dactylogyrus minor, *Pseudomurraytrema ardens*, and *Diplectanum aequans* were used as outgroups, because of their phylogenetic positions relative to the genus *Lamellodiscus* (Boeger and Kritsky 1997; Desdevises 2000; Desdevises et al. 2001).

Coevolution

Several methods to assess the coevolutionary interactions in a host-parasite association have been proposed in the literature. The first method dedicated to the study of

such association is Brooks' Parsimony Analysis (BPA: Brooks 1981; Brooks and McLennan 1991), which consists in building a host phylogeny from characters derived from the parasite phylogeny and comparing this tree to the known host phylogeny. BPA has been widely used in coevolutionary studies (e.g., Paterson et al 1993, Boeger and Kritsky 1997). Another method, Component analysis, popularized by Page (1993b), relies on the comparison of tree topologies, but does not allow the incorporation of host-switching events. This was made possible with TreeMap (Page 1994b), which uses reconciled trees to compute the fit between the host and parasite phylogenies. Ronquist (1995) proposed to use methods based on generalized parsimony to assess the same fit, incorporating a differential cost to the four types of potential events (see Ronquist 1995; Page and Charleston 1998; Paterson and Banks 2001) occurring in a host-parasite association: cospeciation (C), duplication (D), sorting (S), and host switching (H). The optimal reconstruction is the one that minimizes the global cost. This is implemented in the program TreeFitter 1.0 (by F. Ronquist (2001), available at URL http://www.ebc.uu.se/systzoo/research/treefitter/treefitter.html), which also allows the statistical testing of the overall cost and of the occurrence of each type of event. Testing is done through a permutational procedure. By assigning different costs to the events, TreeFitter allows one to carry out analyses equivalent to BPA (but this function is not implemented in the current version of TreeFitter) and TreeMap. We used TreeFitter 1.0 with default settings (C = 0, D = 0, S = 1, H = 2) and TreeMap settings (C = –1, D = 0, S = 0, H = 0). In addition, we varied the host switching cost (H) to study its effect on the reconstruction. TreeMap 1.0b was also used and its results were compared to those of TreeFitter. Comparatively to the current version of TreeFitter, TreeMap allows a graphic display of the results and therefore the identification of coevolutionary events. TreeMap also includes a testing procedure, by generating random trees and comparing the random number of cospeciation events in the association to the observed number, to assess if it is significantly higher that due to chance alone.

These methods preferably require one host-one parasite associations; else the complexity of the problem could become too important to guarantee finding an optimal solution. They all compare the topologies of the host and parasite phylogenies and allow proposing precise evolutionary scenarios for the history of the association. This supposes,

however, that the trees are well known and, if possible, unique. Since a topology is sensitive to the presence or absence of taxa, the methods assume a thorough knowledge and good sampling of the clades under study. But even when all species are known and sampled, consideration of an extinct species could alter the topology of the actual tree and change the proposed coevolutionary scenario (see Brooks and McLennan 1991); this problem unfortunately that cannot be resolved. This is why, in addition to the comparison of the topologies, we found it desirable to use another method especially designed for the assessment of the coevolutionary character of the data.

In addition to TreeFitter and TreeMap, a new method will be used in this study to test the null hypothesis (H_0) that the evolution of the two groups, revealed by the two phylogenetic trees and the set of host-parasite association links, has been independent. This method, called ParaFit (Legendre et al. submitted), combines the information from three data matrices: the first one (matrix **A**, 0-1 data) contains a description of the host-parasite (H-P) association links observed in nature. The two other matrices contain some estimates of the phylogenetic trees or phylogenetic distances among the hosts and parasites, respectively. One may start either with matrices of patristic distance derived from phylogenetic trees, or raw distance matrices issued directly from the comparison of sequences, or distance matrices representing DNA/DNA hybridization data, or even distance matrices computed from morphological character. This is why ParaFit is not affected by polytomies in the tree and it can be used with any number of hosts per parasite or parasites per host. Matrix **B** used in the analysis contains principal coordinates (Gower 1966) representing the parasite phylogenetic tree or phylogenetic distances. Likewise, matrix **C** contains the transpose of the matrix of principal coordinates representing the host phylogenetic tree of phylogenetic distances. Matrices **B** and **C** may be incommensurable: the number of hosts and parasites may be different. Their relationship is mediated by the host-parasite relationships in matrix **A**; using the fourth-corner approach (Legendre et al. 1997), a matrix $\mathbf{D} = \mathbf{C}\,\mathbf{A}'\,\mathbf{B}$ is computed, and from it a trace statistic which is used to test the hypothesis of coevolution through a permutational procedure. A test of significance for each host-parasite link in matrix **A** is also obtained as follows: compute matrix **D** and its trace statistic with and without a given H-P link, calculate the difference between the trace statistics, and test this difference by permutation. Numerical simulations have shown that

the global test as well as the test of individual links have correct type I error and good power under various types of error conditions. No such test was available in the literature. This test is thus complementary to the methods described in the previous paragraphs for studying host-parasite coevolution.

For the three methods used in this paper, all permutational tests of significance were performed using 999 permutations.

RESULTS

Phylogenies

Hosts

The length of the Cyt-b mtDNA sequences was 1102bp. The saturation plot suggested a high level of saturation for the Cyt-b sequences (Fig. 1a). We decided to weight the transversions at the first and third codon positions. Since the Ti/Tv ratio was estimated, by ML estimation, to 14 for these positions (and to 1 for the second position), a weight of 14 was applied to transversions at the first and third codon positions. This value is very close to the highest Ti/Tv value observed between the closest species (*Diplodus puntazzo* and *Oblada melanura*), which is 13.4. The number of parsimony-informative characters was 355. Using a heuristic search algorithm, the tree-bisection-reconnection branch swapping option, and a random addition sequence (10 replicates), the MP analysis led to a single MPT (CI = 0.515), shown in Fig. 3b. The proportion of base changes was estimated from the MPT and led to the pattern presented in Fig. 2a. This observation, combined with a slight difference on the empirical frequencies of the four bases, led us to use a HKY85 model in the ML analysis. α was estimated to 0.17 with 30 rate classes, and Ti/Tv to 5.8. The tree obtained via ML (HKY85 + Γ model, through a heuristic search using the same settings as MP) is shown on Fig. 3c. These trees are roughly similar and differ only by the positions of *D. dentex* and *S. cantharus*. We then combined the 16S (435 bp) and Cyt-b sequences before a "total evidence" analysis (430 parsimony-informative characters). The highly linear saturation plot observed for 16S sequences (Fig. 1b) led us not to weight transversions vs transitions for this fragment. In the MP analysis, all 16S positions were

given a weight of 14, the same weight as cyt-b transversions for first and third codon positions. This analysis led to a single MPT (CI = 0.530) shown in Fig. 3d. The ML analysis (heuristic search with the previous settings) was carried out with a HKY85 model (see base change pattern in Fig. 2b), using a Γ distribution with a shape parameter (α) estimated to 0.17 and a Ti/Tv ratio estimated to 6 from the MPT. It led to a very similar tree, shown in Fig. 3e. The only difference between these two trees is the presence of the clade (*P. erythrinus*(*D. dentex*, *P. pagrus*)) in the MP tree, while it is (*D. dentex*(*P. pagrus*, *P. erythrinus*)) in the ML tree. We decided to keep the ML tree for the subsequent coevolution analysis, because the clade (*D. dentex*(*P. pagrus*, *P. erythrinus*)) is also found in our cyt-b tree and in the Hanel and Sturmbauer (2000) tree.

Parasites

The sequence length of the partial 18S rDNA fragment varied between 524 and 525 bp, with an alignment length of 525 bp. Note that it was not possible to align unambiguously the ITS1 sequences across species. The roughly linear saturation curve observed (Fig. 1c) led us not to use any weighting scheme. The trees were first rooted using the three outgroup taxa. Since the ingroup, formed by *Lamellodiscus* spp. and *Furnestinia echeneis* (hereafter considered to be a *Lamellodiscus*-like species), was always clearly monophyletic in all trees, with the pattern (*Dactylogyrus minor*, *Pseudomurraytrema ardens*, *Diplectanum aequans* (*Lamellodiscus* spp.)) always present, only *D. aequans* was kept as the outgroup. The number of parsimony-informative characters was 66. The MP analysis (using the branch-and-bound algorithm) led to five MPTs (CI = 0.632). All MPTs contain three main clades, hereafter labeled after one of their representative members: the *elegans* clade, the *ignoratus* clade, and the *gracilis* clade. They differ in the placement of *L. ignoratus*, *L. knoeppfleri* and the monophyletic clade formed by *L. baeri* and *L. erythrini*. These taxa are inverted in the five MPTs, but remain together in the *ignoratus* clade. A strict consensus of these five trees is presented in Fig. 4a. This consensus tree is fully compatible with a tree generated by neighbor-joining (NJ, not shown). A HKY85 model was also used in the ML analysis (see base change pattern on Fig. 2c). Using 30 discrete classes, α was estimated to 0.18 and the Ti/Tv ratio to 3.8 by maximum-likelihood evaluation from the MPT. The ML analysis, performed using a heuristic search procedure

with the previous settings, produced the tree presented in Fig. 4b. This tree is roughly similar to the trees generated by MP. They differ by the basal relationship between the three main clades and the placement of *F. echeneis*, which is a sister taxon to *L. drummondi* in all MPTs, whereas it is basal to the (*L. furcosus*, (*L. coronatus*, *L. elegans*)) group in the ML tree.

All these phylogenetic hypotheses will be used in the ParaFit coevolution study of the host-parasite association. TreeFitter and TreeMap require a fully-resolved tree; for these analyses, we used a modified ML tree in which the polytomies were resolved following the NJ tree. This resolution of the *ignoratus* clade is compatible with what was observed in one of the MPTs, and the *gracilis* clade was never fully resolved in any of the MPTs.

Coevolution

The fully-resolved host and parasite phylogenies, with the pattern of observed host and parasite associations, are presented in Fig. 5.

TreeFitter

The analysis performed with TreeFitter 1.0, using default settings, suggests that there is a phylogenetic structure in this association. The fit between the host and parasite phylogenies, tested by permutation, shows that the overall cost is significantly lower than expected by chance alone (P = 0.006) and that the main factor contributing to this is a relatively small number (5 to 6) of switching events (P = 0.0014). No other type of events is common, as evidenced by the non-significant P-values for these events. If cospeciation and sorting are made almost impossible via a very high cost (2000), those significant values disappear. This implies that the significant number of host-switching events observed took place over a cospeciation-sorting background. Thus, this result suggests that some parallel cladogenesis is occurring in the system. However, these results depend highly on the costs associated to each event. If the switching cost is lowered (to 1 or 0, keeping default values for the other events), the global fit between the two trees is no longer significant (P = 0.165 for H = 1, and P = 1.0 for H = 0). A similar result is observed with the TreeMap settings implemented in TreeFitter (P = 0.450 for the global fit between the two trees).

TreeMap

As expected from the previous results, the TreeMap analysis suggests the absence of cospeciation in this association. Without invoking any host switching, reconciliation needs to call upon 5 cospeciation events, 14 duplication events and 62 sorting events to reconcile the two trees. This number of cospeciation events remains the same even if we add host-switching events in the reconstruction (using a heuristic search). This number was statistically tested using TreeMap, by repeatedly permuting the parasite tree and recalculating the number of cospeciation events, generating therefore many realizations of the null hypothesis of no association between the two trees. A thousand random parasite trees were generated using the proportional-to-distinguishable option of the program. A distribution of the number of cospeciation events was generated, and the observed number was tested against this distribution. The resulting histogram (Fig. 6), suggests that the observed number of cospeciation events is not significant (P = 0.317). Thus, according to this criterion, this association does not show coevolution via cospeciation. However, all reconstructions generated by TreeMap (not shown because they are highly complicated) suggest that cospeciation took place between (*Lamellodiscus baeri*, *L. erythrini*) and (*Pagrus pagrus*, *Pagellus erythrinus*).

ParaFit

The ParaFit results are the same for all of the parasite phylogenetic trees; so, only the results obtained for the ML tree are described. Patristic distances were computed from the ML parasite tree and compared with the patristic distances computed from the ML total evidence host tree. The global test performed by ParaFit (Table 2) indicates that there is no global relationship between the host and parasite phylogenies, mediated by the table of host-parasite association links (P = 0.243). This test confirms the TreeFitter and TreeMap result that the phylogenies are not generally congruent. The test computed by ParaFit for individual host-parasite links shows, however, a statistically significant structure brought by the associations *Lamellodiscus baeri–Pagrus pagrus* and *Lamellodiscus erythrini–Pagellus erythrinus* (bottom of Fig. 5), with respective probabilities of 0.028 and 0.018. When the global test is not significant but the test of an individual link is significant,

simulations reported in Legendre et al. (submitted) show that we may be dealing with a mixed structure containing a coevolutionary and a random portion. So, Table 2 suggests that this four-species association has coevolved, confirming the result obtained from TreeMap.

Discussion

Phylogenies

The phylogeny for the Sparidae confirms the results of Hanel and Sturmbauer (2000) obtained using 16S mtDNA only. However, the total evidence analysis increased the resolution of the tree: the basal nodes are better supported and the *Diplodus* clade, containing *Oblada melanura*, is now fully resolved. It also suggests that the taxonomy of the Sparidae family should be revised, as the subfamilies Boopsinae, Pagellinae and Sparinae defined by Fiedler (1991) on the basis of dentition and diet only are not monophyletic in our tree; this cannot be assessed for the Denticinae, since they are only represented by *Dentex dentex* in our dataset. The three lineages mentioned by Hanel and Sturmbauer (2000) are found in our phylogeny. The first one (the *Boops* clade) comprises *Spondyliosoma cantharus*, *Boops boops* and *Sarpa salpa*; the second one (the *Diplodus* clade) contains all *Diplodus* species, *O. melanura*, *Sparus aurata*, *Pagellus acarne*, *P. bogaraveo* and *Lithognathus mormyrus*; the third lineage (the *Pagrus* clade) is composed of *Dentex dentex*, *Pagrus pagrus* and *Pagellus erythrinus*. Several genera appear to be polyphyletic (as *Pagellus*) or paraphyletic (as *Diplodus*). Our phylogenetic hypothesis suggests that the *Pagrus* clade is the most primitive one, while *Diplodus* is a derived genus.

The phylogenetic tree obtained for the *Lamellodiscus* species is supported by the observation of morphology, which was not included in the phylogenetic analysis. The grouping of the species is compatible with the type of their lamellodisc (attachment organ): all species in the *ignoratus* clade possess a non-split lamellodisc, whereas in the other two clades, species harbor a split-type. Similarly, the copulatory organ morphology in the *ignoratus* clade (type *en lyre*, see Oliver 1987) is the same for all species; the types of copulatory organs are also similar in the other two clades. These observations will be

detailed in a further study. The topology is roughly the same, whatever the method employed, showing that there is a strong phylogenetic structure in the molecular dataset.

Coevolution

The phylogenetic structure found by TreeFitter for this host-parasite association is statistically significant only if we consider host switching to be a "difficult" event, by assigning to it a cost higher than for the other three events. In the present situation, where hosts are all sympatric and monogenean larvae are highly mobile, this cost may be overestimated in the situation corresponding to the TreeFitter default settings. Assigning an optimal cost to the various types of events is very difficult, because it may differ in every host-parasite association considered and it may depend on ecological factors. Switching weight is particularly critical (Ronquist 1995), as it greatly influences the inferred reconstruction pattern. This is also what was observed in this study. Since every host-switching event added to the reconstruction decreases the number of required cospeciation events to obtain a fit between the trees, Ronquist (1995) proposed to find the optimal switch cost by finding the cost that leads to the largest reduction in the number of cospeciation events. It could be argued, however, that this cost should be defined from biological instead of statistical data, as it is likely to be different in different types of associations. For example, this cost should be lower for parasites with dispersal stages (like monogeneans or copepods) than for parasites that are more closely dependent on their hosts for transmission (like lice or mites). The high biological difficulty for host switching (which corresponds to a higher cost for this event) has been used to explain the high level of cospeciation in some host-parasite associations (Page and Hafner 1996).

If we consider host switching to have an equal or lower cost to sorting, all methods used — ParaFit, TreeMap and TreeFitter — suggest that there is no coevolution in this association. According to three different criteria (i.e., the overall cost, the number of cospeciation events, and the fit between phylogenetic distance matrices), the level of congruence in this association is not higher than expected by chance. These results confirm what was previously known about fish-monogenean coevolution, that host-switching events are common (Klassen and Beverley-Burton 1987, 1988; Boeger and Kritsky 1997).

Cospeciation events reported in the literature seem to be mainly found at high taxonomic levels (i.e., family or above, Boeger and Kritsky 1997). This suggests that a close phylogenetic association between the hosts and their monogenean parasites is driven by broad historical constraints (e.g, immunological or morphological) acting at large scale (i.e., high taxonomic levels). In the present case, this is consistent with the observation that *Lamellodiscus* are only found on Sparid fishes, whereas several other potential hosts can be found (Whitehead et al. 1986). This could prevent dispersal to distantly related taxa. This may also be due to an ancestral geographic separation of Sparidae leading to important parasite divergence (vicariance), impeding subsequent host-switching events across host families. This is not an absolute rule, since some switching has been hypothesized to occur in monogeneans between distant taxa (see Boeger and Kritsky 1997). At finer scale, as between species, it seems that historical constraints are less important and that dispersal is widespread. However, monogeneans are known to be highly host-specific (Baer 1957; Llewellyn 1957; Rohde 1979; Noble et al. 1989; Sasal et al. 1998), which supposes a close interaction with their hosts. Hosts may have an influence on the genetic and morphological differentiation in monogeneans, but this does not prevent subsequent host switching. This association could be seen as temporary in evolutionary time. Monogeneans seem to have an important potential for polymorphism and may be able to adapt rapidly to various conditions, as suggested by Desdevises et al. (2000) where a single *Lamellodiscus* species was shown to exhibit two slightly different morphologies on two distinct host species.

Specialization in monogeneans seems to be mainly under the influence of ecological factors; this has also been suggested in other studies (Klassen and Beverley-Burton 1988; Bentz et al. 2001). This is supported by the observation that *D. sargus* and *D. vulgaris*, which are not sister taxa (see Fig. 3), harbor a lot of parasites in common (see Table 1). However, these two species are ecologically close, living together in the same schools (Whitehead, 1986). The putative cospeciation event between the clades (*Lamellodiscus baeri*, *L. erythrini*)–(*Pagrus pagrus*, *Pagellus erythrinus*) could be explained by the solitary behavior of *P. pagrus* (Whitehead et al. 1986). It is noteworthy that all solitary species among Sparidae studied here (data from Whitehead et al. 1986, and Hanel and Sturbauer 2000), *Sparus aurata*, *Diplodus cervinus* and *P. pagrus*, possess only one *Lamellodiscus* parasite species. In contrast, the host species with the highest species richness in

Lamellodiscus are the members of the clade containing all *Diplodus* species (except *D. cervinus*), and they are all gregarious species living closely together, especially *D. sargus*, *D. vulgaris*, *D. puntazzo* and *D. annularis* (Whitehead et al. 1986). Therefore, the social behavior of the hosts seems to promote host switching in *Lamellodiscus* monogeneans. The present study strongly suggests that their shared parasites were not acquired via cospeciation.

Pocket gophers and chewing lice, the "model system" to study host-parasite coevolution (Page and Hafner 1996), represent a very special case of biological association, because there is almost no opportunity for contact between hosts of the different species (Nadler and Hafner 1989; Nadler et al. 1990). Parasites are then separated by a strong ecological barrier. Nevertheless, some cases of incongruence interpreted as host-switching events have been observed (Hafner et al. 1994; Page 1993c; Ronquist 1995). In the case studied here, this type of ecological barrier is absent since the hosts live in sympatry. Parasites have many opportunities to switch hosts, and this seems to happen in nature. This suggests that host choices by parasites and subsequent specialization are not driven by historical factors, at least not in an important proportion. Coevolution may be a by-product of host separation, either geographical or behavioral (see Bentz et al. 2001), and it seems to be controlled by allopatric speciation. This was also suggested by Reed and Hafner (1997) for the pocket gopher-chewing lice association.

It has been argued that host specificity is highly linked with cospeciation processes (Poulin 1992; Kearn 1994). In the association under study here, however, many specialist parasites do not exhibit cospeciation patterns with their hosts (e.g., *Lamellodiscus knoeppfleri*, *L. parisi* or *L. drummondi*). This suggests that the processes leading to specificity do not necessary lead to host-parasite coevolution. The presence of no cospeciation associated to the presence of specialist species is interpreted by Brooks (1979) to represent a strong ecological host-parasite association for these species (see Klassen and Beverley-Burton 1988), and suggests adaptive processes. This has been emphasized by Hoberg (1986) who proposed that pronounced host specificity and host-parasite coevolution may not always be associated, especially in groups of great evolutionary age. In the present case, the observation that the ITS1 sequences are not alignable among

species, and that rDNA 18S, a relatively slow-evolving molecule (Hillis and Dixon 1991), contains sufficient phylogenetic signal to infer the phylogeny of the *Lamellodiscus* species, is in favor of an old age for this genus. However, these observations may also be due to a fast evolutionary rate. The *Lamellodiscus* species seem to be able to speciate rapidly, even in sympatric conditions; the host and parasite phylogenies support the hypothesis of sympatric speciation for some species, like *L. bidens* and *L. hilii* on *Diplodus puntazzo*, and *L. mormyri* and *L. verberis* on *Lithognathus mormyrus*. This ability to speciate and the many opportunities for host switching could explain the absence of cospeciation pattern observed here, even for specialist species. Secord and Kareiva (1996) emphasized that the colonization ability of a parasite is a function of its morphological variability (polymorphism): some morphs would allow the use of different hosts and can lead to subsequent speciation. Such intraspecific morphological variability has been observed in monogeneans (e.g., Mo 1991a,b), among which the *Lamellodiscus* species (Desdevises et al., 2000). This supports the hypothesis of an important potential for adaptability in monogeneans, leading to their opportunistic colonization behavior.

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LITERATURE CITED

Baer, J. G., ed. 1957. First Symposium on Host Specificity among Parasites of Vertebrates. Paul Attinger, Neuchatel.

Bakke, T. A., P. D. Harris, P. A. Jansen, and L. P. Hansen. 1992. Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). Dis. Aquat. Org. 13:63-74.

Barker, S. C. 1994. Phylogeny and classification, origins, and evolution of host associations of lice. Int. J. Parasitol. 24:1285-1291.

Basaglia, F. 1991. Interspecific gene differences and phylogeny of the Sparidae family (Perciformes, Teleostei), estimated from electrophoretic data on enzymatic tissue expression. Comp. Biochem. Physiol 99B:495-508.

Bentz, S., S. Leroy, L. du Preez, J. Mariaux, C. Vaucher, and O. Verneau. 2001. Origin and evolution of African *Polystoma* (Monogena: Polystomatidae) assessed by molecular methods. Int. J. Parasitol. 31: 697-705

Boeger, W. A., and D. C. Kritsky. 1989. Phylogeny, coevolution, and revision of the Hexabothriidae Price, 1942 (Monogenea). Int. J. Parasitol. 19:425-440.

——. 1997. Coevolution of the monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. Int. J. Parasitol. 27:1495-1511.

Brooks, D. R. 1979. Testing the context and extent of host-parasite coevolution. Syst. Zool. 28:299-307.

——. 1981. Hennig's parasitological method: A proposed solution. Syst. Zool. 30: 229-249.

——. 1988. Macroevolutionary comparisons of host and parasite phylogenies. Ann. Rev. Ecol. Syst. 19:235-259.

Brooks, D. R., and D. R. Glen. 1982. Pinworms and primates: a case study in coevolution. Proc. Helminthol. Soc. Wash. 49:76-85.

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, Ecology, and Behavior. A research program in comparative biology. Univ. of Chicago Press, Chicago.

Bychowsky, B. E. 1961. Monogenetic Trematodes their systematics and phylogeny. English translation edited by W. J. Hargis Jr. American Institute of Biological Sciences: Washington.

Caro, A., C. Combes, and L. Euzet. 1997. What makes a fish a suitable host for Monogenea

in the Mediterranean? J. Helminthology 71:203-210.

Cataudella, S., P. Perin Riz P, and L. Sola. 1980. A chromosome study of eight Mediterranean species of Sparidae (Pisces, Perciformes). Genetica 54:155–159

Charleston, M.A. 1998. Jungles: a new solution to the host/parasite phylogeny reconciliation problem. Math. Biosci. 149:191-223.

Cressey, R. F., B. B. Collette, and J. L. Russo. 1983. Copepods and scombrid fishes: A study in host-parasite relationships. Fish. Bull. 81:227-265.

Desdevises, Y. 2001. The phylogenetic position of *Furnestinia echeneis* (Monogenea, Diplectanidae) based on molecular data: a case of morphological adaptation? Int. J. Parasitol. 31:205-208.

Desdevises, Y., R. Jovelin, O. Jousson, and S. Morand. 2000. Comparison of ribosomal DNA sequences of *Lamellodiscus* spp, (Monogenea, Diplectanidae) parasitising *Pagellus* (Sparidae, Teleostei) in the north Mediterranean sea: species divergence and coevolutionary interactions. Int. J. Parasitol. 30:741-746.

Desdevises, Y., S. Morand, and G. Oliver. 2001. Linking specialization to diversification in the Diplectanidae Bychowsy 1957 (Monogenea, Platyhelminthes). Parasitol. Res. 87:223-230.

Euzet, L. 1984. Diplectanidae (Monogenea) parasites de poissons des Iles Kerkennah (Tunisie). Arch. Instit. Pasteur Tunis 61:463-474.

Euzet, L., C. Combes, and A. Caro. 1993. A check list of monogenea of mediterranean fish. Second International Symposium on Monogenea, Montpellier/Sète (France).

Euzet, L., and G. Oliver. 1966. Diplectanidae (Monogenea) des Téléostéens de la Méditerranée occidentale. III. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Diplodus* (Sparidae). Ann. Par. Comp. (Paris) 41:573-598.

——. 1967. Diplectanidae (Monogenea) de Téléostéens de la Méditerranée occidentale. IV. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Pagellus* Cuvier, 1829 (Sparidae). Ann. Par. Comp. (Paris) 42:407-425.

Farris, J. S., M. Kallersjo, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. Cladistics 10:315-320.

Fiedler, K. 1991. Familie Sparidae. In: Starck D (ed) Lehbruch der Speziekken Zolologie.

Tejl 2: Fishe. Gustav Fisher Verlag, Jena.

Garrido-Ramos, M. A., M. Jamilena, R. Lozano, S. Càrdenas, C. Ruiz Rejon, and M. Ruiz Rejon. 1995. Phylogenetic relationship of the Sparidae family (Pisces, Perciformes) inferred from satellite-DNA. Hereditas 122:1-6.

Garrido-Ramos, M. A., R. de la Herràn, R. Lozano, C. Ruiz Rejon, and M. Ruiz Rejon. 1998. A satellite DNA of the Sparidae family (Pisces, Perciformes) associated with telomeric sequences. Cytogenet. Cell Genet. 83:3-9.

Garrido-Ramos, M. A., R. de la Herràn, R. Lozano, M. Jamilena, C. Ruiz Rejon, and M. Ruiz Rejon. 1999. Evolution of centromeric DNA and its use in phylogenetic studies of the Sparidae family (Pisces, Perciformes) associated with telomeric sequences. Mol. Phyl. Evol 12:200-204.

Gower, J. C. 1966.Some distant properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325-338.

Guégan, J.-F., and J.-F. Agnèse. 1991. Parasite evolutionnary events inferred from host phylogeny: the case of *Labeo* species (Teleostei, Cyprinidae) and their dactylogyrid parasites (Monogenea, Dactylogiridae). Can J. Zool. 69:595-603.

——. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. Nature 332(6161):258-259.

Hafner, M. S., P. D. Sudman, F. X. Villablanca, T. A. Spradling, J. W. Demastes, and S. Nadler. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science 265:1087-1090.

Hanel, R, and C.Sturmbauer. 2000. Multiple recurrent evolution of trophic types in Northeastern Atlantic and Mediterranean Seabreams (Sparidae, Percoidei). J. Mol. Evol. 50:276-283

Hillis, D. M., and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quaterly Rev. Biol. 66:411-453.

Hoberg, E. P. 1986. Evolution and historical biogeography of a parasite-host assemblage: *Alcataenia* sp. (Cyclophyllidea, Dilepidae) in Alcidae (Charadriiformes). Can. J. Zool. 64:2576-2589.

Huelsenbeck, J. P., B. Rannala, and Z. Yang. 1997. Statistical tests of host-parasite cospeciation. Evolution 51:410-419.

Huelsenbeck, J. P., B. Rannala, and B. Larget. 2000. A Bayesian framework for the analysis of cospeciation. Evolution 54:352-364.

Jousson, O., P. Bartoli, and J. Pawlowski. 2000. Cryptic speciation among intestinal parasites (Trematoda: Digenea) infecting sympatric host fishes (Sparidae). J. Evol. Biol. 13:778-785.

Kearn, G. C. 1967. Experiments on host-finding and host-specificity in the monogenean skin parasite *Entobdella soleae*. Parasitology 57:585-605.

——. 1988. The monogenean skin parasite *Entobdella soleae*: movement of adults and juveniles from host to host (*Solea solea*). Int. J. Parasitol. 18:313-319.

——. 1994. Evolutionary expansion of the Monogenea. Int. J. Parasitol. 24:1227-1271.

Kellogg, V. L. 1913. Distribution and species-forming of ectoparasites. Am. Nat. 47:129-158.

Kennedy, C. R. 1975. Ecological Animal Parasitology. Blackwell Scientific Publication, Oxford.

Klassen, G. J. 1992. Coevolution: A history of the macroevolutionary approach to studying host parasite associations. J. Parasitol. 78:573-587.

Klassen, G. J. and Beverley-Burton, M. 1987. Phylogenetic relationships of *Ligictaluridus* spp. (Monogenea : Ancyrocephalidae) and their ictalurid (Siluriformes) hosts : an hypothesis. Proc. Helminthol. Soc. Wash. 54:84-90.

——. 1988. North American Fresh Water Ancyrocephalids (Monogenea) with articulating haptoral bars: host-parasite coevolution. Syst. Zool. 37:179-189.

Lapointe, F.-J. 1998. How to validate phylogenetic trees? A stepwise procedure. Pp.71-88 *in* C. Hayashi, N. Ohsumi, K. Yajima, Y. Tanaka, H. H. Bock, and Y. Baba, eds. Studies in Classification, Data Analysis, and Knowledge Organization: Data Science, Classification, and Related Methods. Springer, Tokyo.

Legendre, P., Y. Desdevises, and E. Bazin. Submitted. A statistical test for host-parasite coevolution. Syst. Biol.

Legendre, P., R. Galzin, and M. L. Harmelin-Vivien. 1997. Relating behavior to habitat: solutions to the fourth-corner problem. Ecology 78:547-562.

Llewellyn, J. 1957. Host-specificity in monogenetic trematodes. Pp. 199-212 *in* Premier Symposium sur la spécificité parasitaire des parasites de Vertébrés. Institut de

Zoologie, Université de Neuchâtel, Imprimerie Paul Attinger S.A., Neuchâtel.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209-220.

Mo, T. A. 1991a. Variations of opisthohaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in South-eastern Norway. Syst. Parasitol. 20:1-9.

Mo, T. A. 1991b. Variations of opisthohaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. Syst. Parasitol. 20: 11-19.

Nadler, S. A., and M. S. Hafner. 1989. Genetic differentiations in sympatric species of chewing lice (Mallophaga: Trichodectidae). Ann. Entomol. Soc. Am. 82:109-113.

Nadler, S. A., M. S. Hafner, J. C. Hafner, and D. J. Hafner. 1990. Genetic differentiation among chewing louse population (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomydae). Evolution 44:942-951.

Noble, E. R., G. A. Noble, G. A. Schad, A. J. MacInnes. 1989. Parasitology. The Biology of Animal Parasites. 6th Edition. Lea & Febiger, Philadelphia.

Oliver, G. 1968. Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. II. Lamellodiscinae nov. sub. fam. Vie Milieu. 19:95-138.

Oliver, G. 1973. *Lamellodiscus obeliae* n. sp. une nouvelle espèce de Diplectanidae (Monogenea, Monopisthocotylea) parasite de *Pagellus centrodontus* (Delaroche, 1809) (Pisces, Sparidae). Z. Parasitenk. 41:103-108.

Oliver, G. 1974. Nouveaux aspects du parasitisme des Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea) chez les Téléostéens Perciformes des côtes de France. C.-R. Acad Sci. Paris 279:803-805.

Oliver, G. 1987. Les Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea, Dactylogyridea). Systématique. Biologie. Ontogénie. Écologie. Essai de phylogénèse. Thèse d'état. Université des Sciences et Techniques du Languedoc.

Page, R. D. M. 1993a. COMPONENT 2.0. Tree comparison software for the use with Microsoft Windows. The Natural History Museum, London.

——. 1993b. Genes, organisms, and areas : the problem of multiple lineages. Syst. Biol.

42:77-84.

——. 1993c. Parasites, phylogeny and cospeciation. Int. J. Parasitol. 23:499-506.

——. 1994a. Maps between trees and cladistic analysis of historical associations among genes, organisms, and areas. Syst. Biol. 43:58-77.

——. 1994b. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics 10:155-173.

Page, R. D. M., and M. A. Charleston. 1998. Trees within trees - phylogeny and historical associations. TREE 13:356-359.

Page, R. D. M., D. H. Clayton, and A. M. Paterson. 1996. Lice and cospeciation: a reponse to Barker. Int. J. Parasitol. 26:213-218.

Page, R. D. M., and M. S. Hafner. 1996. Molecular phylogenies of host-parasite cospeciation: gophers and lice as a model system. *in* P. H. Harvey, A. J. L. Brown, J. Maynard Smith, and S. Nee, eds. New Uses for New Phylogenies. Oxford Univ. Press, Oxford.

Page, R. D. M., P. L. M. Lee, S. A. Becher, R. Griffiths, and D. H. Clayton. 1998. A different tempo of mitochondrial DNA evolution in birds and their parasitic lice. Mol. Phyl. Evol. 9:276-293.

Paterson, A. M., and J. B. Banks. 2001. Analytical approaches to measuring cospeciation of host and parasites: through a glass, darkly. Int. J. Parasitol. 31:1012-1022.

Paterson, A. M., R. D. Gray, and G. P. Wallis. 1993. Parasites, Petrels and Penguins: does louse presence reflect seabird phylogeny? Int. J. Parasitol. 23:515-526.

Poulin, R. 1992. Determinants of host specificity in parasites of freshwater fishes. Int. J. Parasitol. 22:753-758.

Reed, D. L. and M. S. Hafner. 1997. Host specificity of chewing lice on pocket gophers: a potential mechanism for cospeciation. J. Mammal. 78:655-660.

Reina, J., G. Martinez, A. Amores, and M. C. Alvarez. 1994. Interspecific genetic differentiation in Western Mediterranean sparid fish. Aquaculture 125:47-57.

Rohde, K. 1979. A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. Am. Nat. 114:648-671.

——. 1982. Ecology of Marine Parasites. Univ. of Queensland Press. St Lucia.

Ronquist, F. 1995. Reconstructing the history of host-parasite associations using

generalised parsimony. Cladistics 11:73-89.

Ronquist, F. 1997. Phylogenetic approaches in coevolution and biogeography. Zool. Scr. 26:313-322.

Sasal, P., Y. Desdevises, and S. Morand. 1998. Host-specialization and species diversity in fish parasites: phylogenetic conservatism? Ecography 21:639-643.

Secord, D., and P. Kareiva. 1996. Perils and pitfalls in the host specificity paradigm. Bioscience 46:448-453.

Simkova, A., Y. Desdevises, M. Gelnar, and S. Morand. 2000. Coexistence of nine gill ectoparasites (*Dactylogyrus* : Monogenea) parasitising the roach (*Rutilus rutilus* L.): history and present ecology. Int. J. Parasitol. 30:1077-1088.

Sinnappah, N. D., L. S. Lim, K. Rohde, R. Tinsley, C. Combes, and O. Verneau. 2001. A paedomorphic parasite associated with a neotenic amphibian host: phylogenetic evidence suggests a revised systematic position for Sphyranuridae within Anuran and Turtle Polystomatoideans. Mol. Phyl. Evol. 18:189-201.

Smith, J. L. B., and M. M. Smith. 1986. Family No 183: Sparidae. *in* M. M. Smith, and P. C. Heemstra, eds. Smith's sea fishes. Springer, New York, Berlin, Heidelberg.

Sullivan, J., K. E. Holsinger, and C. Simon. 1996. The effect of topology on estimation of among-site rate variation. J. Mol. Evol. 42:308-312.

Swofford, D. L. 2001. *PAUP**: Phylogenetic analysis using parsimony and other methods. Version 4.0d8. Champaign, Illinois: Illinois Natural History Survey.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, D. G. Higgins. 1997. The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Ac. Res. 24:4876-4882.

Toft, C. A., and A. J. Karter. 1990. Parasite-host coevolution. TREE 5:326-329.

Verneau, O., F. Renaud, and F. Catzeflis. 1997. Evolutionary relationships of sibling tapeworm species (Cestoda) parasitizing teleost fishes. Mol Biol. Evol. 14:630-636.

Whitehead, P. J. P., M. L. Bauchot, J. C. Hureau, J. Nielsen, and E. Tortonese. 1986. Checklist of the fishes of the North-eastern Atlantic and of the Mediterranean. Paris, UNESCO, Vol. III.

Yang, Z. 1994. Maximum likelihood phylogenetics estimation from DNA sequences with variable rates over sites: approximate methods. J. Mol. Evol. 39:306-314.

Yang, Z. 1995. A space-time process model for the evolution of DNA sequences. Genetics 139:993-1005.

Yang, Z. 1996. Among-site rate variation and its impact on phylogenetic analyses. TREE 11:367-372.

Yang, Z., N. Goldman, and A. E. Friday. 1994. Comparison of models for nucleotide substitution used in maximum likelihood phylogenetic estimation. Mol. Biol. Evol. 11:308-312.

Table 1: *Lamellodiscus* – Sparidae associations and GenBank Accession numbers. (o): outgroup taxa. *: sequence obtained during this study

Lamellodiscus and outgroup species	Sparidae species	GenBank Accession numbers	
		Parasites (18S)	Hosts (16S/Cyt-b)
L. baeri	*Pagrus pagrus*	AY038187*	AJ247277/AJ319815*
L. bidens	*Diplodus puntazzo*	AY038188*	AJ247291/AJ277368
L. coronatus	*Diplodus annularis*	AY038189*	AJ247286/AJ277366
	Diplodus cervinus		AJ247290/AJ277367
	Diplodus sargus		AJ247293/AJ277369
L. drummondi	*Pagellus acarne*	AJ276441	AJ247281/AJ276879
L. elegans	*Diplodus annularis*	AF294956	
	Diplodus sargus		
	Diplodus vulgaris		AJ247294/AJ277370
	Oblada melanura		AJ247296/AJ319813*
	Spondyliosoma cantharus		AJ247280/AJ319811*
L. ergensi	*Diplodus annularis*	AY038190*	
	Diplodus puntazzo		
	Diplodus sargus		
	Diplodus vulgaris		
L. erythrini	*Pagellus erythrinus*	AJ276440	AJ247284/AJ276881
L. fraternus	*Diplodus annularis*	AY038191*	
	Diplodus vulgaris		
L. furcosus	*Diplodus annularis*	AY038192*	
	Diplodus sargus		
L. gracilis	*Diplodus annularis*	AY038193*	
	Diplodus sargus		
	Oblada melanura		
L. hilii	*Diplodus puntazzo*	AY038194*	
L. ignoratus	*Diplodus annularis*	AF294957	
	Diplodus puntazzo		

	Diplodus sargus		
	Diplodus vulgaris		
	Lithognathus mormyrus		AJ247285/AJ277371
	Sarpa salpa		AJ247269/AJ319812*
L. impervius	*Diplodus puntazzo*	AY038195*	
L. knoepfffleri	*Spondyliosoma cantharus*	AY038196*	
L. mirandus	*Diplodus sargus*	AY038197*	
L. mormyri	*Lithognathus mormyrus*	AF294954	
L. parisi	*Sarpa salpa*	AY038198*	
L. verberis	*Lithognathus mormyrus*	AF294955	
L. virgula	*Pagellus acarne*	AJ276442	
	Pagellus bogaraveo		AJ247283/AJ276880
Furnestinia echeneis	*Sparus aurata*	AF294953	AJ247279/AJ319809*
Not parasitised	*B. boops*		AJ247268/AJ319810*
Not parasitised	*D. dentex*		AJ247271/AF143197
D. aequans (o)	*Dicentrarchus labrax* (o)	AJ276439	
P. ardens (o)		AJ228793	
D. minor (o)		AF294952	
	Spicara maena (o)		AJ247298

Table 2: Results from ParaFit. Probabilities are computed after 999 random permutations. The null hypothesis (H_0) of the global test (bottom of the table) is that the evolution of the two groups, as revealed by the two phylogenetic trees and the set of host-parasite association links, has been independent. In the tests of individual host-parasite association links, the null hypothesis is that the link under test is random. *: significant association ($P \leq 0.05$).

Parasite	Host	Probability
F. echeneis	*S. aurata*	0.170
L. baeri	*P. pagrus*	0.028*
L. bidens	*D. puntazzo*	0.775
L. coronatus	*D. annularis*	0.079
L. coronatus	*D. cervinus*	0.230
L. coronatus	*D. sargus*	0.220
L. drummondi	*P. acarne*	0.130
L. elegans	*D. annularis*	0.058
L. elegans	*D. sargus*	0.220
L. elegans	*D. vulgaris*	0.172
L. elegans	*O. melanura*	0.240
L. elegans	*S. cantharus*	0.892
L. ergensi	*D. annularis*	0.549
L. ergensi	*D. puntazzo*	0.692
L. ergensi	*D. sargus*	0.766
L. ergensi	*D. vulgaris*	0.548
L. erythrini	*P. erythrinus*	0.018*
L. fraternus	*D. annularis*	0.672
L. fraternus	*D. vulgaris*	0.635
L. furcosus	*D. annularis*	0.095
L. furcosus	*D. sargus*	0.258
L. gracilis	*D. annularis*	0.970
L. gracilis	*D. sargus*	0.791

L. gracilis	*O. melanura*	0.729
L. gracilis	*S. cantharus*	0.431
L. hilii	*D. puntazzo*	0.802
L. ignoratus	*D. annularis*	0.545
L. ignoratus	*D. puntazzo*	0.670
L. ignoratus	*D. sargus*	0.723
L. ignoratus	*D. vulgaris*	0.523
L. ignoratus	*L. mormyrus*	0.633
L. ignoratus	*S. salpa*	0.631
L. impervius	*D. puntazzo*	0.794
L. knoeppfleri	*S. cantharus*	0.724
L. mirandus	*D. sargus*	0.885
L. mormyri	*L. mormyrus*	0.238
L. parisi	*S. salpa*	0.279
L. verberis	*L. mormyrus*	0.246
L. virgula	*P. acarne*	0.096
L. virgula	*P. bogaraveo*	0.090
Global test		0.243

FIGURES LEGENDS

Fig. 1: Saturation plots showing distances (% differences) computed from transitions (Ti) versus distances computed from transversions (Tv). (a) Cyt-b mtDNA sequences from Sparidae. (b) 16S mtDNA sequences from Sparidae. (c) 18S rDNA sequences from *Lamellodiscus*.

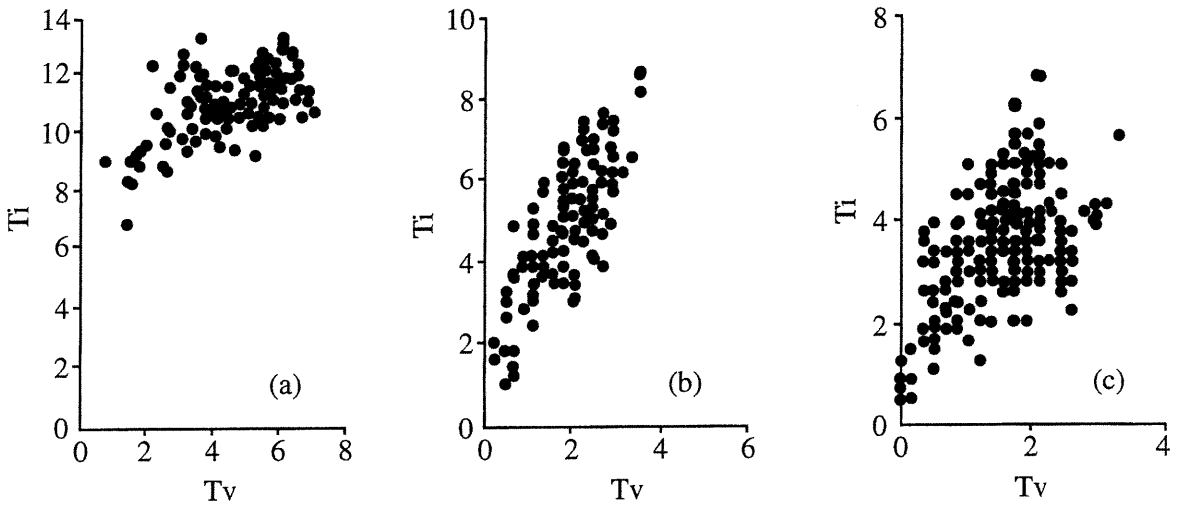
Fig. 2: Frequencies of base changes, proportional to circle diameters, estimated from most-parsimonious trees, (a) for Sparidae cyt-b mtDNA sequences; (b) for Sparidae cyt-b and 16S mtDNA (total evidence) sequences; (c) for *Lamellodiscus* 18S rDNA sequences. Transitions are represented by dark circles, transversions by light circles.

Fig. 3: Host phylogenetic trees estimated from mtDNA partial sequences; numbers are bootstrap values. (a) Consensus tree published by Hanel and Sturmbauer (2000), estimated from 16S mtDNA. (b) MP tree estimated from Cyt-b mtDNA sequences. (c) ML tree computed from Cyt-b mtDNA sequences. (d) MP tree computed from Cyt-b and 16S mtDNA sequences (total evidence). (e) ML tree estimated from Cyt-b and 16S mtDNA sequences (total evidence).

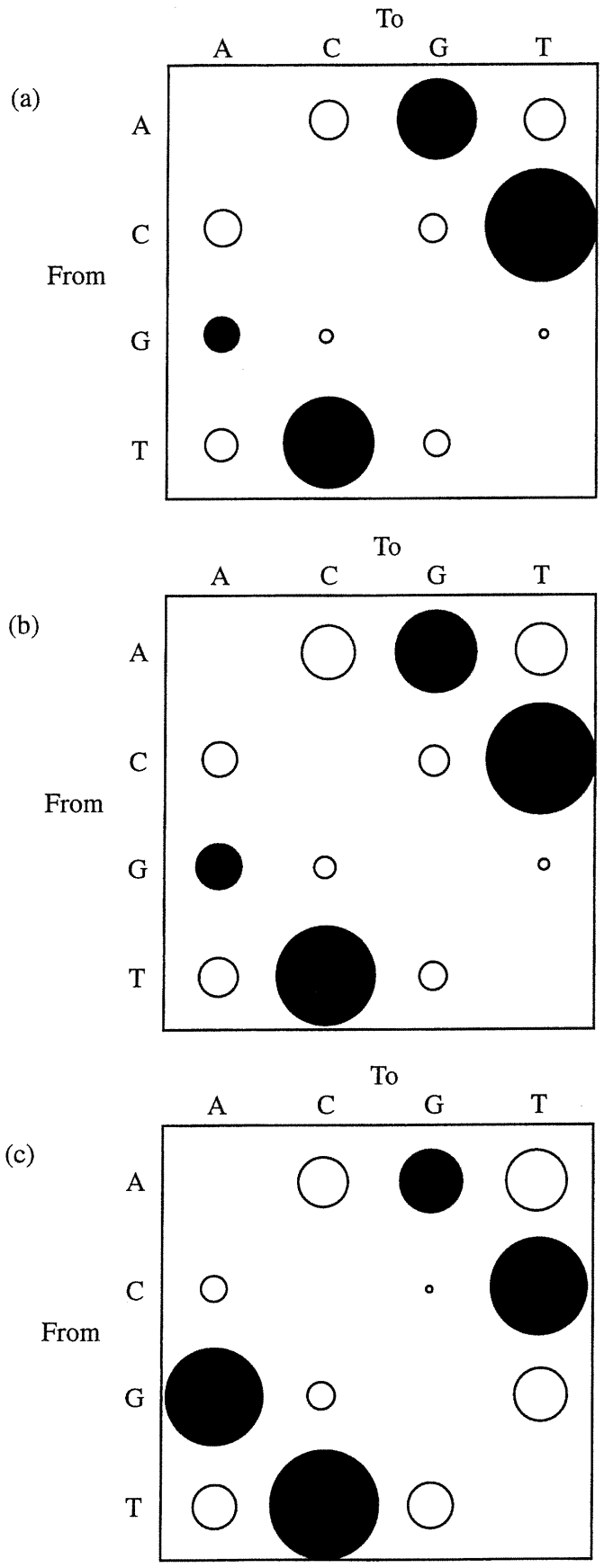
Fig. 4: Parasites phylogenetic trees estimated from 18S rDNA partial sequences; numbers are bootstrap values. (a) Strict consensus of five MP trees. (b) ML tree.

Fig. 5: Pattern of host and parasite associations, with ML trees estimated for the hosts and parasites. Lines depict the observed host-parasite associations.

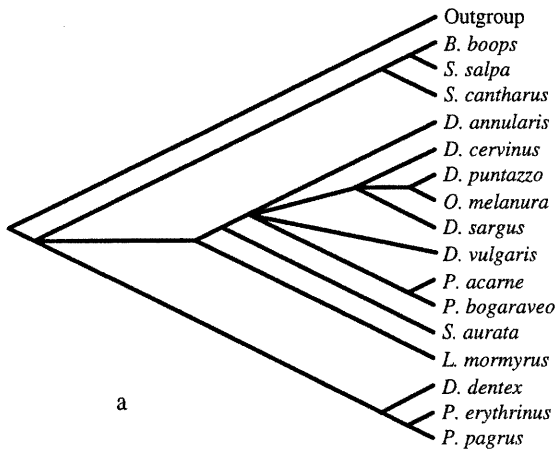
Fig. 6: Histogram generated by TreeMap: distribution of the number of cospeciation events in random associations. *: number of cospeciation events inferred by TreeMap for the *Lamellodiscus*-Sparidae association.



Desdevises et al., Fig. 1



Desdevises et al., Fig. 2



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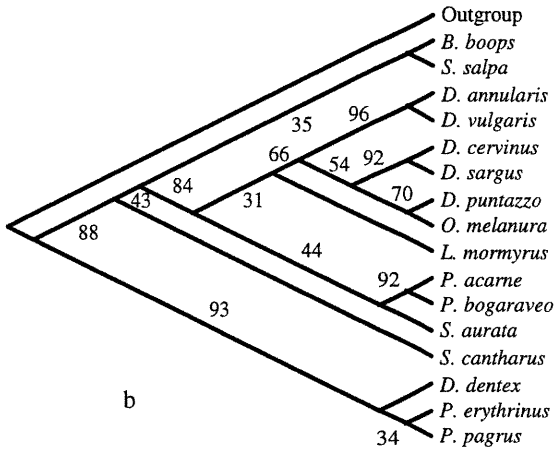

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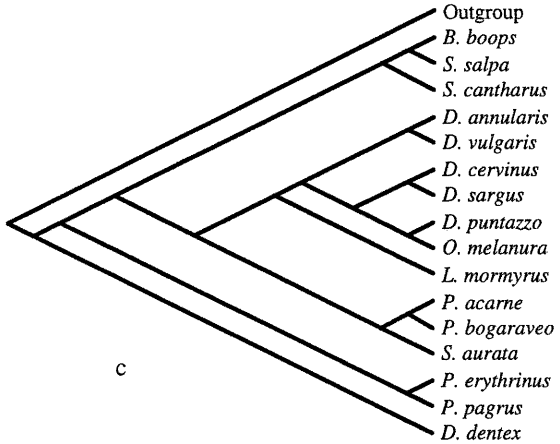

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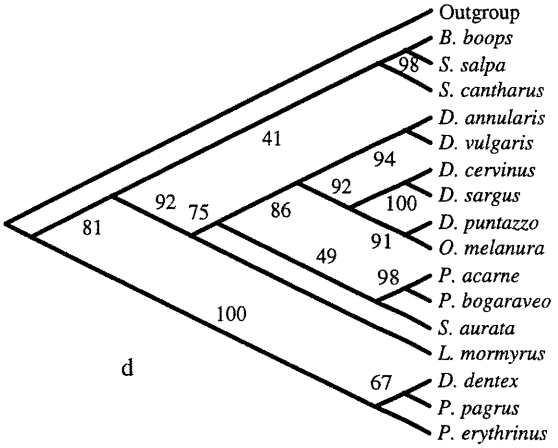

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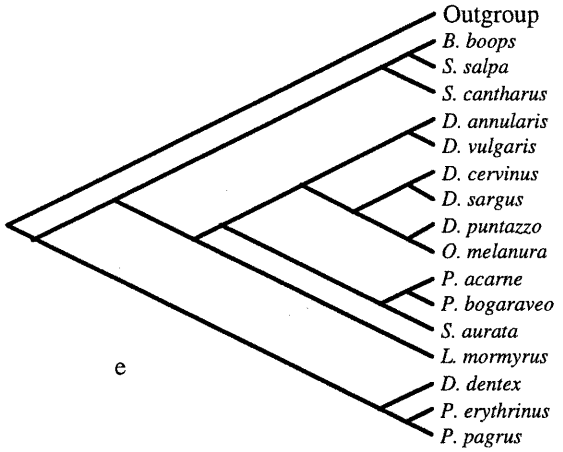

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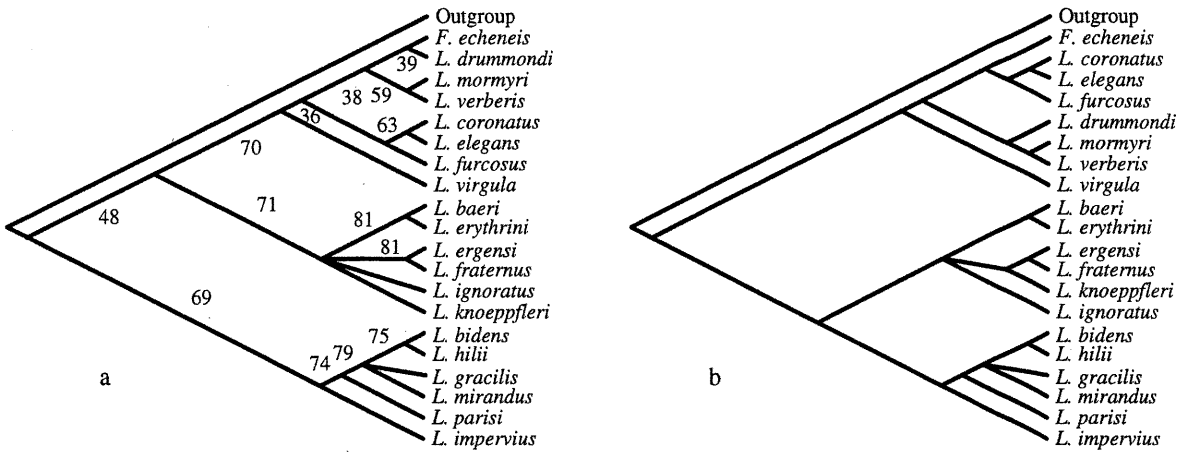
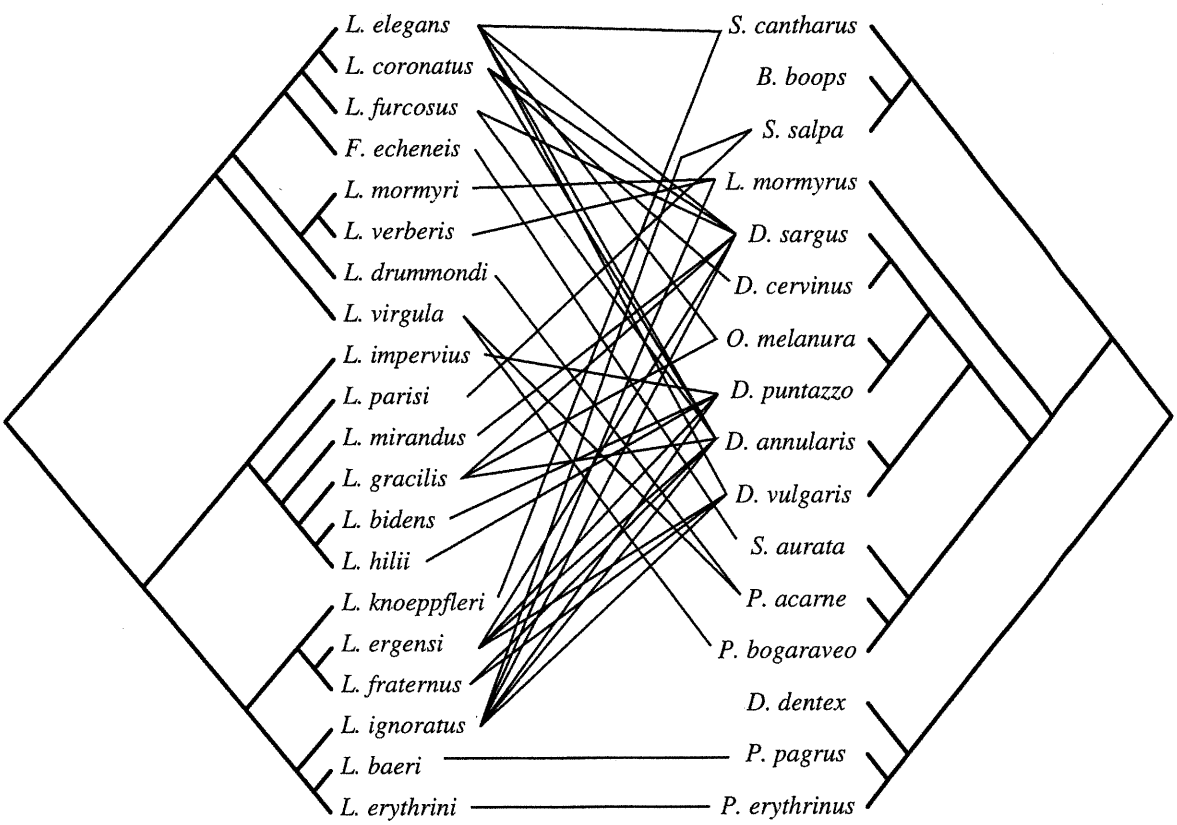
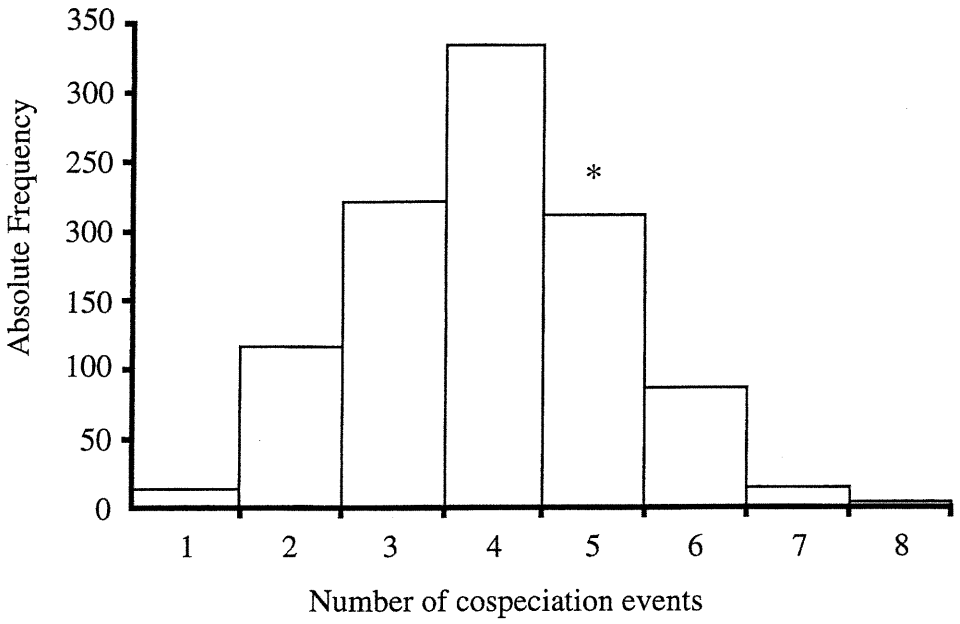
Desdevises et al., Fig. 3



Desdevises et al., Fig. 4



Desdevises et al., Fig. 5



Desdevises et al., Fig. 6

D. Déterminants de la spécificité dans le système *Lamellodiscus*-Sparidae

Article 6

DESDEVISES Yves, Serge MORAND and Pierre LEGENDRE. Evolution and determinants of specificity in *Lamellodiscus* (Monogenea). Soumis.

Objectif g et objectif principal (synthèse)

Dans cette publication, nous essayons d'identifier les déterminants écologiques de la spécificité chez les *Lamellodiscus*, ainsi que l'évolution de cette caractéristique. La spécificité semble être contrôlée en partie pas la taille de l'hôte : les monogènes étudiés ici semblent se spécialiser sur les hôtes les plus grands. Cela est interprété comme une spécialisation sur des hôtes prédictibles. La spécificité est également très liée à la phylogénie, suggérant par là qu'elle est liée à des caractéristiques transmissibles des parasites. La partie commune de la variation de la spécificité due à la phylogénie ainsi qu'à la taille de l'hôte est quantifiée et s'avère importante. Une analyse d'optimisation de caractère sur l'arbre phylogénétique des *Lamellodiscus* suggère que la spécificité ne semble pas conduire à un cul-de-sac évolutif chez ces parasites. En outre, comme chez les Diplectanidae, la spécificité ne semble pas associée à une plus grande diversification taxonomique chez les *Lamellodiscus*.

Participation du thésard :

- Recherche bibliographique
- Analyse comparative
- Analyses statistiques
- Rédaction

EVOLUTION AND DETERMINANTS OF HOST SPECIFICITY IN THE GENUS *LAMELLODISCUS* (MONOGENEA)

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Running head: determinants of specificity in *Lamellodiscus*

Abstract

The evolution and determinants of host specificity in *Lamellodiscus* species (Monogenea, Diplectanidae) were investigated. The twenty known Mediterranean species were studied. They are all parasites of fishes from the family Sparidae (Teleostei). An index of specificity was defined, which takes into account the phylogenetic relationships of their fish hosts species. The link between specificity and its potential determinants was investigated in a phylogenetic context using the method of independent contrasts. Host specificity in *Lamellodiscus* species appeared to be highly constrained by the phylogeny, but it is also linked to host size. Mapping specificity onto the parasite phylogenetic tree suggests that specialist species do not represent an evolutionary dead end, nor that specialization is a derived condition. It is suggested that the ability to be generalist or specialist in *Lamellodiscus* is controlled by intrinsic phylogenetically-related characteristic of the parasites, and that specialist species tend to use large hosts, which are hypothesized to be more predictable.

Introduction

What controls specialization, the process leading to specificity for a given resource, is not fully understood in ecology. Some organisms are restricted to a narrow set of resources while others seems to be far less selective. One might think that the more an organism is able to exploit different niches, the greater should its evolutionary success be. However, Timms and Read (1999) pointed out the non-existence of an ideal organism able to use all ecological niches. On the contrary, many very specialized organisms are found in nature. Using theoretical models, many studies have tried to understand what explains the appearance of specificity, e.g., Fox and Morrow (1981), Futuyma and Moreno (1988), Wilson and Yoshimura (1994), Fry (1996), Gemmill et al. (2000). Ward (1992) suggested, via a simple mathematical model, that organisms tend to specialize on predictable resources, i.e., those that are stable through time, thus minimizing extinction risks.

Since specificity is commonly believed to be the result of an adaptive process (Brooks and McLennan 1991; Begon et al. 1996), it is important to take the phylogenetic relationships of the parasite species under study into account when studying its causes and evolution (Brooks and McLennan 1991, Harvey and Pagel 1991). This allows one to discriminate between the relative effects of the past (phylogenetic) and present-day (ecological) influences (Futuyma and Moreno 1988; Brooks and McLennan 1991).

Host-parasite systems are good models to study these evolutionary questions (see Price 1980; De Meeûs et al. 1998; Paterson and Banks 2001). The ecological niche of a parasite is generally easier to define than that of a free-living organism (Timms and Read 1999), since its main environment, its habitat and its food are represented at least in part by its host. In addition, a phylogeny of the hosts provides a hypothesis about the evolution of the habitat of the parasites. Understanding the determinants of host specificity is a key issue in evolutionary parasitology (Rohde 1994; Thompson 1994); it is essential for the control of parasitic zoonoses (Secord and Kareiva 1996).

As in the study of Poulin (1992, 1997), only host specificity on the part of parasites will be considered here. Host specificity is defined as the number of host species used by a given parasite species. It is the same as host range (Lymbery 1989). A parasite species

using a single host species is usually defined as a specialist (e.g., Euzet and Combes 1980; Ludwig 1982), whereas if it uses several host species, it is said to be a generalist. It should be reminded that specificity is inversely related to host range, so it decreases while host range increases. These concepts are relative (e.g., Kitahara and Fuji 1994): sometimes, parasite species using a single genus or family are defined as specialists (Ludwig 1982). According to Futuyma and Moreno (1988), "specialization must lie in the eye of the beholder".

Parasites depend on their hosts to survive. Several hosts can be involved in the parasite's life cycle, complicating therefore the pattern of specificity and its potential determinants. A parasite with a direct life cycle permits to overcome these difficulties. This is why a group of monogeneans was chosen for the present study. Monogeneans are almost entirely dependent upon their hosts, which is their unique environment, and they are known to be generally highly host-specific (Baer 1957; Kennedy 1975; Rohde 1979, 1982; Noble et al. 1989; Sasal et al. 1998). Barker (1991), Poulin (1992), and Kearn (1994) suggested that their high degree of host specificity is explained by tight coevolutionary interaction with their hosts, as hypothesized by Tinsley and Jackson (1998) for Polystomatid monogeneans found in amphibians. Humphery-Smith (1989) listed characteristics favoring host-parasite coevolution by cospeciation: the parasites should be highly specific and non pathogenic to their hosts. This description matches that of monogenan parasites. However, some authors, like Brooks and McLennan (1991), believe that monogenans possess ideal characteristics to experience numerous host-switching events. This would suggest that other determinants than host evolutionary history control parasite specificity. In addition, several studies suggest that many monogeneans do not exhibit cospeciation patterns with their hosts (Klassen and Beverley-Burton 1987, 1988; Desdevises et al. 2000; Desdevises et al. submitted(b)). Sasal et al. (1999) have suggested that specialist monogenans tend to use larger fish species than generalists do. This supposes the existence of an adaptive strategy, which differs from a strict and passive phylogenetic tracking. As a potential factor limiting host specificity, competition does not seem to be an important determinant of specificity in monogeneans (Euzet and Combes 1998). Indeed, most monogeneans are skin or gill ectoparasites, and they live in an environment where the number of available niches seems to be very high (Rohde 1978). It is only when space is limiting (e.g., for endoparasitic

species) that competition could play a role in monogenans (Jackson et al. 1998). This has led some authors to propose the "mating hypothesis" to explain the high host specificity of some parasite species: in low-density populations, individuals tend to meet on a single resource to mate (Rohde 1979; Colwell 1986), but this hypothesis has been questioned by other authors (e.g., Adamson and Caira 1994). This also supports the idea that there are ecological determinants for host specificity.

Futuyma and Moreno (1988) insist that the causes and consequences of specialization should not be confounded, even if the distinction may not be straightforward, since a single factor can be both a cause and a consequence of specificity, i.e., be the product of specificity via an adaptive process while it also constrains subsequent specialization.

The host-parasite association studied here is formed by fishes from the family Sparidae (Teleostei) and their gill monogenean parasites from the genus *Lamellodiscus* (Diplectanidae). This study was carried out in the northwestern Mediterranean Sea near the Golfe du Lion, on all known *Lamellodiscus* species (20) in this area (see Oliver 1987; Desdevises et al. submitted(b)). *Furnestinia echeneis* will be considered to belong to the *Lamellodiscus* genus (Desdevises 2001). This host-parasite system has been well studied, and its current pattern of host specificity can be considered to be one of the best-known in the world (Caro et al. 1997). The sampling bias, if any, can then be considered to be very small. Phylogenies are available for the hosts and parasites (Desdevises et al. submitted(b)). A wide range of specificity can be found in this genus (from 1 to 6 hosts, see Table 1), which is relatively rare for monogeneans, and makes this host-parasite system of special interest for the study of specificity. Studying host-parasite coevolution in *Lamellodiscus*, Desdevises et al. (submitted) suggested that the host and parasite phylogenetic trees were not generally congruent, and that almost no cospeciation seems to have occurred between these two species complexes. Therefore, choice of hosts on the part of the parasites and specialization of the parasites on their hosts do not seem to be controlled by the host evolutionary history. Desdevises et al. (submitted) also observed that ecologically- but not phylogenetically-related hosts may have one or several parasite species in common, and that solitary species are less parasitized than gregarious species. This suggests that the

choice of hosts and the subsequent specialization in *Lamellodiscus* monogeneans is driven by opportunities for colonization.

In this paper, we will investigate the evolution and determinants of host specificity in *Lamellodiscus* species. First, we want to know if specificity is constrained by the phylogeny of the parasites. In other words, are the parasites with the same level of specificity more related to one another than they are to other congeneric species? If this is the case, it will support the hypothesis that host specificity in *Lamellodiscus* is controlled by intrinsic heritable characteristics of the parasites. Second, we want to investigate if specificity is a derived condition (*sensu* Thompson 1994). This would support an old belief that specialization is "an evolutionary dead end" (Huxley 1942; Simpson 1953, see Futuyma and Moreno 1988). Third, in the search for potential determinants, we will look for ecological variables that are significantly related to specificity, using comparative analysis (see Harvey and Pagel 1991). Fourth, we will examine if specificity is linked to taxonomic diversification, as this hypothesis as been proposed in the literature (Brooks and McLennan 1991, 1993). Specialization could promote species diversification (1) by reducing gene flow (Futuyma and Moreno 1988) and (2) because specialists are less tolerant to host changes and would then be more subject to host selective pressures after a host switch or a cospeciation event (Brooks and McLennan 1991). This would lead more frequently to parasite speciation. No such trend was observed among genera in the Diplectanidae (Desdevises et al. 2001), but this hypothesis will be tested again here at the species level. Finally, we will look for a correlation between host size and parasite body length. Such a link would represent a mechanism optimizing the morphological adaptation of parasites to their hosts, and would therefore be related to host specificity.

Materials and methods

Sampling

Sparid fishes and their *Lamellodiscus* parasites were sampled in several locations in the northwestern Mediterranean Sea, following the protocol described in Desdevises et al. (submitted(b)). Data on *Lamellodiscus* monogeneans and their pattern of specificity were found in Oliver (1968, 1969a,b, 1973, 1974, 1987), Euzet and Oliver (1966, 1967), Euzet

(1984), Euzet et al. (1993) and Kouider El Ouahed-Amine (1998). Parasite body lengths were measured with an optical micrometer ; they were comparable to measures reported in the literature. Note that *L. virgula* and *L. obeliae* are considered to be the same species (*L. virgula*) on the basis of molecular evidence (Desdevises et al. 2000) and that *Furnestinia echeneis* is considered to be a *Lamellodiscus* species because of its phylogenetic position inside the *Lamellodiscus* genus (Desdevises 2001). One of the *Lamellodiscus* species can be found parasitizing a non-Sparidae fish: in addition to its main host *Spondyliosoma cantharus* (Sparidae), *L. knoeppfleri* is also found on two Centracanthidae, *Spicara maena* and *S. smaris.*

Link between specificity and phylogeny

Specificity is represented here by the number of hosts that a parasite is using (as in Poulin 1992; see Lymbery 1989). This can lead to classify as "specialists" parasites that are using a single host, and as "generalists" parasites that are using two or more hosts. However, this distinction could be considered to be rather arbitrary. If there is no ambiguity for specialist parasites, and there is no doubt that some parasites are "true" generalists (i.e., parasitizing several distantly related hosts across genera or families), others species parasitize several closely-related hosts and could also be said to be generalists. An extreme case would be a parasite that uses two very closely related hosts, which could be labeled a generalist. But it can also be argued that a classification of specificity based only on the number of hosts may lead to arbitrary distinctions between species using, for instance, 5 or 6 host species. On the other hand, a parasite species using two hosts may not be considered the same way depending on whether these two hosts are closely phylogenetically related or not.

We decided to use four semi-quantitative classes to account for specificity: 1- *specialists* using a single host; 2- *intermediate specialists* using two closely related hosts; 3- *intermediate generalists* using two or more hosts in the same clade; 4- *generalists* using two or more hosts across several clades. This index will be hereafter named NSI for NonSpecificity Index, because, as number of hosts, the more NSI is high, the more host specificity is low. The definition of the four classes requires a host phylogeny, to define

host clades. Eight clades were defined in the host phylogeny (see Fig. 1). For that, we considered only well-supported clades from the phylogenies presented in Desdevises et al. (submitted(b)). The pattern of host-parasite association for *Lamellodiscus* species is presented in Table 1. We used the host and parasite phylogenetic trees, estimated by maximum likelihood (Figs. 1 and 2 respectively), that have been proposed by Desdevises et al. (submitted(b)).

The study of the evolution of host specificity was carried out by parsimony character mapping (Farris 1970; Brooks and McLennan 1991) of NSI onto the parasite phylogenetic tree. A statistical test was also conducted using the patristic distance matrix calculated from the phylogenetic tree to assess if NSI was significantly linked to the phylogeny. Instead of using directly the distance matrix to compare it to specificity through a Mantel test, which would require to transform NSI into a distance matrix, we decided to transform instead the patristic distance matrix into principal coordinates, a technique which has been shown to be efficient to represent phylogenetic inertia (Diniz-Filho et al 1998). Vector NSI was regressed onto the resulting principal coordinates. In more detail: a principal coordinate analysis (PCoA) was first performed on the phylogenetic distance matrix using THE R PACKAGE V.4.0 (Casgrain, P. and P. Legendre. 2000. The R Package for Multivariate and Spatial Analysis, version 4.0 d3 - User's Manual. Département de sciences biologiques, Université de Montréal. Freeware available at URL http://www.fas.umontreal.ca/BIOL/legendre/). Together, the computed principal coordinates (PCs) fully represented the phylogenetic variance. Since up to (n–1) PCs can be computed for n species, this high number of explanatory variables does not allow one to test the significance of the partial regression coefficients. On the other hand, the PCs are linearly independent of one another by definition; so, they can be divided into subsets, before carrying out multiple regression, without modifying the estimated regression coefficients. The PCs that are not significant in the subsets can be eliminated from the study. The existence of significant PCs would indicate a statistical link between specificity and phylogeny. Following this method, we performed two regression analyses, each one on half of the 18 PCs obtained. The regression parameters were tested by permutation using the program PERMUTE 3.4 (freeware written by P. Casgrain, available at URL http://www.fas.umontreal.ca/BIOL/Casgrain/en/labo/permute).

Is specificity a derived condition?

Character mapping was used to visually assess if specificity is a derived condition. To investigate this point statistically, we regressed NSI against the number of nodes separating each species from the root of the tree. The higher this number, the more derived a species is, being the product of many prior speciation events. A simple linear regression was performed and tested by permutation using the program PERMUTE 3.4 described in the previous paragraph.

Determinants of specificity

We used the method of independent contrasts (Felsenstein 1985), which takes the phylogeny into account, to investigate the determinants of specificity. The independent contrasts method consists of estimating the differences (contrasts) between sister-taxa of the phylogeny, and implementing statistical tests using those phylogenetically-independent values; see Felsenstein (1985) for more details. To be used, contrasts must be standardized across the phylogenetic tree (Felsenstein 1985), and the regression performed on the independent contrasts must be forced through the origin (Garland et al. 1992). The software CAIC 2.6.7b (Purvis and Rambault 1995) was used to compute the contrasts for NSI and the explanatory variables (below). We tried to find which variables were statistically linked to NSI via multiple regression forced through the origin using the independent contrasts. The environmental variables chosen were:

- Maximum host size (from Whitehead et al. 1986).
- Host abundance (from Whitehead et al. 1986). Abundance was coded in three semi-quantitative classes: 1: rare; 2: intermediate; 3: common.
- Host social behavior (binary variable: gregary or solitary, data from Whitehead et al. 1986 and Caro et al. 1997).

These three variables were considered to be linked to host predictability (see Winemiller and Rose 1992).

- Number of potential hosts (see Poulin 1992), defined as the total number of species in the host clade(s) containing the parasitized host(s). For example, a parasite species using only *Diplodus sargus* possesses 6 potential host species.

As in Poulin (1992), this variable was chosen because it can be supposed that closely related hosts will share genetic and physiological characteristics allowing their colonization by the same parasite species. Poulin (1992) found a significant relationship between host specificity and the number of potential hosts for some monogenean family.

All variables were ln-transformed before calculating the contrasts in order to obtain linearisation and normality of contrasts (Kolmogorov-Smirnov test, a = 5 %). Normality tests were performed using THE R PACKAGE V.4.0.

In addition, the estimation of the phylogenetic inertia by principal coordinate analysis (above) allowed the partitioning of the respective influences of the phylogeny and the environmental variables on the variation of specificity (NSI). The effects of phylogeny and environment may not be independent; these effects may act simultaneously on the response variable (Fig. 3; see Westoby et al. 1995). What we are trying to identify is that portion of the total variance of NSI that may contain a phylogenetic effect related to ecology, which Harvey and Pagel (1991) called "phylogenetic niche conservatism". This includes the shared attributes that related species may have acquired because they tended to occupy similar niches during evolutionary history. For that purpose, three multiple regressions were performed:

1. A regression of NSI on the significant PCs (calculated above).
2. A regression of NSI on the important environmental variables, selected via a backward elimination procedure.
3. A regression of NSI on the significant PCs and the selected environmental variables, without using any further selection procedure.

The coefficient of determination (R^2) was calculated for each multiple regression. This allowed us to partition the variation of NSI between the environmental and phylogenetic components (Fig. 3). Fraction [a] is the purely environmental component, fraction [c] is the purely phylogenetic component, while fraction [b] represents the variation explained by the common part of phylogeny and environment (Harvey and Pagel's phylogenetic niche conservatism; see Desdevises et al submitted(a)). R^2 computed following regression 1 is equal to [a+b], R^2 computed following regression 2 is equal to [b+c], R^2 computed

following regression 3 is equal to [a+b+c]. [a], [b] and [c] are then easily found by subtraction.

Is specificity correlated to taxonomic diversification?

To find out whether or not NSI is linked to taxonomic diversification, we used the software MacroCAIC 0.8.2 (freeware written by P.-M. Agapow, available at http://www.bio.ic.ac.uk/evolve/software/macrocaic/), which is especially designed to find such correlation (see Desdevises et al. 2001). MacroCAIC allows one to use species richness as a variable in a comparative analysis to estimate if traits (such as host specificity in the present study) are associated with high speciation rates represented by clade species richness. Comparison of species richness is made between sister-clades at each node of the phylogeny. Barraclough et al. (1998) consider this approach to be the best to study the potential causes of taxonomic diversification. Species richness cannot be used as any other continuous variable, through independent contrasts, because, in that case, its estimated value at the nodes of the phylogeny is not the average of the values in lower phylogenetic positions, but their sum. No contrasts were calculated for polytomies. Linear regressions were assessed by testing the Pearson correlation coefficient. The tested variable representing species richness is the natural log (ln) of the clade ratio. This is the ratio, for each node of the phylogeny, of the number of species in the sister clade where the estimated value of specificity (at the node) is the lowest, to the number of species in the other sister clade. When this ratio is smaller than one (negative ln), the clade with the lowest NSI contains more species; when the ratio is 1 (null ln), the number of species in each sister clade is the same; and when it is greater than one (positive ln), the clade with more species has the lowest specificity (highest NSI). The analysis is performed between sister clades at each node of the phylogeny. Clade ratios were regressed against standardized contrasts for ln(NSI). If the null hypothesis is false, we should observe a significantly increased diversification with specificity, which in turn produces a negative relationship with NSI.

Is specificity linked to morphological adaptation?

We looked for a link between host size and parasite body length. A positive relationship between parasite and host body sizes may be due to the necessity to develop large attachment organs to large hosts (see Sasal et al. 1999). For instance, more important mechanical constraints in larger fish could lead ectoparasites to develop large attachment organs in order to remain fixed. We tested the hypothesis that parasite body length is related to host size, which should result in a positive relationship between the parasite size (strongly correlated to haptor size in *Lamellodiscus*, $r = 0.875$) and the size of the hosts. We assessed this relationship separately for specialists and generalists. For that purpose, we divided the species into specialists (having a single host) and generalists (having several hosts). We did not use the previously defined classes of specificity (NSI) because the small number of parasites in three of the classes (except the specialists) would have resulted in low statistical power. For generalists, several host are involved, and to use the mean of all hosts sizes could bias the analysis since certain host species are much more parasitized than others. Instead, for generalists, we used the size of the main host species (Table 1), the one where the recorded parasite abundance is the largest. When there are several main host species, the mean size was used. Simple linear regressions were computed on independent contrasts to control for phylogenetic effects. We used maximum sizes for hosts and parasites. Host sizes were taken from Whitehead et al. (1986).

Results

Is specificity a derived condition?

Mapping specificity onto the parasite phylogenetic tree (Fig. 4) does not indicate that there are more specialists among the derived than among the primitive species. From Fig. 4, the specialist condition is suggested to be the ancestral state in genus *Lamellodiscus*. No statistical link can be found between NSI and the number of nodes separating the species from the root of the tree (Fig. 5, $r = 0.06$, $P = 0.800$). These results support the hypothesis that host specificity in *Lamellodiscus* is not a derived condition.

Link between specificity and phylogeny

Multiple regression of NSI against the PCs extracted from the phylogenetic distance matrix found PC1, PC2 and PC5 to be highly correlated to specificity (P = 0.001); PCs are presented in order of decreasing eigenvalues, therefore in decreasing order of the amount of variance of the phylogeny that they represent. These three PCs account for 50.4% of the phylogenetic variance and explain together 72.6% of the variation of NSI ($R^2 = 0.726$). This suggests that specificity is linked to phylogeny, i.e., that specialists, as well as generalists, tend to be grouped in the same clades. This can also be observed in the character mapping (Fig. 4).

Determinants of specificity

Only host size was retained by a backward elimination procedure after multiple regression of NSI on the environmental variables using independent contrasts (r = -0.631, P = 0.005). The simple linear regression of NSI on host size, after removal of the non-significant environmental variables, is shown in Fig. 6. This result suggests that specialists are found on larger hosts.

The variation partitioning results (Fig. 7) are striking: as in the analysis of contrasts, host size was the only environmental variable linked to specificity, but fraction [a], the purely environmental component, explained only 4% of the variation in host specificity, while fraction [c], the purely phylogenetic component (from PC1, PC3, and PC5) explained 45% of this variation, and the fraction common to host size and phylogeny ("phylogenetic niche conservatism") explained 24 % of the variation of NSI.

Is specificity correlated to taxonomic diversification?

The simple linear regression of ln(clade ratio) on ln(NSI) suggests that specificity is not linked to taxonomic diversification in *Lamellodiscus* (Fig. 8, r = 0.331, P = 0.320). To have a low or high mean host specificity, a clade does not have to contain more or fewer species.

Is specificity linked to morphological adaptation?

We found a significant positive correlation between parasite body length and host size in the case of all species (Fig. 9, $r = 0.719$, $P < 0.001$), the specialist species ($r = 0.644$, $P = 0.030$), as well as the generalist species ($r = 0.841$, $P = 0.004$). Similar results were observed without controlling for the phylogeny.

Discussion

Our results show that host specificity in *Lamellodiscus* monogeneans is linked to host size. Specialist *Lamellodiscus* tend to use larger hosts than generalist species. The same kind of relationship was found by Sasal and Morand (1998), Sasal et al. (1999) and Simková et al. (2001). Since large-bodied fish live longer and are usually found at the top of the food chain (Winfield and Nelson 1991; Winemiller and Rose 1992), they can be thought of as being more predictable. This supports the hypothesis that specialization occurred on a predictable resource (Ward 1992). Host size may be selected by specialists for other reasons than predictability, however. Large fish may contain more available niches for parasite specialization than smaller fish (Dogiel et al. 1961; Kuris et al. 1980). In any case, there is no evidence of interspecific competition in monogeneans (Rohde 1979, 1994; Simková et al. 2000). Host abundance could also be seen as an indication of predictability, but it is not statistically linked to specificity. Perhaps this characteristic is more labile in evolutionary time than size, or that the semi-quantitative variable used to account for abundance resulted in a decrease of statistical power leading to a non-significant relationship. Norton and Carpenter (1998) pointed out that more generalists exist when the hosts are unpredictable, and that the key to host specificity is the relative host abundance- i.e., the abundance of a host species relatively to the others. They suggest that a threshold in relative host abundance may explain the appearance of specificity in parasites: generalism is favored under this threshold because the relative abundance of one host species is too low to maintain a parasite population. Thus, this is around the value of this threshold that host abundance could play a role in the specificity of the parasite. It is possible that this hypothetical threshold is reached in our case. The impact of relative host abundance on the determinism of specificity should also be linked to parasite dispersal

abilities (see Reed and Hafner 1997), which should be taken into account in the assessment of this threshold. The number of potential host species is not linked to specificity, contrarily to the observation of Poulin (1992) for *Gyrodactylus* monogenean from Canada. However, the absence a phylogeny did not allow Poulin to control his results for that influence, which may explain the discrepancies with our results. The colonization strategy of *Gyrodactylus* may also be different from that of *Lamellodiscus*. Our result does not implies that the most suitable hosts (if phylogenetically related hosts are considered in this way) are not more colonized by the same *Lamellodiscus* species but, if this colonization is followed by speciation, as suggested by Desdevises et al. (submitted(b)) for *Lamellodiscus* species, no decrease of specificity will result. It is also possible that the potential hosts should be more broadly defined, and not only by phylogenetic proximity, but this would require thorough physiological and ecological studies.

Links between specificity and ecological factors have been encountered in other host-parasite associations. For a plant-phytophagous insect association, Smiley (1978) suggested that the appearance of specialization is due more to ecological factors (like predation or host abundance) than to genetically-controlled compatibility with the host. The appearance of biochemical or metabolic adaptations only comes after this specialization, and it prevents the possibility to colonize other plant species. This implies that this type of compatibility is only a proximate factor to specialization.

We did not find an increase in specificity for derived species. This has also been reported by Thompson (1994) in a review of many studies. This supports the hypothesis that specificity is not an evolutionary dead-end, as proposed by Simpson (1953), and that "there is no intrinsic direction to the evolution of specialization" (Thompson, 1994). The hypothetical specialist ancestral state followed by the appearance of generalism seems to indicate that specialization is not an irreversible condition.

The significant link between specificity and phylogeny suggests that host specificity is under the influence of historically constrained characteristics. Therefore, only some groups of *Lamellodiscus* species, that are phylogenetically related, may be able to develop a specialist or generalist behavior. The existence of such phylogenetic constraints is in

accordance with what can be observed at a deeper taxonomic level (e.g., between classes or phyla) where some groups, like the monogeneans, show marked preferences for a type of host specificity (Sasal et al. 1998). This suggests the appearance of a feature in an ancestral species constraining its descendants into a type of specificity. This hypothetical feature may not be the same in all clades. Specificity in *Lamellodiscus* monogeneans seems then to be determined by a mixture of historical and ecological influences. The variation partitioning results suggest that an important fraction of specificity is controlled by host size and phylogeny. It seems then that related parasites tend to specialize on large hosts. Everything happens as if some *Lamellodiscus* species have acquired through the phylogeny the ability to be generalists (which seems to be the derived state in this genus) and then they became able to use more hosts and not only the largest species. This ability could be related to morphological, physiological or immunological factors.

The absence of link between specificity and taxonomic diversification for *Lamellodiscus* species was previously found at a deeper taxonomic level, for the Diplectanidae (Desdevises et al. 2001). It could be explained by the absence of a higher speciation rate or a higher extinction rate among the specialist species (see Slowinsky and Guyer 1993). The single host that specialist species have may increase the risk of extinction, even if its larger size makes it more predictable. It can also be due to a lack of statistical power, since only ten contrasts were used in the analysis, because of the relatively low number of species considered and the presence of polytomies in the phylogenetic tree. However, even when using a fully-resolved tree (data not shown), there is no statistical link between specificity and diversification. Moreover, the trend observed, even if not significant, would favor the inverse hypothesis, that of an increase in the number of host species with taxonomic diversification.

The significant correlation between parasite body length and host size suggests the existence of a selective pressure exerted by the hosts on the parasites (Poulin 1996). The fact that this link was found with or without controlling for the phylogeny also indicates an absence of strong historical constraints on parasite body size, therefore suggesting an adaptive nature for *Lamellodiscus* body length. In other studies, parasite body size has been found to be positively correlated to host body size, mostly in a context of high host

specificity (Morand et al. 1996). For endoparasite species, Morand and Sorci (1998) hypothesized, and supported by a comparative analysis, that parasite body size can be related to host longevity, which is correlated to host body size. Long-lived hosts would provide more energy and would harbor more long-lived parasites, and consequently larger parasite species (Morand 1996; Morand et al. 1996). However, in the Monopisthocotylean monogeneans, the small size (relative to the host), the probable absence of competition, and the short generation time (Rohde 1982) suggest that resources are not limiting. Morphological adaptation to the host, perhaps for better attachment (see Sasal et al. 1999), is a more plausible explanation. Simková et al. (2001) observed that specialist dactylogyrids monogenans seem to be more tightly adapted to their hosts than generalists, highlighting the influence of adaptive processes for the attachment to the host. This leads to the question: do *Lamellodiscus* parasites use mostly hosts for which their size is compatible, or is their size modified by the host they use the most for some other reason? This highlights the difficulty of disentangling causes and consequences, as pointed out by Futuyma and Moreno (1988). These authors argued that morphological and physiological adaptations may be seen to be consequences rather than causes of specialization, and that the determinants of specificity may be more behavioral. The fact that generalist *Lamellodiscus* species use hosts with a wide range of sizes and that this relationship is significant, for generalist species, only if the size of the main host is used, suggest that this correlation is more likely to be a consequence than a cause of host specificity. This is also supported by the absence of link between parasite body length and phylogeny, which should be expected if such a link existed, because specificity is significantly linked to host size. Tompkins and Clayton (1999) suggested that feather size is a determinant of host specificity of lice parasitizing swiftlets. The same kind of relationship has been suggested by Reed and Hafner (1997) and supported by Morand et al. (2000) for pocket gophers and their chewing lice. In the case of gophers, there is a match between the size of the parasite's attachment organ and barb size.

Experimental studies (e.g., Gemmill et al. 2000) can bring insight on the intrinsic factors limiting the colonization of new hosts by *Lamellodiscus* parasites. The question remains: are those observations more likely to be causes than consequences of host specificity? Such morphological adaptations would intuitively limit further dispersal of

specialist species, and, even if caused by an adaptive process, would also be a determinant of specificity for the descendant species. A study of the polymorphism and genetic variability of attachment organs is needed to reach a better understanding of this mechanism. The genetic variability of hosts should also be taken into account (Secord and Kareiva 1996). The variance of these components of host-parasite interaction may be the important factors determining the potential for colonization on the part of the parasites.

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Literature cited

Adamson, M. L., and J. N. Caira. 1994. Evolutionary factors influencing the nature of parasite specificity. Parasitology 109:S85-S95.

Baer, J. G, ed. 1957. First Symposium on Host Specificity among Parasites of Vertebrates. Paul Attinger, Neuchatel.

Barker, S. C. 1991. Evolution of host-parasite associations among species of lice and rock-wallabies: coevolution? International Journal for Parasitology 21:497-501.

Barraclough, T. G., A. P. Vogler, and P. H. Harvey. Revealing the factors that promote speciation. Philosophical Transactions of the Royal Society of London B 333:241-249.

Begon, M., J. L. Harper, and C. R. Townsend. 1996. Ecology: Individuals, population and communities. 3^{d} ed. Oxford: Blackwell Science.

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, Ecology, and Behavior. A Research Program in Comparative Biology. The University of Chicago Press, London.

Brooks, D. R., and D. A. McLennan. 1993. Parascript. Smithsonian series in Comparative Evolutionary Biology, Washington and London.

Caro, A., C. Combes, and L. Euzet. 1997. What makes a fish a suitable host for Monogenea in the Mediterranean? Journal of Helminthology 71:203-210.

Colwell, R. K. 1986. Population structure and sexual selection for host fidelity in the speciation of hummingbird flower mites. Pages 475-495 in S. Karlin, and E. Nevo, eds. Evolutionary Processes and Theory. New York: Academic Press.

De Meeûs, T., Y. Michalakis, and F. Renaud F. 1998. Santa Rosalia revisited: or why are there so many kind of parasites in "The Garden of Earthly Delights"? Parasitology Today 14:10-13.

Desdevises, Y. 2001. The phylogenetic position of *Furnestinia echeneis* (Monogenea, Diplectanidae) based on molecular data: a case of morphological adaptation? International Journal for Parasitology 31:205-208.

Desdevises, Y., R. Jovelin, O. Jousson, and S. Morand. 2000. Comparison of ribosomal DNA sequences of *Lamellodiscus* spp, (Monogenea, Diplectanidae) parasitizing *Pagellus* (Sparidae, Teleostei) in the north Mediterranean sea: species divergence and coevolutionary interactions. International Journal for Parasitology. 30:741-746.

Desdevises, Y., L. Azouzi, P. Legendre, and S. Morand. Submitted(a). Partitioning the phylogenetically-structured environmental variation. Journal of Evolutionary Biology.

Desdevises, Y., S. Morand, O. Jousson, and P. Legendre. Submitted(b). Coevolution between *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): the study of a complex host-parasite system. Evolution.

Desdevises, Y., S. Morand, and G. Oliver. 2001. Linking specialization to diversification in the Diplectanidae Bychowsky 1957 (Monogenea, Platyhelminthes). Parasitology Research 87:223-230.

Diniz-Filho, J. A. F., C. E. R. de Sant'Ana, and L. M. Bini. 1998. An eigenvector method for estimating phylogenetic inertia. Evolution 52:1247-1262.

Dogiel, V. A., G. K. Petrushevsky, and Y. Polyanski. 1961. Parasitology of Fishes, English translation. Oliver and Boyd, London.

Euzet, L. 1984. Diplectanidae (Monogenea) parasites de poissons des Iles Kerkennah (Tunisie). Archives de l'Institut Pasteur de Tunis 61(4):463-474.

Euzet, L., and C. Combes. 1980. Les problèmes de l'espèce chez les animaux parasites. In C. Boquet, J. Génermont, and M. Lamotte, eds. Les problèmes de l'espèce dans le règne animal. Tome III. Mémoires de la Société Zoologique Française 40:239-285.

Euzet, L., and Combes C. 1998. The selection of habitats among the Monogenea. International Journal for Parasitology 28:1645-1652.

Euzet, L., C. Combes, and A. Caro. 1993. A checklist of Monogenea of Mediterranean fish. Second International Symposium on Monogenea. Montpellier/Sète - 5-8 Juillet 1993.

Euzet, L., and G. Oliver. 1966. Diplectanidae (Monogenea) des Téléostéens de la Méditerranée occidentale. III. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Diplodus* (Sparidae). Annales de Parasitologie Comparée (Paris) 41(6):573-598.

Euzet, L., and G. Oliver. 1967. Diplectanidae (Monogenea) de Téléostéens de la Méditerranée occidentale. IV. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Pagellus* Cuvier, 1829 (Sparidae). Annales de Parasitologie Comparée (Paris) 42(4):407-425.

Farris, J. S. 1970. Methods for computing Wagner trees. Systematic Zoology 19:83-92.

Felsenstein, J. 1985. Phylogenies and the comparative method. The American Naturalist 125:1-15.

Fox, L. R., and P. A. Morrow. Specialization: species property or local phenomenom? Science 211:887-893.

Fry, J. D. 1996. The evolution of host specialization: are trade-offs overrated. The American Naturalist 148:S84-S107.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematic 19:207-233.

Garland, T, Jr, P. H. Harvey, and R. A. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic Biology 41:18-32.

Gemmill, A. W., M. E. Vinet, and A. F. Read. 2000. The evolutionary ecology of host-specificity: experimental studies with *Strongyloides ratti*. Parasitology 120:429-437.

Harvey, P. H. and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford University Press.

Humphery-Smith, I. 1989. The evolution of phylogenetic specificity among parasitic organisms. Parasitology Today 5:385-87.

Huxley, J. 1942. Evolution: The modern synthesis. George Allen & Unwin, London.

Jackson, J. A., R. C. Tinsley, and H. H. Hinkel. 1998. Mutual exclusion of congeneric monogenean species in a space-limited habitat. Parasitology:563-569.

Kearn, G.C. 1994. Evolutionary expansion of the Monogenea. International Journal for Parasitology 24:1227-1271.

Kennedy, C. R. 1975. Ecological Animal Parasitology. Blackwell Scientific Publication, Oxford.

Kitahara, M., and K. Fujii. 1994. Biodiversity and community structure of temperate butterfly species within a gradient of human disturbance: an analysis based on the concept of generalist vs. specialist strategies. Researches on Population Ecology 36:187-199.

Klassen, G. J., and M. Beverley-Burton. 1987. Phylogenetic relationships of *Ligictaluridus* spp. (Monogenea : Ancyrocephalidae) and their Ictalurid (Siluriformes) hosts : and hypothesis. Proceedings of the Helminthological Society of Washington 54:84-90.

Klassen, G. J., and M. Beverley-Burton. 1988. North American Fresh Water Ancyrocephalids (Monogenea) with articulating haptoral bars: host-parasite coevolution. Systematic Zoology 37:179-189.

Kouider El Ouahed-Amine, F. 1998. Contribution à l'étude des Monogènes parasites de Poissons Sparidae (Téléostéens) du littoral algérois, approche taxonomique et

écologique. Thèse. Université des Sciences et de la Technologie Houari Boumediene, Alger.

Kuris, A. M., A. R. Blaustein, and J. J. Alio. 1980. Hosts as islands. The American Naturalist 116:570-586.

Ludwig, H. W. 1982. Host specificity in anoplura and coevolution of anoplura and mammalia. Mémoires du Muséum National d'Histoire Naturelle de Paris 123:145-152.

Lymbery, A. J. 1989. Host specificity, host range and host preference. Parasitology Today 5:298.

Morand, S. 1996. Biodiversity of parasites in relation with their life cycle. Pages 243-260 in M. Hochberg, J. Clobert, and R. Barbault, eds. The Genesis and Maintenance of Biological Diversity. Oxford University Press.

Morand, S., P. Legendre, and S. L. Gardner and J.-P. Hugot. 1996. Body size evolution of oxyurid parasites: the role of hosts. Oecologia 107:274-281.

Morand, S., R. D. M. Page, M. S. Hafner, and D. L. Reed. 2000. Comparative evidence of host-parasite coadaptation: body-size relationships in Pocket Gophers and their Chewing Lice. Biological Journal of the Linnean Society 70:239-249.

Morand, S., and G. Sorci. 1998. Determinants of life-history evolution in nematodes. Parasitology Today 14:193-196.

Noble, E. R., G. A. Noble, G. A. Schad, and A. J. MacInnes. 1989. Parasitology. The Biology of Animal Parasites. 6th Edition. Lea & Febiger, Philadelphia.

Norton, D. A., and M. A. Carpenter. 1998. Mistletoe as parasites: host specificity and speciation. Trends in Ecology and Evolution 13:101-105.

Oliver, G. 1968. Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. II. Lamellodiscinae nov. sub. fam. Vie et Milieu 19:95-138.

Oliver, G. 1969a. Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. II. Lamellodiscinae nov. sub. fam. Vie Milieu 20(1-A):43-72.

Oliver, G. 1969b. Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. III. Biologie. Vie Milieu 20(2-A):397-420.

Oliver, G. 1973. *Lamellodiscus obeliae* n. sp. une nouvelle espèce de Diplectanidae (Monogenea, Monopisthocotylea) parasite de *Pagellus centrodontus* (Delaroche, 1809) (Pisces, Sparidae). Zeitschrift für Parasitenkunde 41:103-108.

Oliver, G. 1974. Nouveaux aspects du parasitisme des Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea) chez les Téléostéens Perciformes des côtes de France. Comptes-Rendus de l'Académie des Sciences de Paris 279:803-805.

Oliver, G. 1987. Les Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea, Dactylogyridea). Systématique. Biologie. Ontogénie. Écologie. Essai de phylogénèse. Thèse d'état. Université des Sciences et Techniques du Languedoc.

Paterson, A. M., and J. B. Banks. 2001. Analytical approaches to measuring cospeciation of host and parasites: through a glass, darkly. International Journal for Parasitology 31:1012-1022.

Poulin, R. 1992. Determinants of host specificity in parasites of freshwater fishes. International Journal for Parasitology 22:753-58.

Poulin, R. 1996. The evolution of body size in the Monogenea: The role of host size and latitude. Canadian Journal of Zoology 74:726-32.

Poulin, R. 1997. Parasites faunas of freshwater fish: the relationship between richness and the specificity of parasites. International Journal for Parasitology 27:1091-1098.

Price, P. W. 1980. Evolutionary Biology of Parasites. Princeton University Press, Princeton.

Purvis, A., and A. Rambaut. 1995. Comparative analysis by independent contrasts (CAIC): and Apple Macintosh application for analysis comparative data. Computer Applied Biosciences 11:247-251.

Reed, D. L., and Hafner M. S. 1997. Host specificity of chewing lice on pocket gophers: a potential mechanism for cospeciation. Journal of Mammalogy 78:655-660.

Rohde, K. 1978. Latitudinal differences in host-specificity of marine Monogenea and Digenea. Marine Biology 47:125-134.

Rohde, K. 1979. A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. The American Naturalist. 114:648-671.

Rohde, K. 1982. Ecology of Marine Parasites. University of Queensland Press. St Lucia.

Rohde, K. 1994. Niche restriction in parasites: proximate and ultimate causes. Parasitology 109:S69-S84.

Sasal, P., and S. Morand. 1998. Comparative analysis: a tool for studying Monogenean ecology and evolution. International Journal for Parasitology 28:1637-1644.

Sasal, P., Y. Desdevises, and S. Morand. 1998. Host-specialization and species diversity in fish parasites: phylogenetic conservatism? Ecography 21:639-645.

Sasal, P., S. Trouvé, C. Müller-Graf, and S. Morand. 1999 Specificity and host predictability: a comparative analysis among monogenean parasites of fish. Journal of Animal Ecology 68:437-444.

Secord, D., and P. Kareiva. 1996. Perils and pitfalls in the host specificity paradigm. Bioscience 46:448- 453.

Simková, A., Y. Desdevises, M. Gelnar, and S. Morand. 2000. Coexistence of nine gill ectoparasites (*Dactylogyrus* : Monogenea) parasitising the roach (*Rutilus rutilus* L.): history and present ecology. International Journal for Parasitology 30:1077-1088.

Simková, A., Y. Desdevises, M. Gelnar, and S. Morand. 2001. Morphometric correlates of host specificity in *Dactylogyrus* species (Monogenea) parasites of European Cyprinid fish. Parasitology 123:169-177.

Simpson, G. G. 1953. The major features of evolution. Columbia University Press, New York.

Slowinski, J. B., and Guyer C. 1993. Testing whether certain traits have caused amplified diversification: an improved method based on a model of random speciation and extinction. The American Naturalist 142(6):1019-1024.

Smiley, J. 1978. Plant chemistry and the evolution of host specificity: new evidence from *Heliconius* and *Passiflora*. Science 201(25):745-747.

Thompson, J. D. 1994. The coevolutionary process. The University of Chicago Press. Chicago and London.

Timms, R., and A. F. Read. 1999. What makes a specialist special? Trends in Ecology and Evolution 14:333-334.

Tinsley, R. C., and J. A. Jackson. 1998. Correlation of parasite speciation and specificity with host evolutionary relationships. International Journal for Parasitology 28:1573-1582.

Tompkins, D. M., and D. H. Clayton. 1999. Host resource govern the specificity of swiftlet lice: size matters. Journal of Animal Ecology 68:489-500.

Ward, S.A. 1992. Assessing functional explanations of host specificity. The American Naturalist 139:883-891.

Westoby, M., M. R. Leishmann, and J. M. Lord. 1995. On misinterpreting the 'phylogenetic correction'. Journal of Ecology 83:531-534.

Whitehead, P. J. P., M. L. Bauchot, J. C. Hureau, J. Nielsen, and E. Tortonese. 1986. Checklist of the fishes of the North-eastern Atlantic and of the Mediterranean. Paris, UNESCO, Vol. III.

Wilson, D. S., and J. Yoshimura. 1994. On the coexistence of specialists and generalists. The American Naturalist 144:692-707.

Winfield, I. J., and J. S. Nelson. 1991. Cyprinid fishes. Systematics, biology and exploitation. Chapman & Hall. Fish and Fisheries Series 3, London.

Winemiller, K. O., and K. A. Rose. 1992. Patterns of life history diversification in North Americam Fishes: implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences 49:2196-2218.

Winfield, I. J., and J. S. Nelson. 1991. Cyprinid fishes. Systematics, biology and exploitation. Chapman & Hall. Fish and Fisheries Series 3, London.

Tables

Table 1: Data on *Lamellodiscus* species (including *Furnestinia echeneis*): parasite maximum size (in µm), host species, host maximum size (in mm), and specificity (classes). The main hosts for generalist parasites are in bold.

Parasite species	Parasite size	Host	Host size	Specificity
F. echeneis	**1100**	***Sparus aurata***	**700**	**1**
L. baeri	1000	*Pagrus pagrus*	750	1
L. bidens	1020	*Diplodus puntazzo*	600	1
L. coronatus	1000	*Diplodus annularis*	240	3
		Diplodus cervinus	550	
		Diplodus sargus	400	
L. drummondi	420	*Pagellus acarne*	360	1
L. elegans	520	*Diplodus annularis*	240	4
		Diplodus sargus	400	
		Diplodus vulgaris	450	
		Oblada melanura	300	
		Spondyliosoma cantharus	600	
L. ergensi	600	*Diplodus annularis*	240	3
		Diplodus puntazzo	600	
		Diplodus sargus	400	
		Diplodus vulgaris	450	
L. erythrini	580	*Pagellus erythrinus*	600	1
L. fraternus	430	***Diplodus annularis***	240	2
		Diplodus vulgaris	450	
L. furcosus	670	*Diplodus annularis*	240	3
		Diplodus sargus	400	
L. gracilis	700	*Diplodus annularis*	240	3
		Diplodus sargus	400	
		Oblada melanura	300	

L. hilii	1050	*Diplodus puntazzo*	600	1
L. ignoratus	640	*Diplodus annularis*	240	4
		Diplodus puntazzo	600	
		Diplodus sargus	400	
		Diplodus vulgaris	450	
		Lithognathus mormyrus	550	
		Sarpa salpa	460	
L. impervius	550	*Diplodus puntazzo*	600	1
L. knoepffleri	730	***Spondyliosoma cantharus***	600	4
		Spicara maena	250	
		Spicara smaris	200	
L. mirandus	800	*Diplodus sargus*	400	1
L. mormyri	480	*Lithognathus mormyrus*	600	1
L. parisi	550	*Sarpa salpa*	460	1
L. verberis	500	*Lithognathus mormyrus*	600	1
L. virgula	470	***Pagellus acarne***	360	2
		Pagellus bogaraveo	700	

Figure legends

Figure 1: Host phylogenetic tree from Desdevises et al. (submitted(b)). Host clades are numbered 1 through 8.

Figure 2: Parasite phylogenetic tree from Desdevises et al. (submitted(b)).

Figure 3: Variation partitioning of phylogenetic and ecological influences, for a dependent variable represented by the thick horizontal line.

Figure 4: Mapping of specificity (NSI, 4 classes) onto the parasite phylogenetic tree.

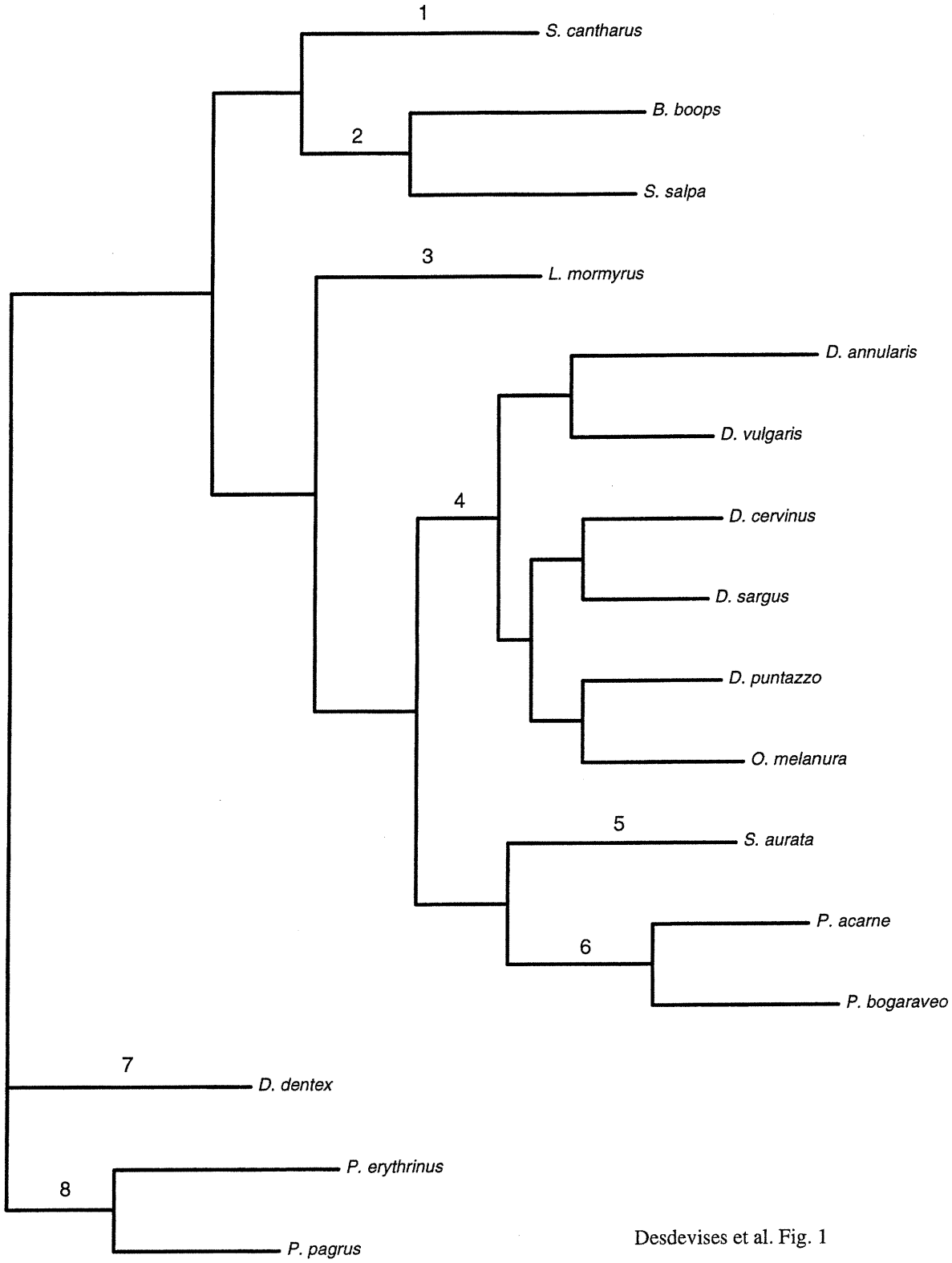
Figure 5: Dispersion diagram of host specificity against the number of nodes from the root of the tree for each species.

Figure 6: Simple regression of independent contrasts on host size (selected by a backward elimination procedure) against independent contrasts on specificity.

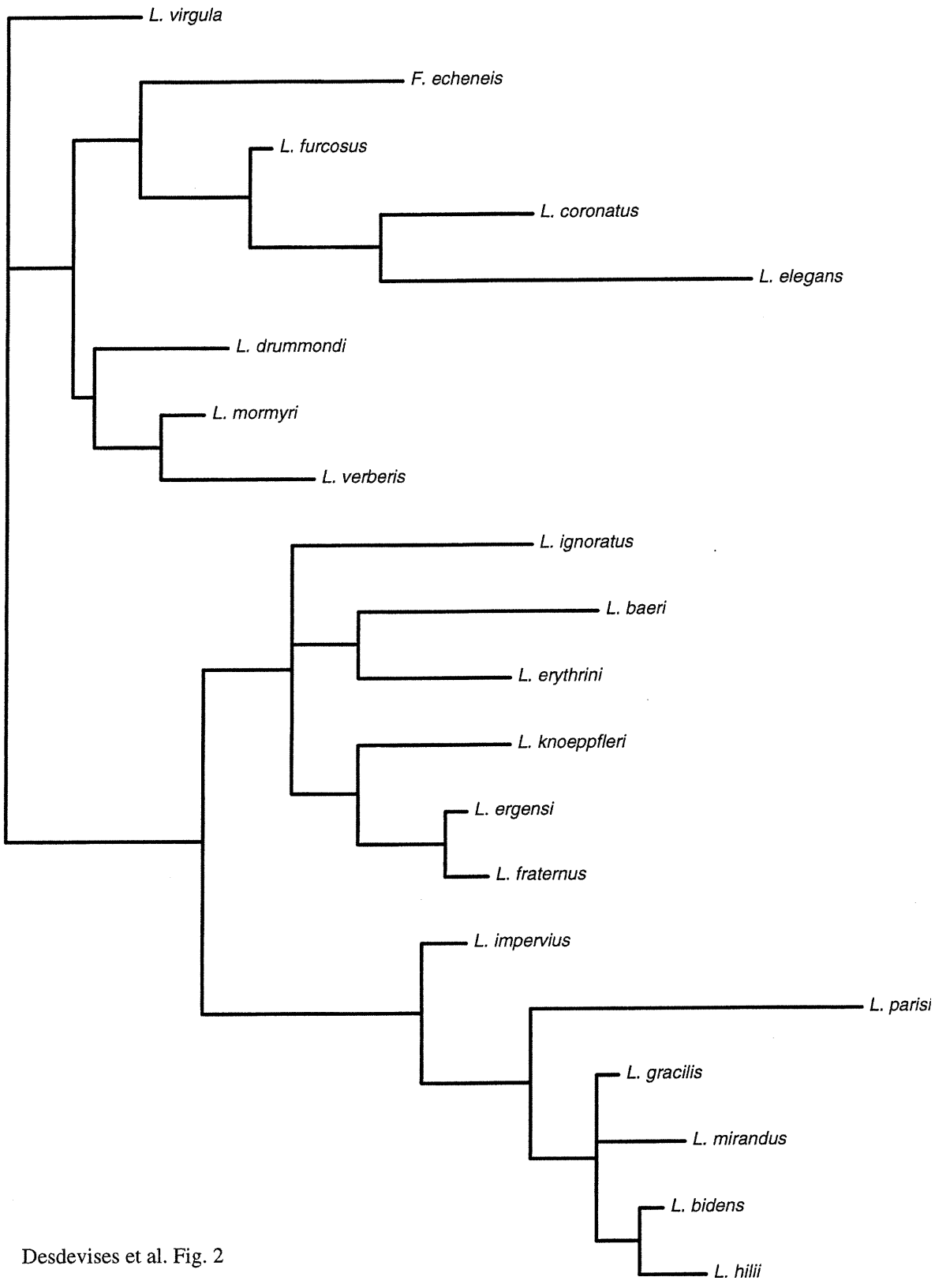
Figure 7: Variation partitioning of host specificity by host size and phylogenetic constraints (PC1, PC2 and PC5). [a] represents the variation of host specificity due to host size alone, [b] is the variation due to the common influence of host size and phylogeny, [c] represents the variation strictly due to phylogeny, and [d] is the unexplained part of the variation.

Figure 8: Dispersion diagram of independent contrasts of ln(clade ratio) against independent contrasts on host size.

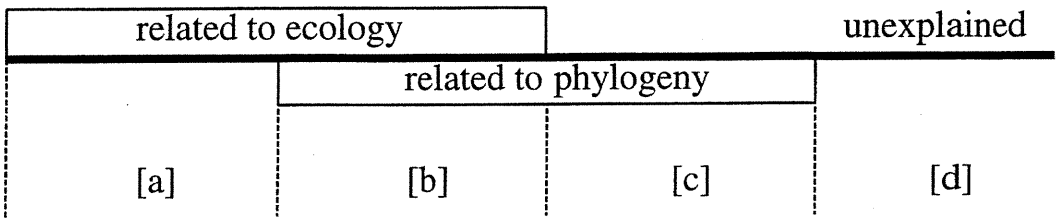
Figure 9: Simple linear regression of independent contrasts of parasite body size against independent contrasts on host size



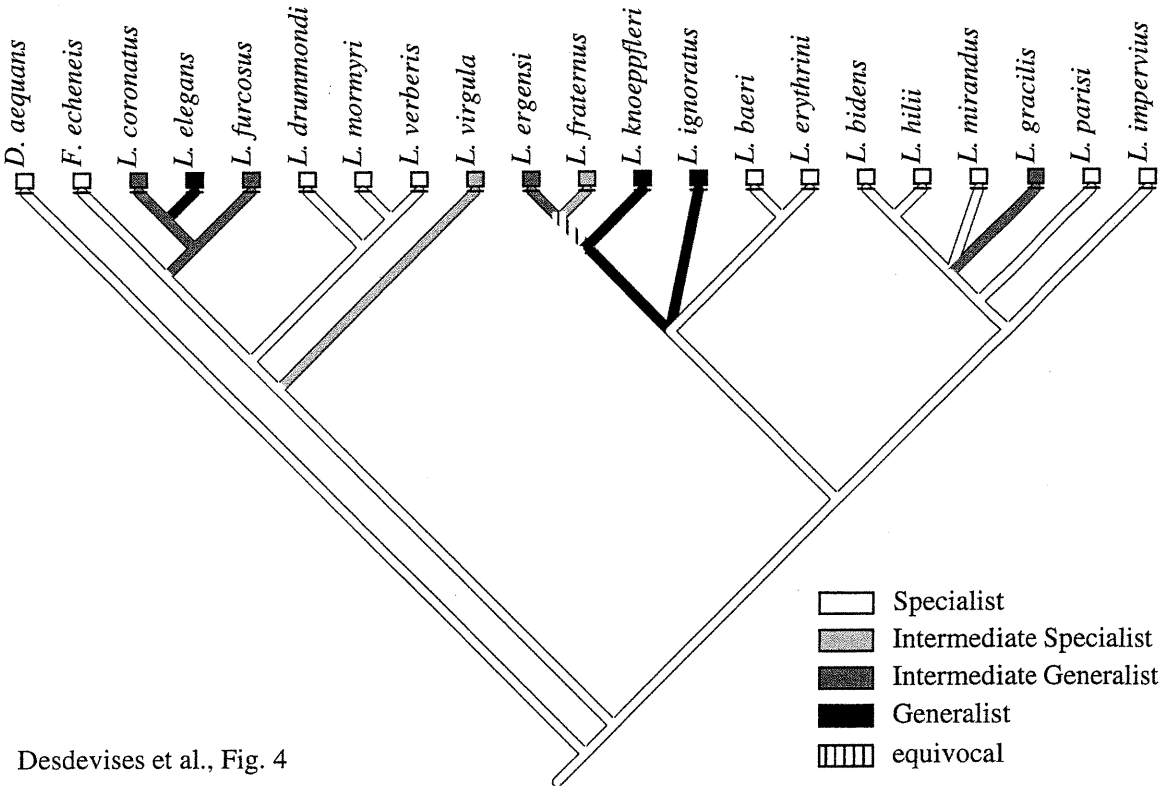
Desdevises et al. Fig. 1



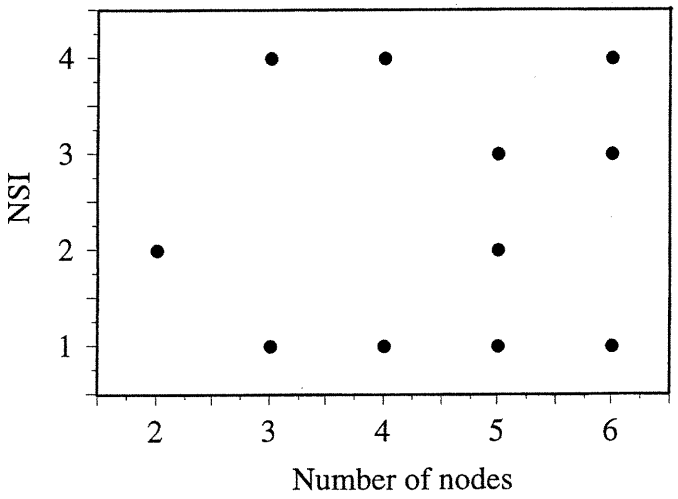
Desdevises et al. Fig. 2



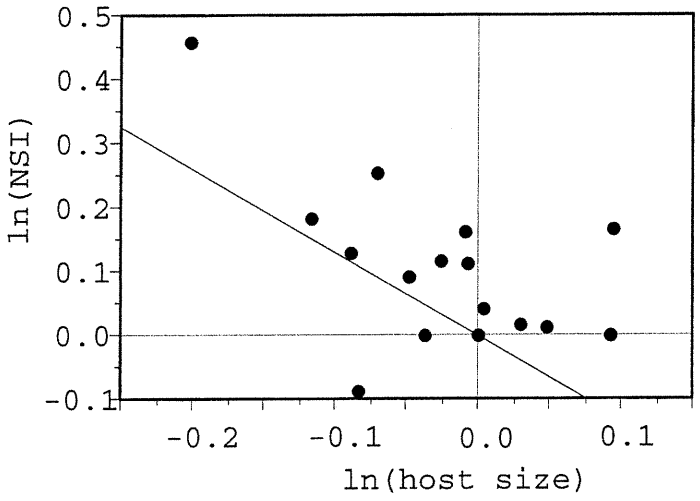
Desdevises et al. Fig. 3



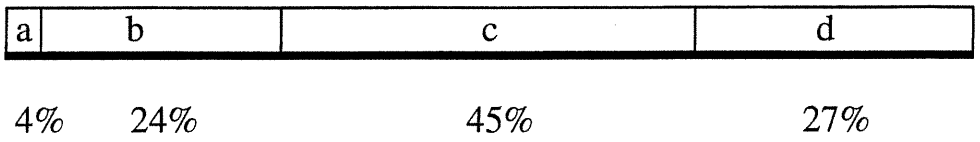
Desdevises et al., Fig. 4



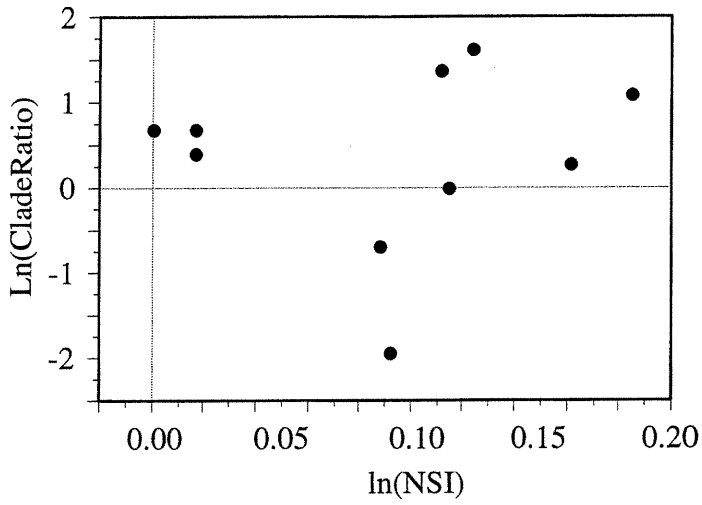
Desdevises et al. Fig. 5



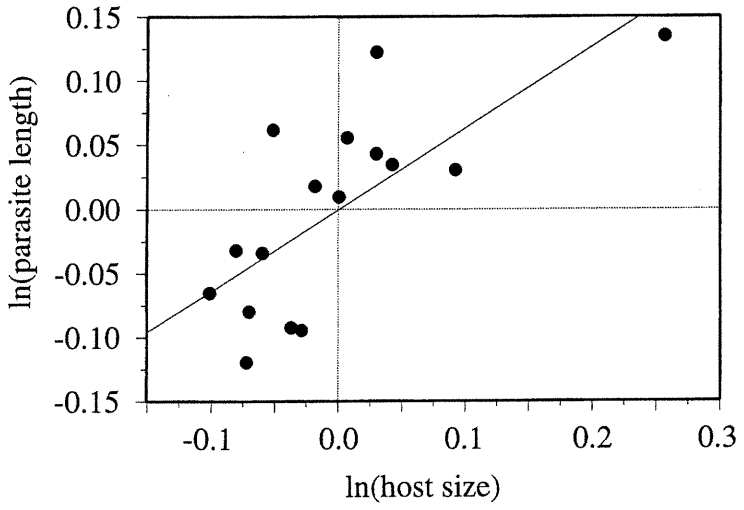
Desdevises et al. Fig. 6



Desdevises et al., Fig. 7



Desdevises et al. Fig. 8



Desdevises et al. Fig. 9

IV. SYNTHÈSE

A. Principaux résultats

Les résultats détaillés, ainsi que les précisions concernant la méthodologie pour chaque analyse, se trouvent dans les publications (Chapitre III). Ici, je ne donne qu'un résumé des principaux résultats, ainsi que quelques précisions ou résultats qui ne sont pas dans les articles.

1. Lien entre spécificité et diversification taxonomique (Article 1, 2, 6)

Au niveau des principaux groupes de parasites (Monogènes, Digènes, Cestodes, Nématodes, Copépodes, Acanthocéphales), il y a un lien significatif entre le nombre d'espèces par groupe et le nombre moyen d'hôtes par espèce (Figure IV.1), donc une corrélation entre spécificité et diversification taxonomique. En ce qui concerne cette même relation au niveau des genres dans la famille des Diplectanidae, aucune relation significative n'a pu être mise en évidence (Fig. IV.2). Le même résultat a été observé entre les espèces de *Lamellodiscus* (Figure IV.3). Ainsi, la relation entre spécificité et diversification taxonomique n'a pu être observée qu'à un niveau taxonomique profond (i.e, classes ou embranchements).

2. Phylogénie des Diplectanidae (Article 2)

L'analyse de la matrice de caractères morphologiques par parcimonie a produit 64 arbres également parcimonieux (indice de cohérence, IC = 0.65). Ce nombre a été réduit à quatre après repondération des caractères par la valeur maximale de l'indice de rétention (Figure IV.4, IC = 0.81). L'arbre 3 (Figure IV.4) étant également le consensus strict de ces quatre arbres, il a été choisi comme hypothèse phylogénétique des relations entre les genres de Diplectanidae. Cet arbre est conservatif et montre notamment que les genres *Lamellodiscus* et *Furnestinia* sont des groupes-frères, dans un clade non résolu comportant également le genre *Telegamatrix*. Les relations entre les genres composant les Lamellodiscinae ne sont pas supportées par de fortes valeurs de bootstrap, mais cela peut être dû au nombre relativement faible de caractères utilisés. Nous choisirons *Furnestinia echeneis*, la seule espèce du genre *Furnestinia*, et *Diplectanum aequans*, de la sous-famille des Diplectaninae, comme extra-groupes pour enraciner la phylogénie des *Lamellodiscus*.

Furnestinia echeneis s'avérera faire probablement partie du genre *Lamellodiscus* (voir plus loin) ; par conséquent, seul *Diplectanum aequans* sera utilisé comme extra-groupe.

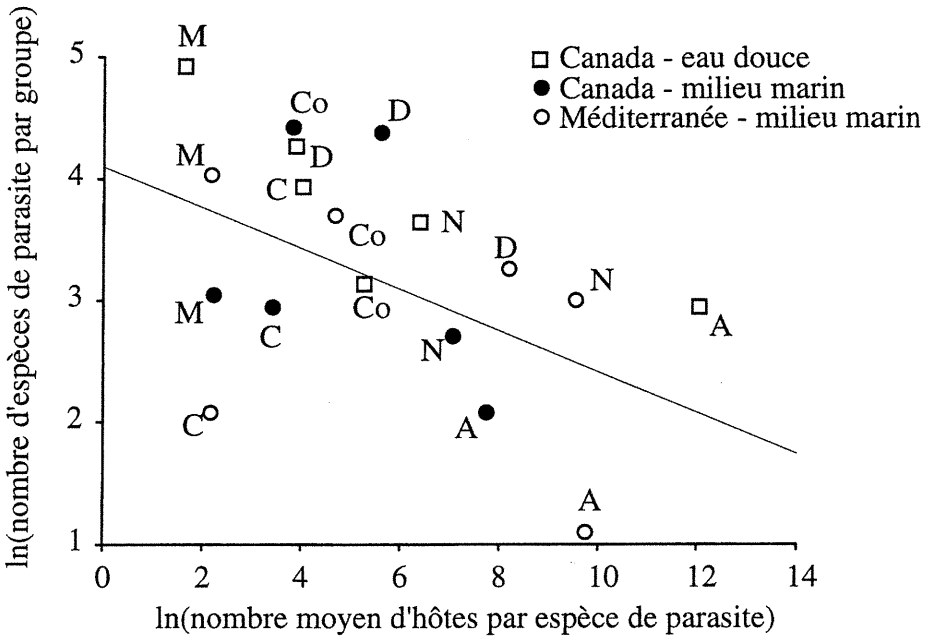


Figure IV.1 : Lien entre la spécificité (nombre d'hôtes) et la diversification taxonomique (nombre d'espèces par groupe) pour les principaux groupes de parasites (r = 0.510, P = 0.027).

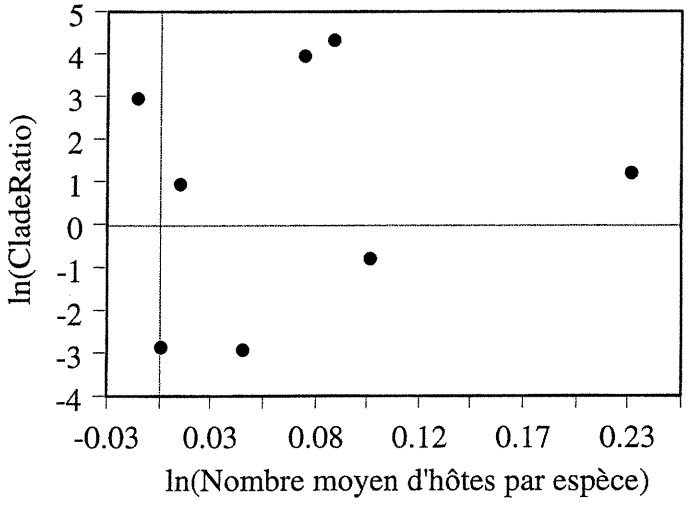


Figure IV.2 : Lien entre la spécificité (nombre d'hôtes) et la diversification taxonomique chez les Diplectanidae (r = 0.321, P = 0.400).

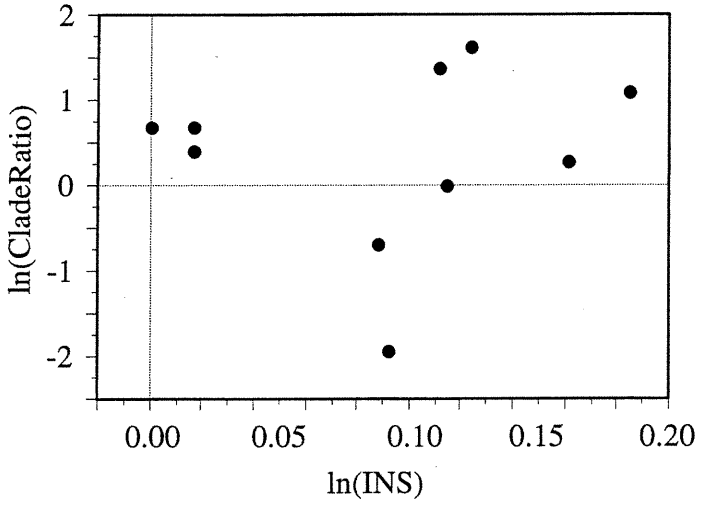


Figure IV.3 : Lien entre la spécificité (INS) la diversification taxonomique chez les *Lamellodiscus* (r = 0.331, P = 0.320).

3. Phylogénie des *Lamellodiscus* (Articles 3, 4, 5)

L'analyse du 18S et de l'ITS1 révèle que l'ITS1 ne peut s'aligner entre la plupart des espèces. Cela plaide en faveur d'une évolution rapide de ce groupe, ou encore de son âge important. Les espèces *Lamellodiscus virgula* et *L. obeliae* possèdent une séquence pratiquement identique pour leur ITS1 (Tableau IV.1). Cela, comparé aux divergences habituelles rencontrées entre les espèces du genre *Lamellodiscus* (voir la comparaison avec *L. drummondi* et *L. erythrini* dans le Tableau IV.1), nous a conduit à considérer ces deux espèces comme une seule espèce, *L. virgula* (la première décrite).

Plusieurs espèces nouvelles de *Lamellodiscus* ont été proposées dans les thèses de doctorat de San Filippo (1978) et Kouider El Ouahed-Amine (1998). Sur la base des divergences des séquences d'ITS1 et de 18S (données non montrées), nous ne considérons pas ces individus comme de nouvelles espèces, mais plutôt comme des variations morphologiques d'espèces déjà existantes. Par ailleurs, l'analyse phylogénétique basée sur le 18S suggère que *Furnestinia echeneis* se place à l'intérieur du clade formé par les *Lamellodiscus* (voir plus loin). Cela suggère que cette espèce devrait être transférée au genre *Lamellodiscus*. Dans la suite de cette discussion, *F. echeneis* sera considéré comme

un *Lamellodiscus*. Les espèces sur lesquelles portent ce travail sont donc celles officiellement décrites dans la littérature, à l'exception de *L. obeliae*, et auxquelles s'ajoute *F. echeneis*.

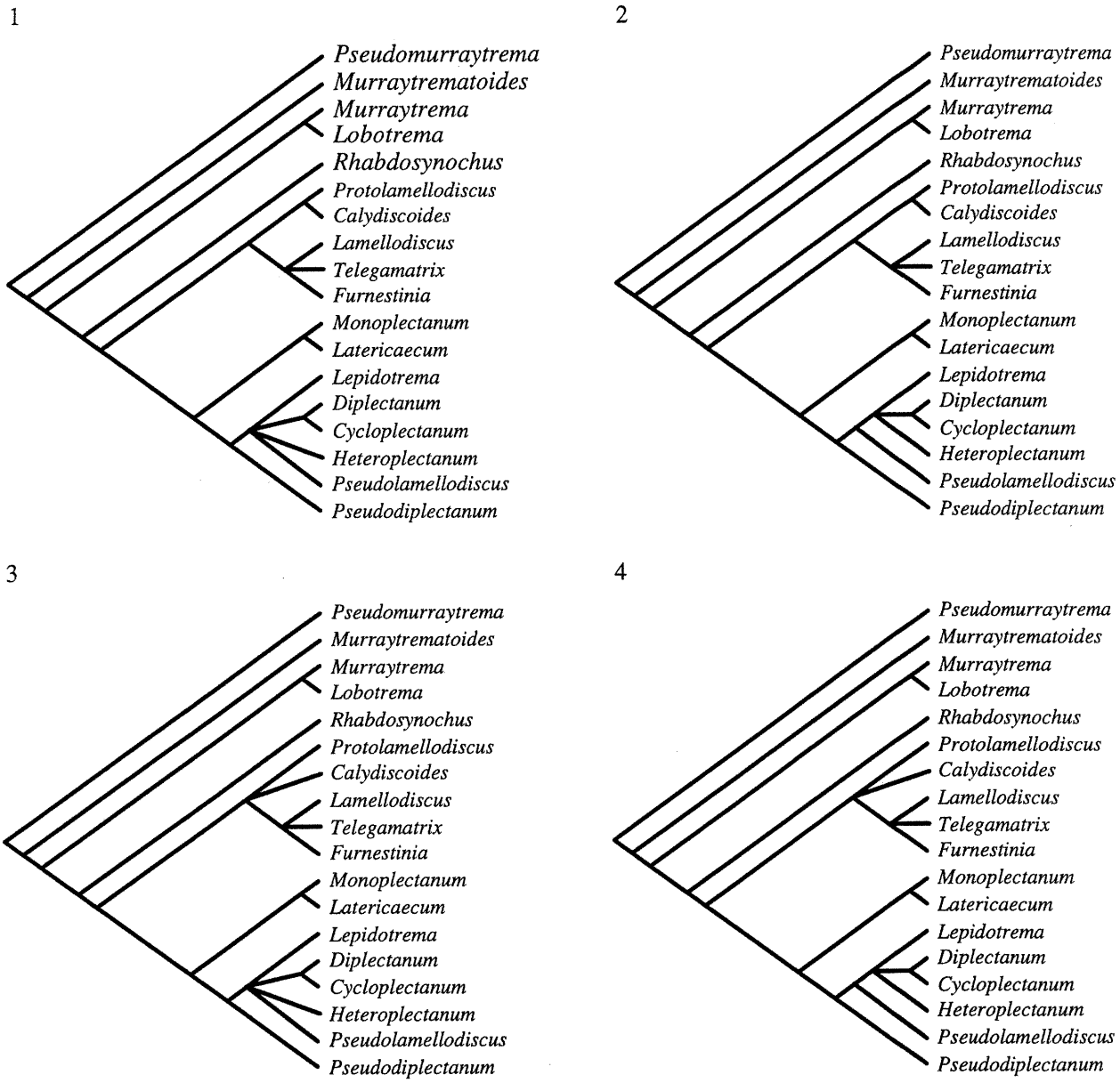


Figure IV.4 : Arbres phylogénétiques des Diplectanidae obtenus par parcimonie après repondération des caractères.

Tableau IV.1 : Longueur de l'ITS1 (entre parenthèses après le nom de l'espèce) et distances entre espèces (en %) pour les séquences de l'ITS1 (premier chiffre) et de l'ADNr 18S (second chiffre) pour les quatre espèces de *Lamellodiscus* parasites de *Pagellus* spp. NA : non alignable.

Espèces (longueur de l'ITS1 en pb)	*L. drummondi*	*L. virgula*	*L. obeliae*
L. erythrini (397)	NA/6.54	NA/5.42	NA/5.42
L. drummondi (469)	0	NA/3.55	NA/3.55
L. virgula (370)	-	0	0.27/0
L. obeliae (371)	-	-	0

Les divergences interspécifique basées sur le 18S ne sont pas très importantes (Tableau IV.2), mais ont néanmoins permis de proposer des hypothèses phylogénétiques basées sur plusieurs méthodes de reconstruction. Les fréquences des bases pour les espèces de *Lamellodiscus* se trouvent au Tableau IV.3. Elles ne présentent pas de différences significatives entre les taxa.

L'analyse par parcimonie a conduit à cinq arbres également parcimonieux (Figure IV.5 ; IC = 0.632). Ils ont une structure assez similaire à celle des arbres obtenus par la méthode NJ ("neighbour-joining") (Figure IV.6) et maximum de vraisemblance (Figure IV.7). Ces arbres sont soutenus par la morphologie des *Lamellodiscus* (voir l'optimisation des caractères par parcimonie à la Figure IV.8) : les morphologies de même type sont groupées dans la phylogénie, et cela quel que soit l'arbre choisi parmi tous ceux qui ont été obtenus.

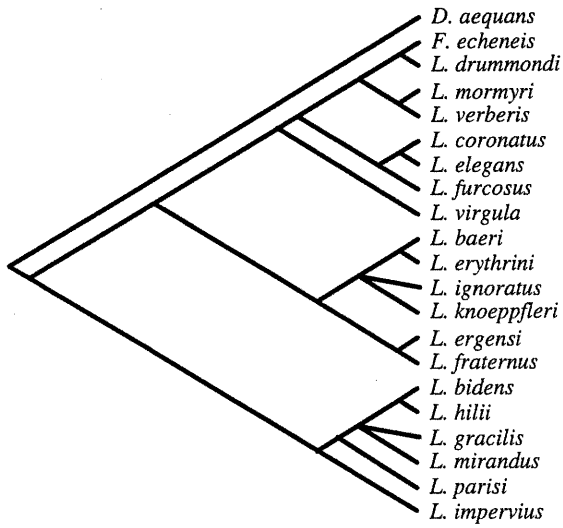
Tableau IV.2 : Pourcentage de différences entre les 18S chez les *Lamellodiscus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1- *F. echeneis*	0.00																			
2- *L. baeri*	7.44	0.00																		
3-*L. bidens*	6.67	6.17	0.00																	
4-*L. coronatus*	5.71	6.69	7.62	0.00																
5-*L. drummondi*	3.43	7.62	6.95	5.33	0.00															
6-*L. elegans*	5.71	7.64	7.81	4.57	5.52	0.00														
7-*L. ergensi*	6.48	4.20	5.14	5.71	5.52	6.67	0.00													
8-*L. erythrini*	6.48	3.44	5.52	6.29	6.29	7.48	3.43	0.00												
9-*L. fraternus*	5.95	3.63	4.95	5.14	4.95	6.95	0.57	2.86	0.00											
10-*L. furcosus*	3.43	6.11	5.95	2.67	3.48	4.57	4.57	5.33	4.00	0.00										
11-*L. gracilis*	6.29	5.73	0.76	7.24	5.71	7.81	4.76	5.14	4.57	5.52	0.00									
12-*L. hilii*	6.67	6.30	0.76	7.81	6.29	7.62	5.33	5.71	5.14	6.95	1.14	0.00								
13-*L. ignoratus*	6.29	4.58	4.95	6.86	6.95	7.62	3.62	3.62	3.48	5.14	4.95	5.14	0.00							
14-*L. impervius*	5.52	4.96	2.48	6.95	4.95	6.86	4.00	4.38	3.81	4.19	2.95	2.67	4.57	0.00						
15-*L. knoeppfleri*	6.87	4.58	5.92	7.26	6.30	7.83	2.29	3.44	2.48	5.16	5.54	6.19	4.10	4.77	0.00					
16-*L. mirandus*	6.48	5.73	1.33	7.43	5.95	7.24	4.95	5.33	4.76	5.71	0.95	1.71	5.52	2.67	5.73	0.00				
17-*L. mormyri*	3.24	5.53	5.73	4.40	2.99	4.58	4.39	5.16	4.20	2.13	5.35	5.92	5.35	4.58	5.15	5.16	0.00			
18-*L. parisi*	7.81	8.21	4.00	9.33	7.43	9.14	6.48	7.62	6.67	7.24	3.62	4.00	7.43	4.19	6.87	4.19	7.66	0.00		
19-*L. verberis*	4.20	6.49	6.49	5.73	3.53	6.30	5.53	5.92	5.35	3.44	6.18	6.68	6.49	5.35	6.17	5.92	1.72	8.21	0.00	
20-*L. virgula*	4.39	5.92	6.18	4.78	3.53	4.97	4.20	4.97	3.82	2.87	5.73	5.92	5.35	4.96	5.34	5.92	2.29	7.26	3.82	0.00

Tableau IV.3 : Fréquences de bases du 18S chez les *Lamellodiscus*.

	A	C	G	T	Nombre de sites
D. aequans	0.248	0.230	0.272	0.250	525
F. echeneis	0.259	0.217	0.267	0.257	525
L. baeri	0.260	0.212	0.267	0.261	524
L. bidens	0.251	0.219	0.274	0.255	525
L. coronatus	0.248	0.215	0.265	0.272	525
L. drummondi	0.251	0.215	0.272	0.261	525
L. elegans	0.250	0.217	0.272	0.261	525
L. ergensi	0.263	0.217	0.267	0.253	525
L. erythrini	0.257	0.208	0.269	0.267	525
L. fraternus	0.261	0.213	0.269	0.257	525
L. furcosus	0.253	0.211	0.269	0.267	525
L. gracilis	0.251	0.215	0.274	0.259	525
L. hilii	0.250	0.215	0.274	0.261	525
L. ignoratus	0.250	0.211	0.272	0.267	525
L. impervius	0.257	0.213	0.270	0.259	525
L. knoeppfleri	0.263	0.208	0.265	0.263	524
L. mirandus	0.253	0.211	0.272	0.263	525
L. mormyri	0.252	0.218	0.273	0.258	524
L. parisi	0.257	0.211	0.269	0.263	525
L. verberis	0.250	0.219	0.273	0.258	524
L. virgula	0.258	0.218	0.267	0.258	524
Moyenne	0.254	0.215	0.270	0.260	525

Figure IV.5 : Arbres phylogénétiques des *Lamellodiscus* obtenus par parcimonie (page suivante).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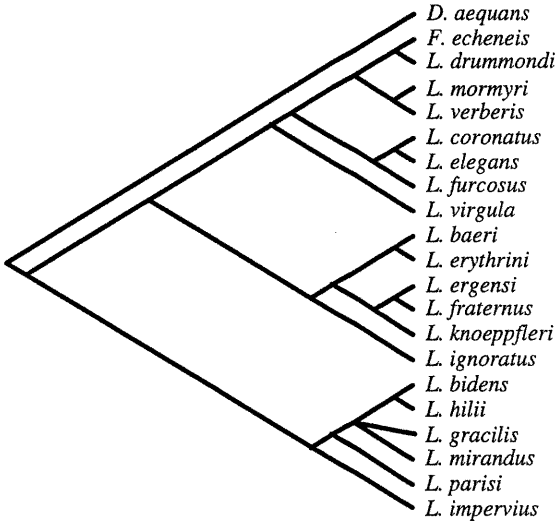
L. drummondi
L. mormyri
L. verberis
L. coronatus
L. elegans
L. furcosus
L. virgula
L. baeri
L. erythrini
L. ignoratus
L. knoeppfleri
L. ergensi
L. fraternus
L. bidens
L. hilii
L. gracilis
L. mirandus
L. parisi
L. impervius


D. aequans
F. echeneis
L. drummondi
L. mormyri
L. verberis
L. coronatus
L. elegans
L. furcosus
L. virgula
L. baeri
L. erythrini
L. ergensi
L. fraternus
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L. ignoratus
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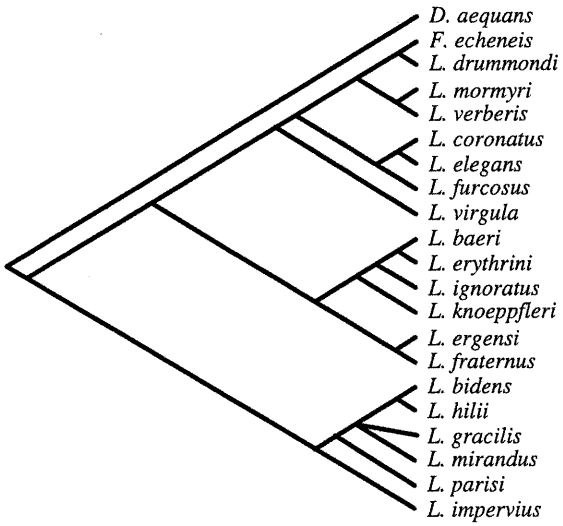
L. hilii
L. gracilis
L. mirandus
L. parisi
L. impervius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L. coronatus
L. elegans
L. furcosus
L. virgula
L. baeri
L. erythrini
L. ignoratus
L. knoeppfleri
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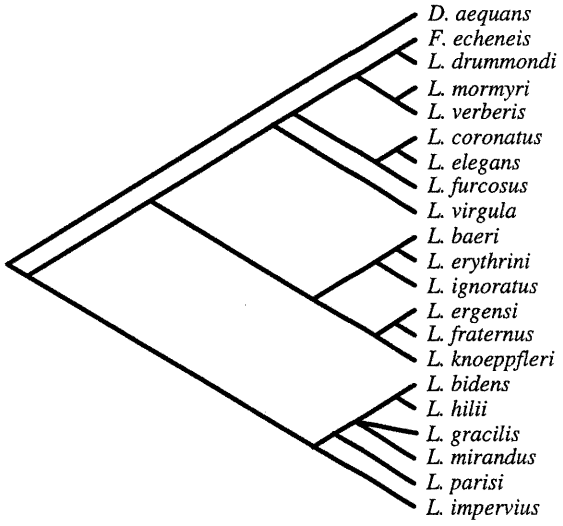
L. gracilis
L. mirandus
L. parisi
L. impervius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L. coronatus
L. elegans
L. furcosus
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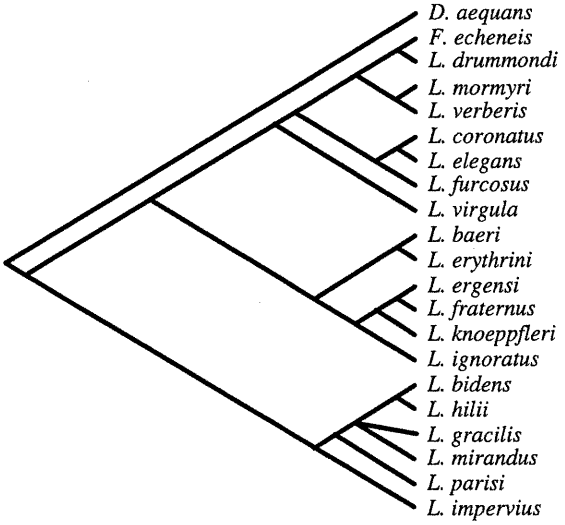
L. baeri
L. erythrini
L. ignoratus
L. ergensi
L. fraternus
L. knoeppfleri
L. bidens
L. hilii
L. gracilis
L. mirandus
L. parisi
L. impervius


D. aequans
F. echeneis
L. drummondi
L. mormyri
L. verberis
L. coronatus
L. elegans
L. furcosus
L. virgula
L. baeri
L. erythrini
L. ergensi
L. fraternus
L. knoeppfleri
L. ignoratus
L. bidens
L. hilii
L. gracilis
L. mirandus
L. parisi
L. impervius

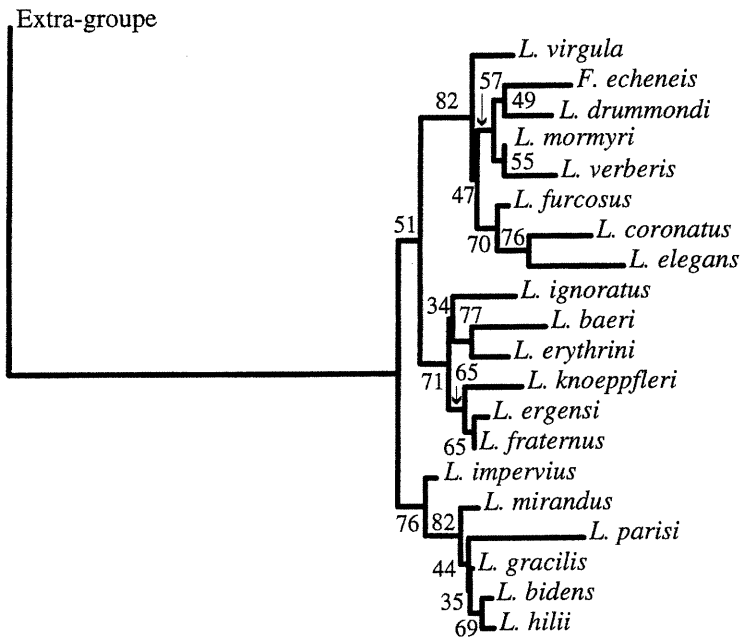


Figure IV.6 : Arbre phylogénétique des *Lamellodiscus* obtenus par "neighbour-joining". Les nombres sont les valeurs de bootstrap, en pourcentage (1000 réplicats).

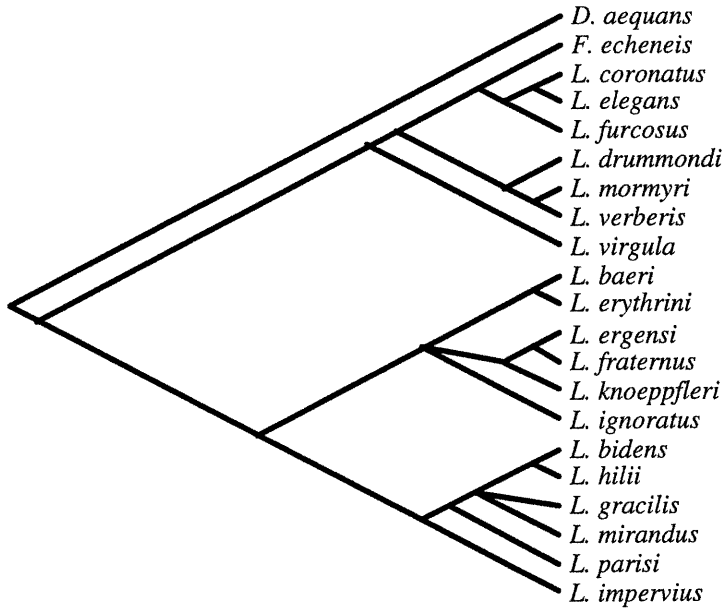


Figure IV.7 : Arbre phylogénétique des *Lamellodiscus* obtenu par maximum de vraisemblance.

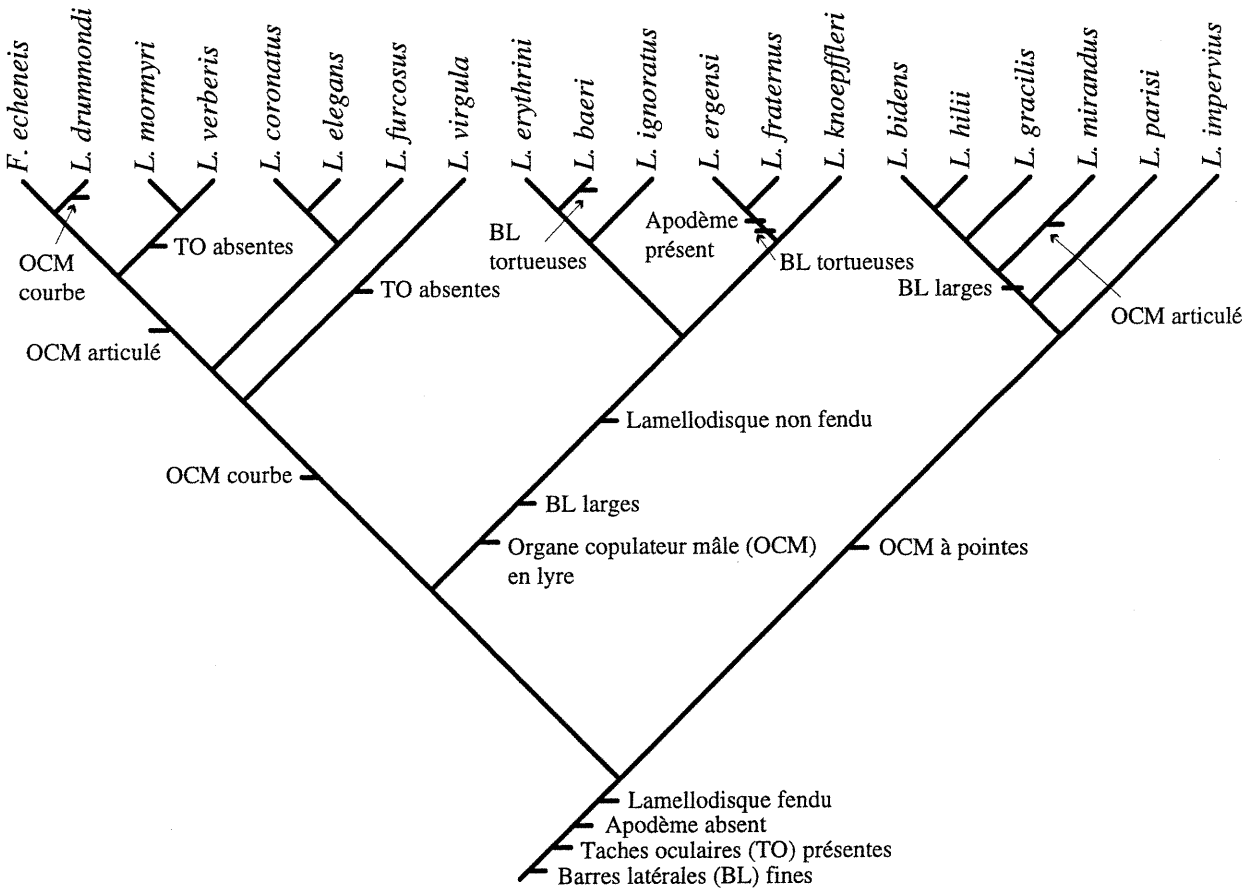


Figure IV.8 : Optimisation des caractères morphologiques des *Lamellodiscus* sur la phylogénie moléculaire obtenue par parcimonie.

4. Phylogénie des Sparidae (Article 5)

Les fragments d'ADN 16S et cyt-b n'ayant pas été déclarés significativement différents par le test d'homogénéité (H_0 : congruence ; P = 0.180) ainsi que le test de Mantel (H_0 : pas de corrélation ; P = 0.001), les analyses phylogénétiques ont été conduites sur les données combinées. Les différences interspécifiques sont présentées au Tableau IV.4 alors que les fréquences des bases pour les différentes espèces de Sparidae figurent au Tableau IV.5. L'analyse par parcimonie a conduit à un seul arbre (IC = 0.53, Figure IV.9) qui est très semblable à celui obtenu par maximum de vraisemblance (Figure IV.10). La seule différence entre ces arbres est la présence du clade (*P. erythrinus*(*D. dentex*, *P. pagrus*)) dans l'arbre le plus parcimonieux, alors qu'on trouve (*D. dentex*(*P. pagrus*, *P. erythrinus*)) dans l'arbre obtenu par maximum de vraisemblance. Comme la seconde possibilité se

retrouve dans l'arbre obtenu par "neighbour-joining", dans l'arbre obtenu par le cyt-b seul ainsi que dans l'arbre publié par Hanel & Sturmbauer (2000) à partir du 16S, l'arbre obtenu par maximum de vraisemblance sera utilisé dans les analyses subséquentes. L'arbre obtenu par "neighbour-joining" est légèrement différent (Figure IV.11), mais les points discordants avec les arbres précédents sont mal supportés par les valeurs de bootstrap.

Tableau IV.4 : Pourcentage de différence entre les séquences d'ADNmt 16S + cyt-b chez les Sparidae.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1- *B. boops*	0.00															
2- *D. annularis*	13.56	0.00														
3- *D. cervinus*	13.05	11.01	0.00													
4- *D. dentex*	14.85	14.77	13.69	0.00												
5- *D. puntazzo*	12.29	10.38	8.29	13.44	0.00											
6- *D. sargus*	12.86	10.51	6.39	14.58	7.41	0.00										
7- *D. vulgaris*	12.99	9.49	8.86	13.63	8.17	8.23	0.00									
8- *L. mormyrus*	13.25	12.98	11.53	14.01	11.53	12.09	12.29	0.00								
9- *O. melanura*	13.37	10.89	8.67	14.07	7.28	8.61	9.11	11.59	0.00							
10- *P. acarne*	13.82	12.92	11.15	15.48	10.83	10.32	11.59	13.12	11.97	0.00						
11- *P. bogaraveo*	13.69	13.42	11.21	14.52	11.08	10.76	11.14	12.09	11.33	8.30	0.00					
12- *P. erythrinus*	15.60	14.01	14.77	11.58	14.70	15.02	14.01	14.20	15.08	16.24	14.58	0.00				
13- *P. pagrus*	14.59	14.83	14.13	10.63	13.56	14.45	13.50	13.81	14.01	15.16	14.90	9.43	0.00			
14- *S. aurata*	13.61	13.35	12.28	15.65	12.59	11.33	12.21	12.54	12.47	12.53	13.29	14.38	14.64	0.00		
15- *S. cantharus*	13.31	15.02	12.17	15.41	13.37	13.06	13.43	14.63	14.07	13.51	13.18	15.35	14.27	14.19	0.00	
16- *S. salpa*	11.20	13.49	13.56	14.59	13.05	12.99	12.54	13.63	13.37	14.12	12.99	14.33	13.95	13.23	12.93	0.00

Tableau IV.5 : Fréquences de bases d'ADNmt 16S + cyt-b chez les Sparidae.

	A	C	G	T	Nombre sites
Extra-groupe	0.256	0.266	0.189	0.289	1583
B. boops	0.254	0.299	0.179	0.268	1581
D. annularis	0.259	0.291	0.178	0.272	1580
D. cervinus	0.264	0.291	0.178	0.267	1580
D. dentex	0.257	0.289	0.178	0.276	1580
D. puntazzo	0.259	0.289	0.178	0.273	1580
D. sargus	0.258	0.292	0.180	0.270	1580
D. vulgaris	0.267	0.277	0.173	0.284	1580
L. mormyrus	0.249	0.291	0.185	0.275	1580
O. melanura	0.259	0.291	0.178	0.271	1580
P. acarne	0.257	0.287	0.182	0.274	1581
P. bogaraveo	0.256	0.285	0.180	0.280	1581
P. erythrinus	0.258	0.277	0.182	0.284	1580
P. pagrus	0.258	0.285	0.182	0.275	1580
S. aurata	0.252	0.289	0.183	0.275	1581
S. cantharus	0.253	0.291	0.182	0.273	1580
S. salpa	0.261	0.294	0.174	0.271	1581
Moyenne	0.258	0.287	0.180	0.275	1580

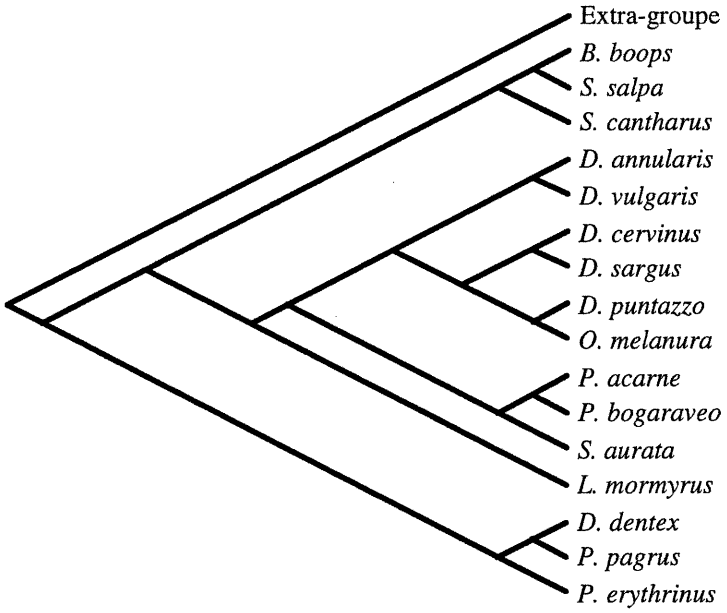


Figure IV.9 : Arbre phylogénétique des Sparidae obtenu par parcimonie à partir des séquences 16S + cyt-b.

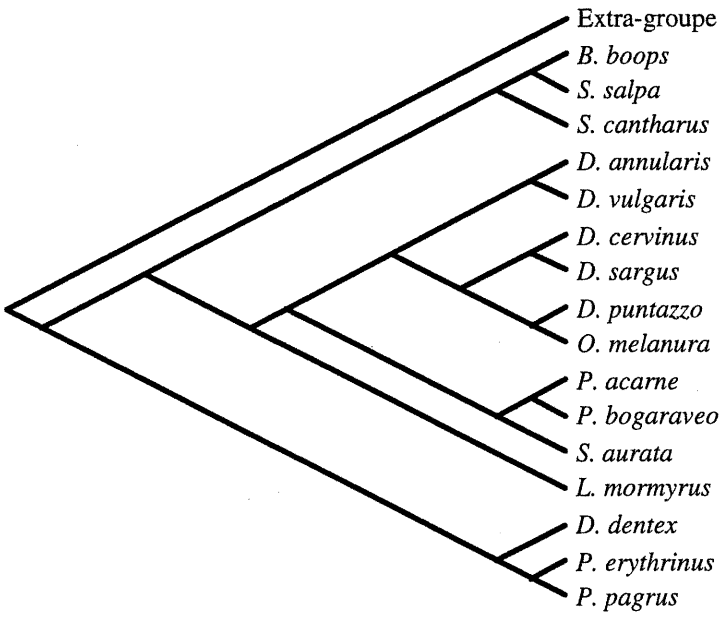


Figure IV.10 : Arbre phylogénétique des Sparidae obtenu par maximum de vraisemblance à partir des séquences 16S + cyt-b.

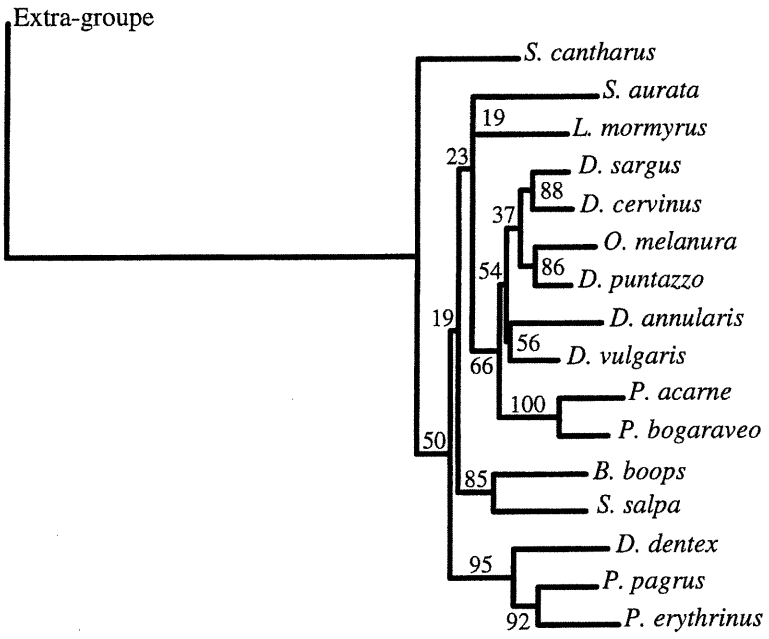


Figure IV.11 : Arbre phylogénétique des Sparidae obtenu par "neighbour-joining" à partir des séquences 16S + cyt-b. Les nombres sont les valeurs de bootstrap, en pourcentage (1000 réplicats).

5. Coévolution *Lamellodiscus*-Sparidae (Article 5)

Le profil de coévolution a été analysé à l'aide de ParaFit en utilisant l'arbre des hôtes obtenu par maximum de vraisemblance ainsi que les arbres des parasites obtenus par parcimonie et maximum de vraisemblance. TreeMap et TreeFitter nécessitent l'utilisation d'arbres complètement résolus, ce qui n'est pas le cas des phylogénies des *Lamellodiscus* élaborées par parcimonie et maximum de vraisemblance et qui ont été présentées dans cette thèse. Pour ces analyses, l'arbre estimé par maximum de vraisemblance a été résolu à l'aide de l'arbre obtenu par "neighbour-joining" puisque leur topologie est compatible. Toutes les analyses faites à l'aide de ParaFit ont produit les mêmes résultats. Ceux qui sont présentés ici concernent donc les mêmes arbres que ceux qui ont été soumis à TreeFitter et à TreeMap, c'est-à-dire avec la phylogénie totalement résolue des parasites.

L'association entre les arbres phylogénétiques des hôtes et des parasites est très complexe (Figure IV.12). La congruence globale entre les deux arbres n'est pas significative avec ParaFit ($P = 0.243$). Le nombre de cospéciations inféré par TreeMap n'est pas significatif quand on le compare à une distribution aléatoire de ce nombre généré à l'aide de phylogénies aléatoires (Figure IV.13), même si on ajoute des événements de capture à la reconstruction. TreeFitter indique la présence d'une structure significativement similaire entre les deux phylogénies, mais seulement si on considère que le coût d'un événement de capture est élevé (deux fois plus élevé que le coût d'une extinction, alors que les événements de cospéciation et de duplication ont un coût nul). Par contre, quand ce coût est abaissé et rendu égal à celui d'une extinction, cette ressemblance significative entre les deux arbres disparaît. Étant donné la stratégie de colonisation des hôtes par les monogènes et le fait que tous les hôtes dans ce système sont sympatriques, il est raisonnable de penser que les événements de capture ne sont peut-être pas aussi "coûteux" que la valeur postulée par défaut par TreeFitter. Ainsi, avec cette réserve, les méthodes utilisées s'accordent sur le fait qu'il n'y a pas de cospéciation généralisée dans ce système hôte-parasite : la phylogénie des parasites semble globalement indépendante de celle des hôtes.

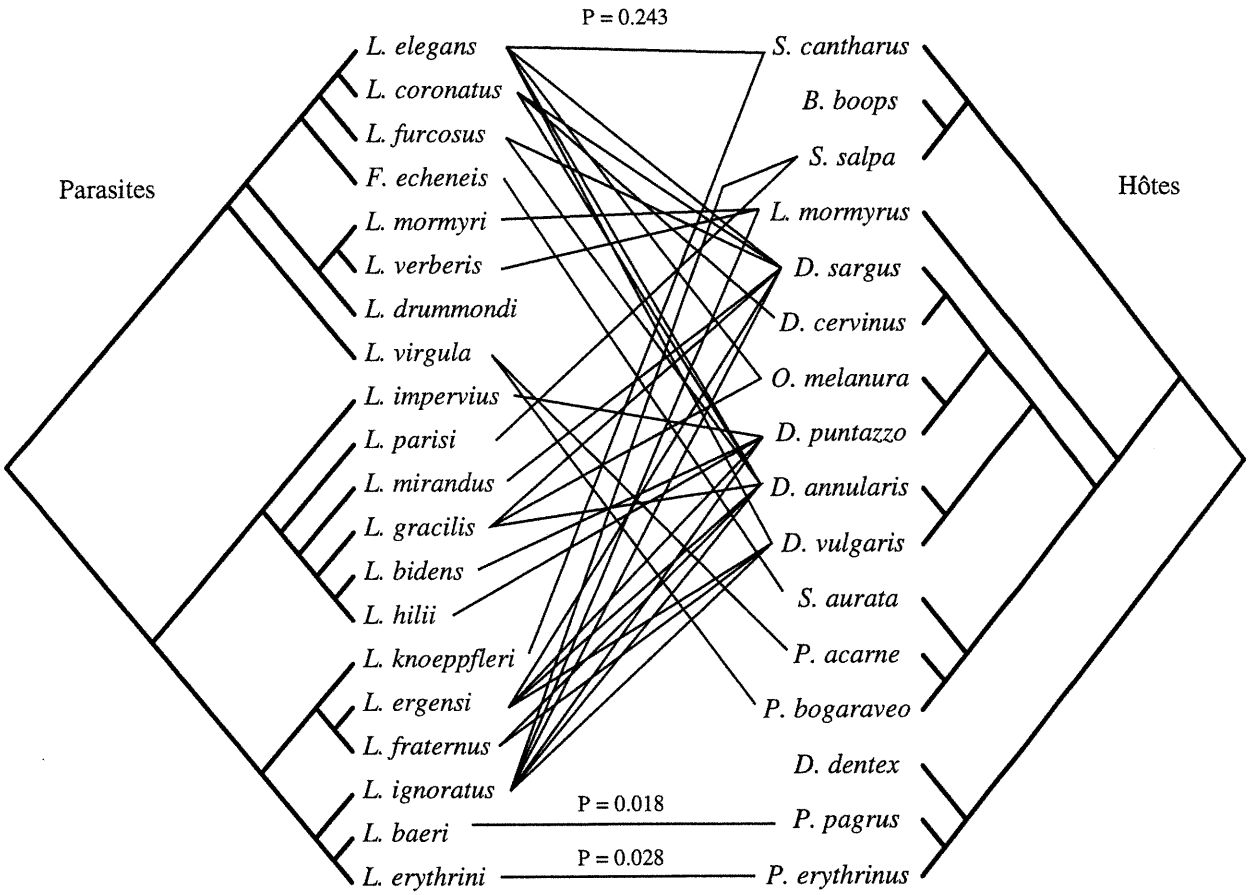


Figure IV.12 : Profil d'association coévolutive entre Sparidae et *Lamellodiscus*.

L'analyse des liens hôte–parasite individuels à l'aide de ParaFit indique que les liens *P. parus-L. baeri* et *P. erythrinus-L. erythrini* sont significatifs. Ces quatre espèces forment chacune deux clades. Sur la base de ce résultat, on peut suggérer l'existence d'un événement de cospéciation. En effet, les simulations rapportées dans l'article décrivant ParaFit (Annexe A) autorisent cette interprétation au niveau des liens particuliers, en dépit du fait que le test global soit non significatif. Il est à noter que cet événement est aussi visible dans toutes les reconstructions proposées par TreeMap (Figure IV.14).

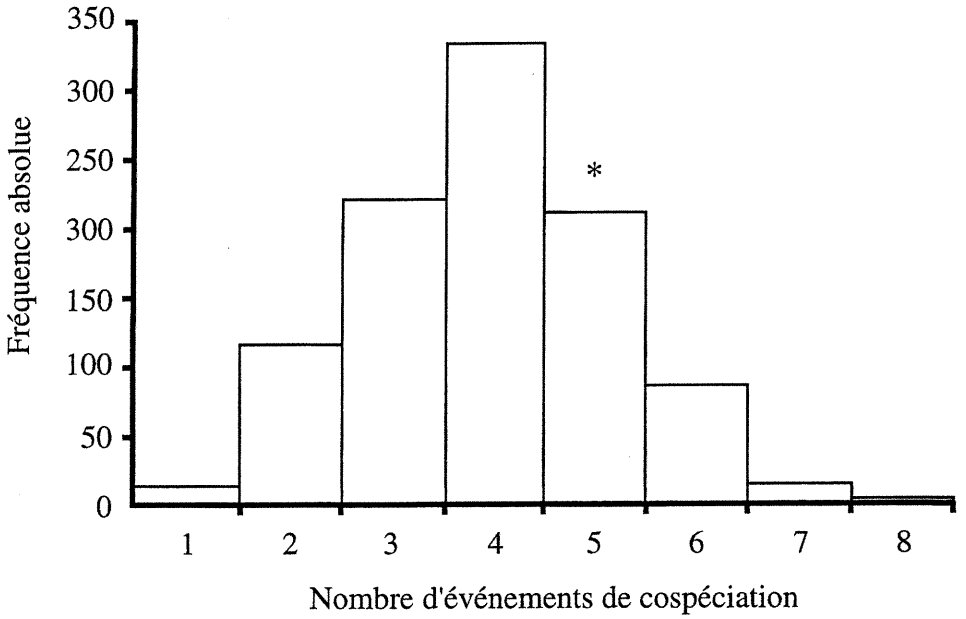


Figure IV.13 : Histogramme généré par TreeMap représentant le nombre d'événements de cospéciations estimé entre la phylogénie des hôtes et 1000 réalisations aléatoires de la phylogénie des parasites.

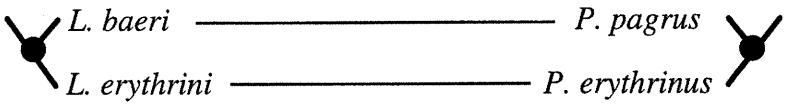


Figure IV.14 : Événement de cospéciation suggéré par les analyses entre *Lamellodiscus baeri-L. erythrinus* et *Pagrus pagrus-Pagellus erythrinus*.

On peut également noter que certains hôtes écologiquement apparentés, mais qui ne sont pas des groupes-frères dans la phylogénie, comme *Diplodus sargus* et *D. vulgaris*, possèdent plusieurs espèces de parasites en commun (Figure IV.15). Si on considère uniquement les espèces spécialistes (Figure IV.16), aucun profil de cospéciation n'est non plus observé, à part le cas mentionné plus haut. Cette observation est confirmée par des analyses réalisées uniquement sur les espèces spécialistes à l'aide de ParaFit (P = 0.600), TreeFitter (P = 0.060) et TreeMap (P = 0.100). On constate également la présence probable d'événements de spéciation intrahôte chez les parasites (Figure IV.17), événements que l'on peut qualifier de spéciation sympatrique, car la reproduction se fait sur les hôtes.

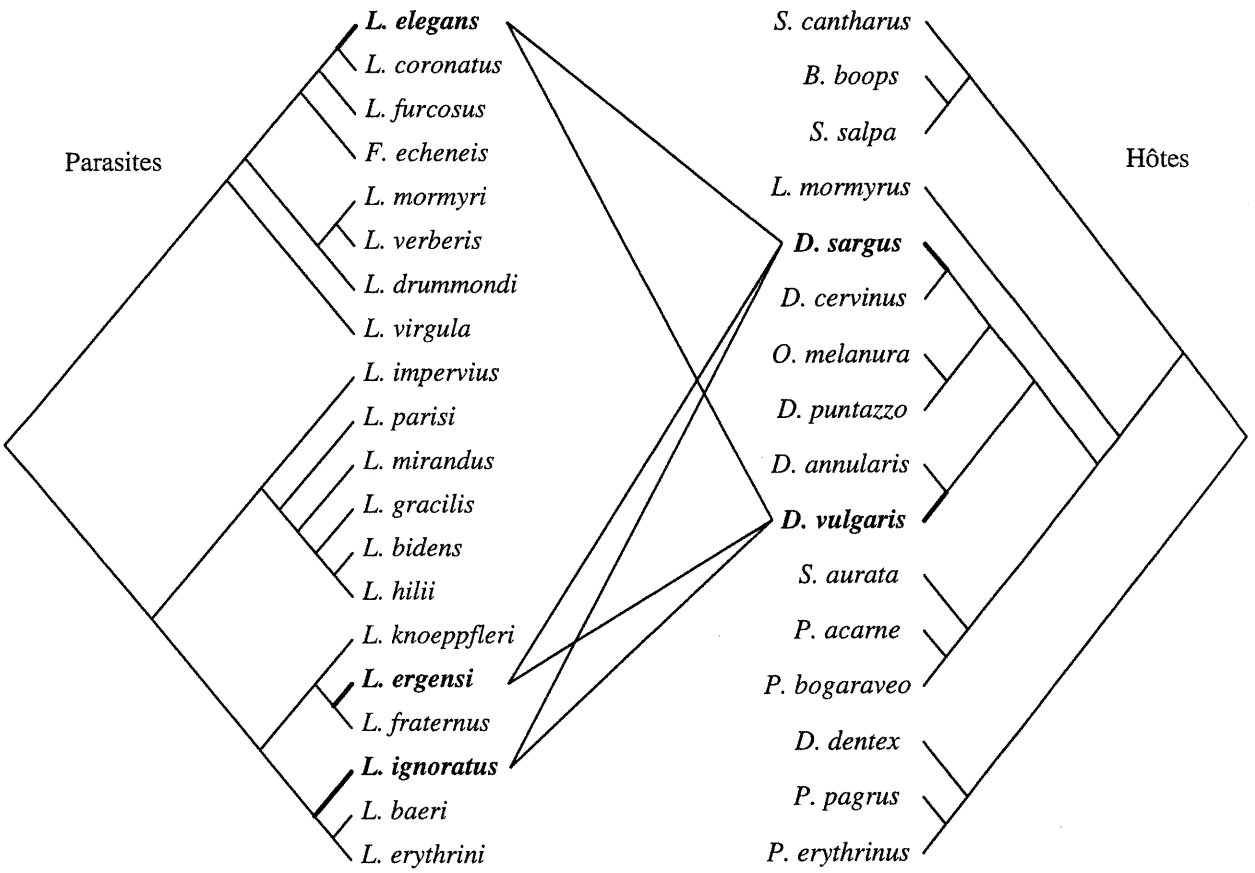


Figure IV.15 : Associations hôte-parasite chez *Diplodus sargus* et *D. vulgaris*.

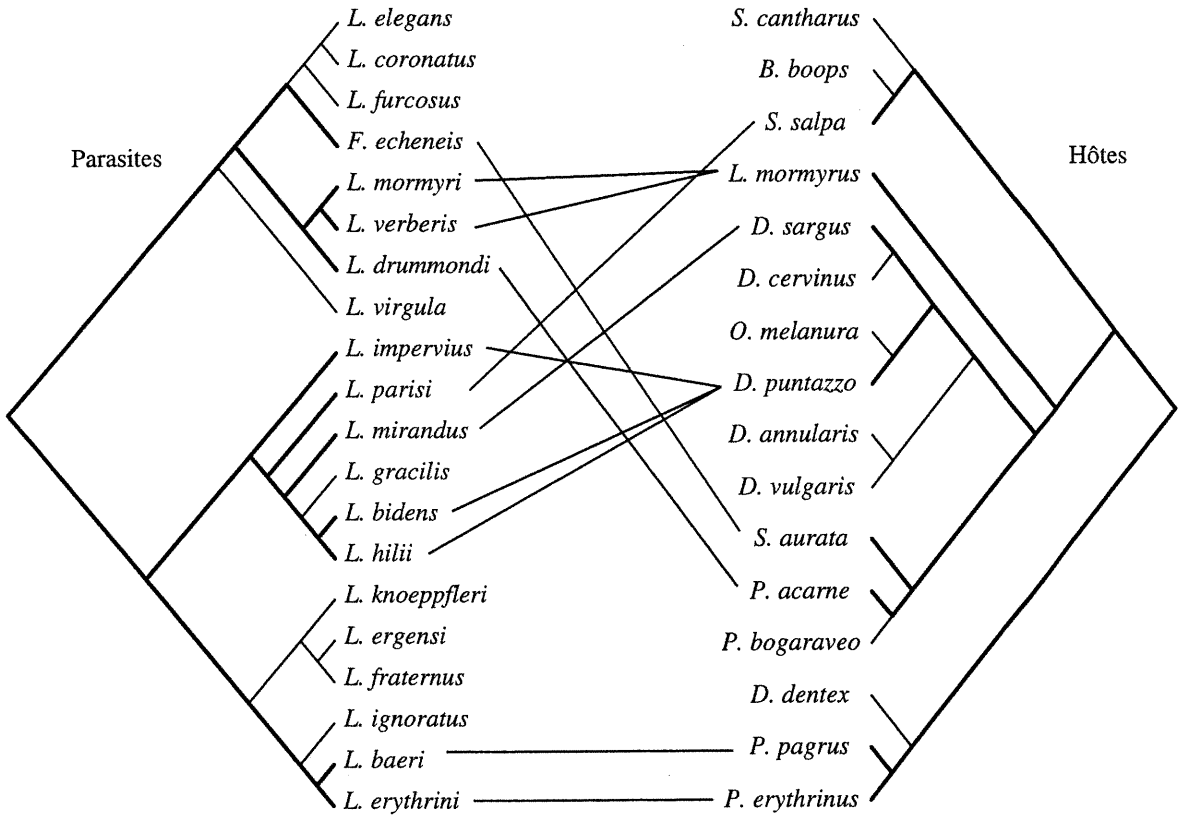


Figure IV.16 : Profil de coévolution hôte-parasite chez les espèces de *Lamellodiscus* spécialistes.

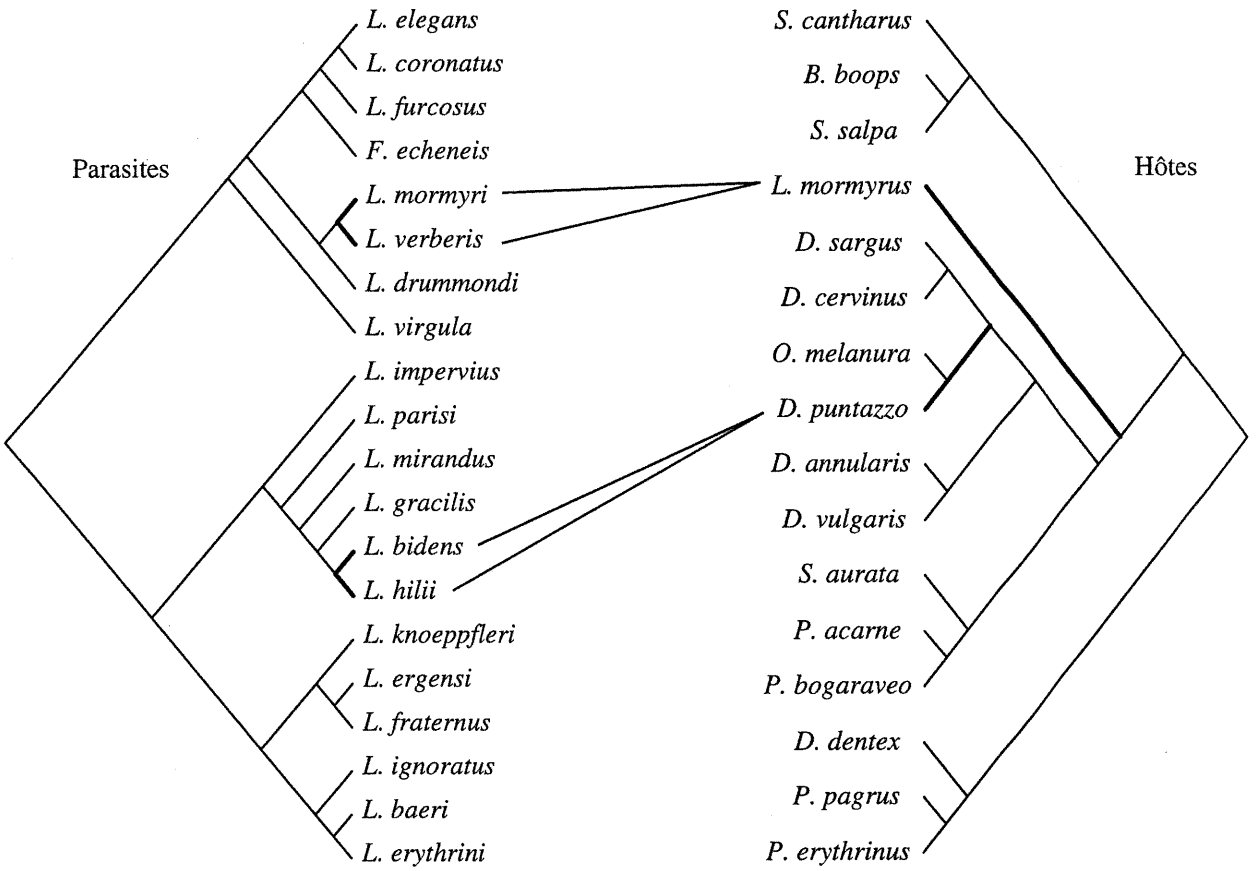


Figure IV.17 : Hypothétiques événements de spéciation sympatrique chez les *Lamellodiscus*.

6. Déterminants de la spécificité (Article 6)

Chez les *Lamellodiscus*, l'optimisation des différents états de l'indice INS sur l'arbre phylogénétique (Figure IV.18) semble indiquer que le fait d'être une espèce spécialiste n'est pas un état dérivé : les branches les plus dérivées correspondent aussi bien à des espèces spécialistes que généralistes. Cela est confirmé par l'analyse statistique de la relation entre la spécificité (INS) et le nombre de nœuds séparant chaque espèce de la racine (pas de relation, Figure IV.19). De plus, dans le genre *Lamellodiscus*, la spécialisation ne semble pas être un "cul-de-sac évolutif" : les transitions spécialiste-généraliste ou généraliste-spécialiste existent toutes les deux (Figure IV.18).

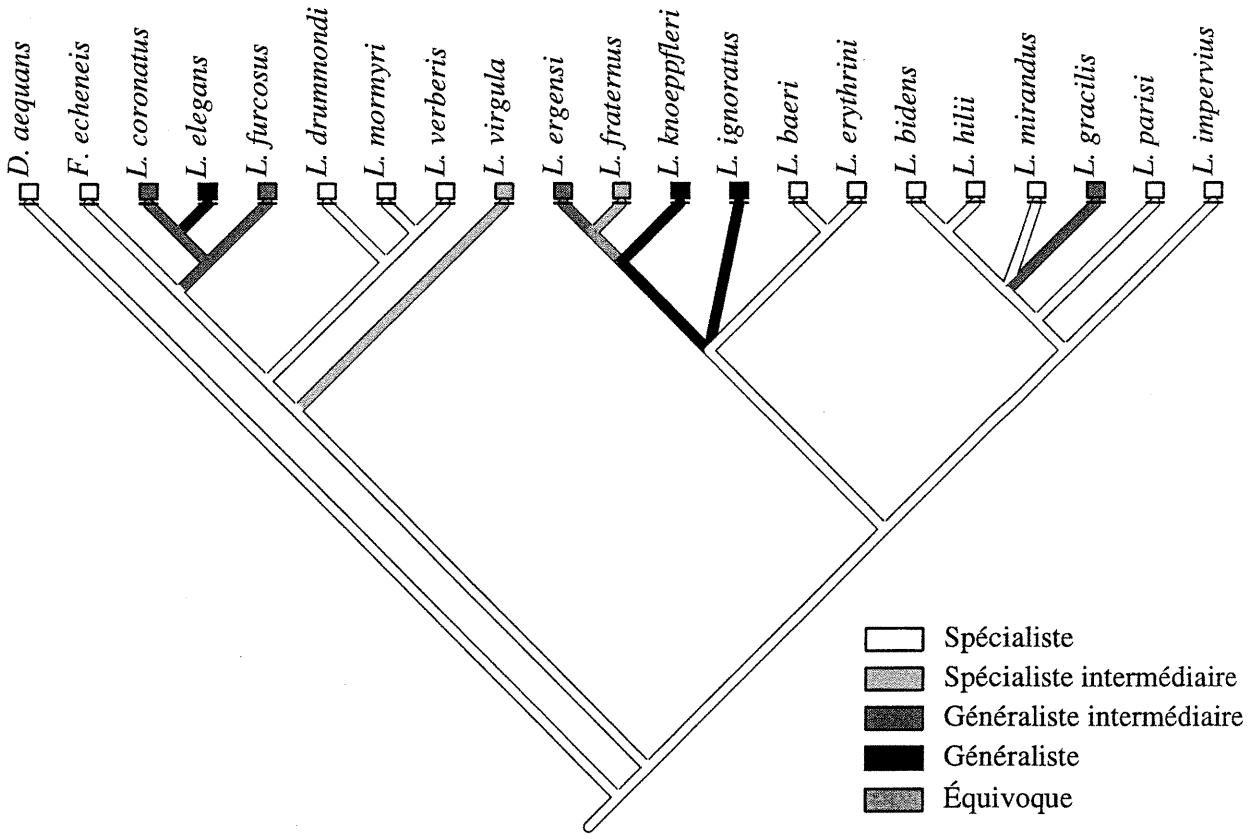


Figure IV.18 : Optimisation des classes de l'indice de spécificité INS sur l'arbre phylogénétique des *Lamellodiscus*.

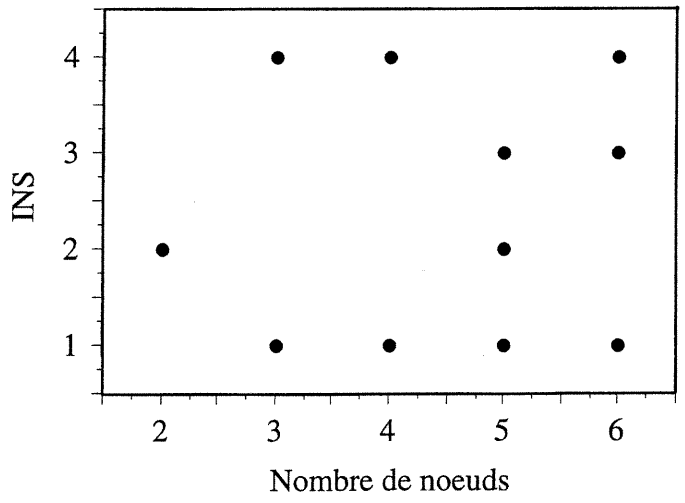


Figure IV.19 : Lien entre la spécificité et le nombre de nœuds séparant chaque espèce de *Lamellodiscus* de la racine de l'arbre (r = 0.060, P = 0.800).

Parmi les variables étudiées (taille de l'hôte, abondance de l'hôte, comportement social des hôtes, nombre d'hôtes potentiels et phylogénie), seules la taille de l'hôte (Figure IV.20) et la phylogénie sont significativement liées à la spécificité. Les spécialistes ont tendance à utiliser les plus grands hôtes. Ni l'abondance, ni le nombre d'hôtes potentiels (c'est-à-dire le nombre d'hôtes contenu dans le clade dans lequel se trouve le ou les hôte(s) utilisé(s)), ni le comportement social des hôtes ne sont liés à la spécificité chez les *Lamellodiscus*. Il est à noter que, pour certaines de ces variables, particulièrement le comportement social et l'abondance, la faible variation contenue dans le jeu de données et le nombre relativement peu élevé d'objets qu'il contient ne permet pas d'écarter l'hypothèse d'un manque de puissance statistique, produisant des relations non significatives.

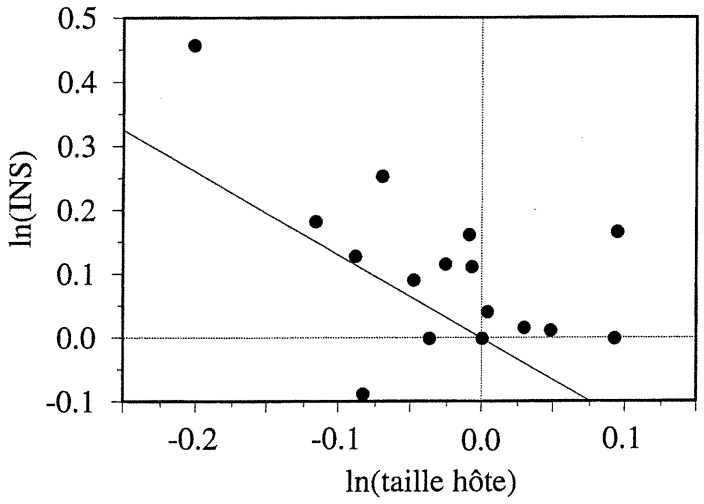


Figure IV.20 : Lien entre l'indice de spécificité et la taille de l'hôte chez les *Lamellodiscus* (r = -0.631, P = 0.005).

Les effets linéaires respectifs de la phylogénie et des variables environnementales (donc la taille de l'hôte ici) sur la spécificité ont été quantifiés, ainsi que la fraction de la variation de la spécificité due à leur influence commune. On constate que l'effet de la taille et de la phylogénie, qui ont été soulignés plus haut, ne sont pas indépendants. Une importante partie de la variation de la spécificité, 24%, est due à leur effet simultané (Figure IV.21). L'effet de la phylogénie seule est très important (45%), alors que l'effet de

la taille seule, indépendamment de la phylogénie, est faible (4%). Cela souligne le rôle apparemment important de contraintes génétiquement transmissibles dans le déterminisme de la spécificité des *Lamellodiscus* pour leurs hôtes.

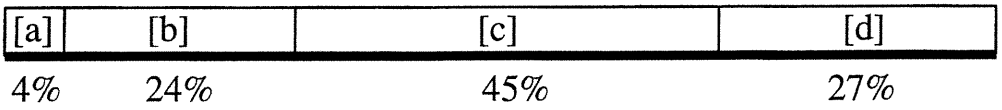


Figure IV.21 : Partition de la variation de la spécificité (INS) entre les influences écologiques et phylogénétiques. [a] représente la fraction de la variation de la spécificité due à la taille de l'hôte, [c] celle due à la phylogénie, [b] la fraction commune à ces deux influences, et [d] la fraction inexpliquée par ces variables.

Un lien significatif a été mis en évidence entre la taille des parasites et celle de leur hôte (Figure IV.22). La taille des parasites étant corrélée à celle de la plupart des structures morphologiques liées à l'accrochage, comme la taille du hapteur, des lamellodisques et des crochets, on peut poser l'hypothèse que cette corrélation est possiblement liée à une adaptation mécanique des parasites à leurs hôtes.

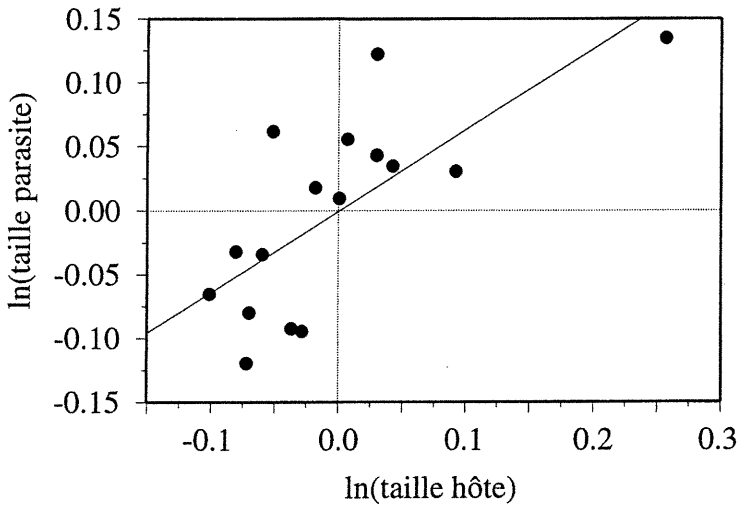


Figure IV.22 : Lien entre la taille des parasites et la taille de l'hôte chez les *Lamellodiscus* ($r = 0.719$, $P = 0.004$).

B. Discussion

1. Lien entre spécificité et diversification taxonomique

À un niveau taxonomique élevé, c'est-à-dire au niveau des grands groupes de parasites (Monogènes, Digènes, Cestodes, Nématodes, Copépodes, Acanthocéphales), la spécificité semble promouvoir la diversification taxonomique : les groupes contenant le plus d'espèces sont ceux dans lesquels la spécificité moyenne est la plus faible. Bien sûr, cela n'implique pas une relation de cause à effet, mais supporte l'hypothèse de Brooks & McLennan (1991, 1993) : les parasites les plus spécifiques, plus sensibles aux changements de leur environnement (leur(s) hôte(s)), seraient soumis à une pression de spéciation plus forte en cas de modification de cet environnement, comme dans le cas d'un changement d'hôte par colonisation ou cospéciation. Cela suppose que la spécificité est due au moins en partie à une caractéristique intrinsèque du parasite. L'hypothèse émise dans l'Article 1 pour expliquer cette relation est que cela pourrait être dû à des mécanismes de cospéciations. Néanmoins, en ce qui concerne les monogènes et au niveau spécifique, cela ne semble pas être le cas pour les *Lamellodiscus* comme cela avait déjà été suggéré pour d'autres types de monogènes (Klassen & Beverley-Burton, 1987, 1988 ; Boeger & Kritsky, 1989). Les événements de cospéciation chez les monogènes semblent être davantage liés aux niveaux taxonomiques plus profonds (au-dessus de la famille), même s'ils sont loin de représenter la règle à ce niveau (Boeger & Kritsky, 1997). La spécificité, en terme de nombre d'hôtes, s'exprime au niveau spécifique. Or nous avons vu qu'à ce niveau, la cospéciation entre les monogènes et leurs hôtes semblait rare, à part dans le cas de quelques systèmes hôtes-parasites très spécifiques dans lesquels la spéciation allopatrique des hôtes due à la séparation géographique joue probablement un rôle important, comme chez les Polystomatidae et leurs hôtes (Sinnappah, 1998 ; Sinnappah et al., 2001). En d'autres termes, le lien spécificité-diversification observé à haut niveau n'est peut-être pas dû aux interactions coévolutives entre les parasites et leurs hôtes, mais davantage aux pressions différentes s'exerçant sur les parasites plus ou moins spécifiques. Les événements de spéciation se produiraient après les événements de colonisation, à cause de la "pression environnementale" importante exercée par l'hôte, ce qui correspond à l'hypothèse de Brooks & McLennan (1991) ainsi qu'à l'opinion de Secord & Kareiva (1996). Par contre, ce lien

spécificité-diversification n'a pas pu être mis en évidence au niveau intergénérique chez les Diplectanidae, ni au niveau interspécifique chez les *Lamellodicus*. Même si l'ampleur de ce jeu de données est insuffisante pour en tirer des conclusions généralisables, cela suggère que, chez les monogènes, ce lien spécificité-diversification ne s'exprime qu'à de hauts niveaux taxonomiques et que les contraintes sur la spécificité parasitaire ne sont pas les mêmes aux niveaux les plus bas. On peut supposer que ces contraintes sont le fruit de différences profondes et générales, aux niveaux immunologique ou physiologique par exemple, agissant uniquement aux niveaux taxonomiques élevés.

2. Phylogénie des *Lamellodiscus*

L'arbre obtenu par l'analyse du 18S pour les *Lamellodiscus* est renforcé par l'observation de la morphologie, ce qui supporte la reconstruction phylogénétique sur la base des données moléculaires. Il est probable que la résolution des arbres obtenus et les légères incongruences constatées entre les arbres obtenus par les différentes méthodes utilisées pourraient être améliorées en séquençant un fragment d'ADN plus grand afin d'obtenir davantage de caractères. Quoiqu'il en soit, l'ADN ribosomique 18S semble être un bon marqueur de l'évolution chez les *Lamellodiscus*. D'autres études montrent qu'il en est de même chez les monogènes en général, aussi bien Monopisthocotylea que Polyopisthocotylea (Cunningham et al., 1995 ; Sinnappah et al., 2001 ; Olson & Littlewood, sous presse).

Une étude moléculaire comme celle-ci a permis d'aider à la clarification de certaines incertitudes taxonomiques difficile à mettre en évidence par l'observation de la seule morphologie (McManus & Bowles, 1996). Chez les *Lamellodiscus*, les espèces spécialistes *Lamellodiscus virgula* (parasite de *Pagellus acarne*) et *L. obeliae* (parasite de *P. bogaraveo*) s'avèrent, sur la base de leur séquence de l'ITS1, former une seule espèce plus généraliste. Leurs différences morphologiques sont minimes. Cette observation rend nécessaire la conduite d'un travail expérimental pour étudier l'influence de l'hôte sur la morphologie des *Lamellodiscus* et d'autres espèces de monogènes (voir Mo, 1991a,b), comme cela a été fait pour des crustacés parasites (Downes, 1990). De même, l'espèce *Furnestinia echeneis* (parasite de *Sparus aurata*), seule espèce du genre *Furnestinia*, ferait partie du genre *Lamellodiscus* selon la reconstruction phylogénétique élaborée à partir du

18S. La différence essentielle entre ce genre et le genre *Lamellodiscus* est le nombre de lamellodisques : deux pour *Lamellodiscus*, un pour *Furnestinia*. Cela suggère de fortes capacités d'adaptation morphologique chez les monogènes, et donc la difficulté potentielle d'utiliser certains caractères morphologiques pour les reconstructions phylogénétiques, surtout dans le cas où ces caractères sont peu nombreux. Cela met également en évidence la puissance des outils moléculaires en taxonomie, permettant d'obtenir des informations difficilement accessibles à la seule étude morphologique. Bien sûr, une telle étude au niveau du gène devrait s'accomplir de concert avec une étude au niveau morphologique.

L'importante divergence observée au niveau des séquences de l'ITS1 chez les *Lamellodiscus* peut paraître surprenante chez des espèces apparemment si proches morphologiquement et dont la divergence pourrait apparaître comme récente. Cela peut s'expliquer par un taux d'évolution rapide, ou par un âge important (Hillis & Dixon, 1991). La question de l'âge pourrait être éclairée par l'étude d'événements de cospéciation avec des hôtes dont la divergence est connue. Mais comme nous l'avons vu, de tels événements semblent rares chez les monogènes. Le taux d'évolution rapide chez les monogènes Monopisthocotylea a été remarqué par Olson & Littlewood (sous presse), spécialement par rapport au même taux chez les Polyopisthocotylea. Concernant le débat entourant la question de la monophylie des monogènes (Justine, 1998 ; Boeger and Kritsky, 2001), on peut noter que cette différence de taux d'évolution peut être vue comme un argument en faveur de l'hypothèse de monophylie (elle biaiserait les études moléculaires) ou comme contradictoire avec cette hypothèse (les deux entités sont différentes).

L'existence d'espèces de monogènes uniques possédant une morphologie polymorphe, comme *Lamellodiscus virgula* et *L. obeliae* dans cette étude, laisse penser que davantage d'espèces de monogènes ont été décrites sur la base de petites différences morphologiques qui ne justifiaient peut-être pas la création de nouvelles espèces. Le mythe de la forte spécificité des monogènes pourrait expliquer en partie cette tendance à créer de nouvelles espèces pour des hôtes différents.

3. Phylogénie des Sparidae

La phylogénie des Sparidae présentée dans ce travail est en contradiction avec la taxonomie classique (Whitehead et al., 1986 ; Fiedler, 1991) : dans la phylogénie présentée ici, les sous-familles Boopsinae, Pagellinae et Sparinae ne sont pas monophylétiques, de même que les genres *Diplodus* et *Pagellus*, les seuls ici composés de plusieurs espèces. Par contre, l'hypothèse proposée par Hanel & Sturmbauer (2000) est supportée, alors que l'arbre proposé dans notre étude est mieux résolu. Cela suggère que la taxonomie des Sparidae devrait être révisée et, comme le suggèrent Hanel & Sturmbauer (2000), que les caractères utilisés pour la classification actuelle, principalement la dentition, répondent essentiellement à des contraintes adaptatives. La phylogénie moléculaire des Sparidae présentée ici est peut-être la plus exacte qui ait été proposée jusqu'ici.

4. Coévolution *Lamellodiscus*-Sparidae

Les méthodes utilisées dans cette thèse s'accordent sur le fait qu'il n'y a pas de profil général de cospéciation dans l'association *Lamellodiscus*-Sparidae ; le choix des hôtes par les parasites n'est pas guidé par les relations phylogénétiques de leurs hôtes. Cela supporte des observations semblables déjà réalisées pour d'autres systèmes monogènes-poissons (Klassen & Beverley-Burton, 1987, 1988 ; Boeger & Kritsky, 1997) et suggère que les événements de transfert d'hôte sont communs chez les monogènes. Les événements de cospéciation observés dans la littérature (Boeger & Kritsky, 1997) sont surtout observés à de hauts niveaux taxonomiques (au-dessus de la famille), même si des événements de captures semblent également exister. Cela supporte l'hypothèse mentionnée plus haut, que les contraintes phylogénétiques à ces niveaux sont différentes de ce qui est observé à l'échelon de l'espèce.

La cospéciation hôte-parasite en général pourrait être principalement contrôlée par la spéciation allopatrique (voir Barker, 1994, mais voir Page et al., 1996) ; les conditions nécessaires à ce mode de spéciation ne sont pas remplies ici, avec des hôtes sympatriques et des parasites possédant une larve mobile. L'observation que des hôtes écologiquement proches partagent en partie les mêmes parasites (Figure IV.15) permet de supposer l'existence de déterminants plus écologiques que phylogénétiques de la colonisation des

hôtes par ces parasites (voir Bentz et al., 2001), qui serait donc davantage basée sur les opportunités offertes au parasite. Cela semble être le cas, par exemple, pour l'association entre les gaufres et leurs poux dans laquelle les hôtes sont séparés les uns des autres par des barrières écologiques fortes (Nadler et al., 1990 ; Hafner et al., 1994 ; Reed & Hafner, 1997). Si c'est le cas, l'histoire coévolutive des deux complexes d'espèces donne des indications sur leur histoire biogéographique, par exemple leurs périodes de contact, car elles expliqueraient les événements de colonisation.

Le lien spécificité-coévolution, proposé par certains auteurs (Noble et al., 1989 ; Poulin, 1992 ; Kearn, 1994), n'est pas confirmé par l'étude du système *Lamellodiscus*-Sparidae. Les espèces spécialistes, prises séparément, ne présentent pas de profil de cospéciation avec leurs hôtes. Cela laisse supposer qu'après un transfert d'hôte, et donc un élargissement de la spécificité, l'espèce ayant colonisé le nouvel hôte va subir une spéciation sous la "pression" de celui-ci. Cela rejoint l'opinion de Hoberg (1986) qui croit que dans les groupes vieux au niveau évolutif, cospéciation et spécificité ne sont pas forcément associés. Cela est interprété par Brooks (1979) comme le résultat d'une forte association écologique entre hôtes et parasites et est en accord avec ce qui est observé ici quant aux déterminants de la spécificité. La présence d'événements probables de spéciation sympatrique supporte l'hypothèse d'une importante capacité à s'adapter et à subir des spéciations chez ces monogènes.

Cet aspect du travail constitue une des rares études de coévolution monogènes-poissons ; aucune étude réalisée à partir de données moléculaires n'a été publiée jusqu'ici pour ces groupes. Très peu d'études de systèmes hôte-parasite aussi complexes ont été réalisées (e.g., Beverley-Burton & Klassen, 1990 ; Roy, 2001). Dans ces études, les analyses ont été limitées par la complexité du problème, alors qu'ici, l'utilisation de la nouvelle méthode ParaFit a permis de proposer des interprétations plus fines aux résultats. L'analyse du système hôtes-parasites *Lamellodiscus*-Sparidae remet en cause un dogme de la coévolution trop souvent accepté sans discussion et contredit la supposition que la forte spécificité des monogènes (et par extension d'autres parasites) est liée à des processus coévolutifs avec leurs hôtes (Noble et al., 1989 ; Poulin, 1992 ; Kearn, 1994).

5. Déterminants de la spécificité

Un lien significatif entre la taille des hôtes et la spécificité a été trouvé chez les *Lamellodiscus*. Cela a déjà été mis en évidence chez les monogènes dans d'autres études (Sasal & Morand, 1998 ; Sasal et al., 1999 ; Simková et al., 2001). Ce lien est interprété comme une spécialisation des parasites sur une ressource prédictible. Les hôtes les plus grands peuvent en effet être considérés comme plus prédictibles (voir Winemiller & Rose, 1992) de par leur position élevée dans la chaîne alimentaire et leur longévité supérieure. Cela supporte l'hypothèse, proposée par Ward (1992), d'une spécialisation sur une ressource stable dans le temps qui a pour effet de limiter les risques d'extinction. L'abondance des hôtes peut également être considérée comme liée à la prédictibilité de la ressource, mais aucun lien significatif n'a pu être mis en évidence entre la spécificité et l'abondance. Peut-être cette variable est-elle trop fluctuante au cours du temps évolutif pour exercer une influence sélective? Norton & Carpenter (1998) suggèrent que ce n'est qu'à partir d'un certain seuil d'abondance relative de leurs hôtes que les parasites vont adopter un comportement spécialiste, la population de l'hôte étant alors assez importante pour supporter celle du parasite. C'est donc autour de ce seuil théorique que la variable abondance peut jouer un rôle dans le déterminisme de la spécificité. Il est possible que ce seuil ne soit pas atteint ici.

La phylogénie est également très liée à la spécificité, ce qui suppose que la spécificité se "transmet" verticalement entre espèces. Cela sous-entend qu'elle est contrôlée par une caractéristique héritable, par exemple un facteur physiologique, immunologique ou morphologique. La complexité des interactions immunologiques entre les monogènes et leur hôtes (Buchmann, 1999) laisse entendre que ce facteur est "coûteux" pour le parasite et pourrait être impliqué dans le déterminisme de la spécificité.

Le partitionnement de la variation de la spécificité entre les influences phylogénétique et écologique indique que la phylogénie et la taille de l'hôte expliquent simultanément une grande part de la variation de la spécificité des *Lamellodiscus*. Cette fraction commune de la variation correspond aux caractéristiques adaptatives développées par des espèces proches, donc vivant dans des niches similaires et soumises aux mêmes pressions environnementales. C'est le "phylogenetic niche conservatism" de Harvey et

Pagel (1991). Dans le cas présent, cela permet de supposer que seuls les parasites pouvant développer une stratégie généraliste, donc ayant tendance à être des espèces proches, pourraient utiliser toute une variété d'hôtes. Le même raisonnement peut être tenu pour les spécialistes : ceux-ci, s'ils sont proches phylogénétiquement, posséderaient une caractéristique leur permettant de se spécialiser sur les hôtes de grande taille. Ce partitionnement de la variation d'une variable entre des facteurs écologiques et phylogénétiques n'avait encore jamais été effectué. Il permet de proposer une interprétation plus fine des données.

L'optimisation de la spécificité sur l'arbre phylogénétique des *Lamellodiscus* suggère que la spécificité n'est pas un "cul-de-sac évolutif", comme cela a été mentionné plus haut, et que l'état spécialiste ne dérive pas forcément d'un état plus généraliste, comme cela est parfois présupposé (Simpson, 1953). Cela supporte l'opinion de Thompson (1994), qui pense que le fait d'être spécialiste ou généraliste s'inscrit dans un équilibre dynamique variable dans le temps et dans l'espace selon les conditions écologiques.

Une corrélation significative entre la taille des parasites et celle des hôtes a été mise en évidence. Chez les monogènes, la petite taille relativement à l'hôte et surtout la non-saturation des niches écologiques (Rohde, 1978) ne sont pas en faveur de l'hypothèse proposée par Morand & Sorci (1998) pour des endoparasites, hypothèse selon laquelle les hôtes les plus grands fournissent plus d'énergie, donc permettent une meilleure croissance à leurs parasites. Cette hypothèse suppose que le milieu est limitant, ce qui ne semble pas être le cas pour les monogènes. Une explication plus probable de ce lien entre la taille du parasite et celle de son hôte est celle de l'adaptation morphologique. La taille des espèces de *Lamellodiscus* est corrélée à plusieurs caractéristiques impliquées dans l'accrochage : largeur du hapteur, taille du lamellodisque. Les *Lamellodiscus* les plus gros sont probablement ceux qui s'accrochent le mieux à leurs hôtes. En posant l'hypothèse que la pression de l'eau dans la cavité branchiale des poissons augmente avec leur taille, on peut proposer que les parasites les plus grands peuvent mieux répondre aux contraintes mécaniques d'accrochement sur les plus grands hôtes, comme cela a été proposé par Sasal et al. (1999). Le fait que le genre *Furnestinia*, sans doute une espèce de *Lamellodiscus* mal nommée, ait développé une très grande taille pour son unique lamellodisque supporte le rôle important que cette structure (dont la taille est très liée à la taille du parasite) joue dans

l'accrochage ; il supporte également l'idée de contraintes importantes quant à l'accrochage. Ce lien entre la taille des hôtes et des parasites peut être considéré comme une cause ou une conséquence de la spécialisation. En effet, Futuyma & Moreno (1988) et Adamson & Caira (1994) suggèrent que les adaptations morphologiques ou physiologiques à une ressource sont davantage des conséquences que des causes de la spécialisation. Le fait que les généralistes parasitent des hôtes d'une gamme de taille importante permettent de favoriser l'hypothèse que ce type d'adaptation morphologique potentielle est davantage une conséquence de la spécialisation ; sinon, les hôtes "choisis" par les généralistes se situeraient dans une gamme de tailles comparables, ce qui n'est pas le cas.

C. Perspectives

À la suite de ce travail, on peut suggérer plusieurs voies pour tenter de mieux comprendre le déterminisme de la spécificité, en particulier chez les monogènes, et d'explorer les points laissés en suspens ici.

Il serait informatif d'effectuer le même type d'étude sur d'autres systèmes monogènes-poissons afin de comparer les résultats à ceux obtenus ici, tout en contrôlant un certain nombre de facteurs car les systèmes hôte-parasite étudiés devraient de même type. En parallèle, l'étude de genres de monogènes possédant des caractéristiques biologiques différentes permettrait d'évaluer l'effet de celles-ci sur les déterminants de la spécificité. Par exemple, le même type d'étude sur des espèces de *Gyrodactylus* mettrait en évidence l'influence d'une moins grande capacité de dispersion larvaire sur la spécificité, étant donné le type particulier de reproduction des parasites de ce genre et la faible capacité de dispersion de leurs larves oncomiracidia. Les monogènes gyrodactylides semblent en effet montrer des réactions particulières à la spécificité (Matejusova et al., 2000), en particulier quant à leur distribution parmi les hôtes. L'étude de Polystomatidae (en admettant que ceux-ci, des Polyopisthocotylea, soient comparables aux Monopisthocotylea, compte tenu du fait que la monophylie des monogènes est sujette à controverse : Justine, 1998) permettrait d'évaluer l'effet d'hôtes généralement mieux séparés géographiquement.

D'autres facteurs potentiels du déterminisme de la spécificité restent à évaluer, particulièrement aux niveaux immunologique et physiologique. Les contraintes

génétiquement transmissibles influençant potentiellement la spécificité chez les *Lamellodiscus* et probablement aussi chez d'autres monogènes sont peut-être à chercher de ce côté. La capacité de dispersion peut également être un facteur important, comme cela a été suggéré au paragraphe précédent.

Quoiqu'il en soit, il est probable que le déterminisme de la spécificité a des causes multiples et que celles-ci dépendent au moins en partie du système hôte-parasite considéré. Une généralisation du travail présenté dans cette thèse permettrait de montrer à quel point les facteurs mis en évidence ici sont spécifiques ou non au système étudié. L'influence de la taille de l'hôte, interprétée comme une spécialisation sur une ressource prédictible, ayant déjà été mise en évidence pour d'autres associations hôte-parasite, on peut supposer que ce facteur, à tout le moins, a une importance dépassant le cadre du complexe d'espèces étudié ici.

Le "dogme" de la coévolution, remis en question par la présente étude (ainsi que par d'autres, e.g., Roy, 2001), tient beaucoup au fait de l'attirance des chercheurs pour des systèmes hôtes-parasites qui suivent ce profil. Par exemple, l'association gaufres-poux est devenue un système modèle pour les études de coévolution (Page & Hafner, 1996) alors qu'il représente sans doute un cas très particulier. D'autres systèmes hôtes-parasites devraient être étudiés à un niveau fin pour tenter de comprendre à quel point le phénomène de la coévolution est courant dans la nature. De nombreuses méthodes analytiques rigoureuses existent maintenant pour ce faire. Une plus grande compréhension du déterminisme de la spécificité chez les parasites permettra de mieux comprendre les mécanismes de la coévolution avec les hôtes.

V. RÉFÉRENCES

A

Adamson M. L. & Caira J. N. 1994. Evolutionary factors influencing the nature of parasite specificity. *Parasitology* 109 : S85-S95.

B

Baer J. G. (Ed.). 1957. *First Symposium on Host Specificity among Parasites of Vertebrates*. Paul Attinger, Neuchatel.

Bakke T. A., Harris P. D., Jansen P. A. & Hansen L. P. 1992. Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* 13(1) : 63-74.

Barker S. C. 1991. Evolution of host-parasite associations among species of lice and rock-wallabies: coevolution? *International Journal for Parasitology* 21 : 497-501.

Barker S. C. 1994. Phylogeny and classification, origins, and evolution of host associations of lice. *International Journal for Parasitology* 24 : 1285-1291.

Barraclough T. G., Vogler A. P. & Harvey P. H. Revealing the factors that promote speciation. *Philosophical Transactions of the Royal Society of London B* 333 : 241-249.

Basaglia F. 1991. Interspecific gene differences and phylogeny of the Sparidae family (Perciformes, Teleostei), estimated from electrophoretic data on enzymatic tissue-expression. *Comparative Biochemistry and Physiology* 99B(3) : 495-508.

Basset Y. 1992. Host specificity of arboreal and free-living insect herbivores in rain forests. *Biological Journal of the Linnean Society* 47 : 115-133.

Begon M., Harper J. L. & Townsend C. R. 1996. *Ecology: Individuals, population and communities*. 3^{d} ed. Oxford: Blackwell Science.

Bentz S., Leroy S., du Preez L, Mariaux J., Vaucher C & Verneau O. 2001. Origin and evolution of African *Polystoma* (Monogena: Polystomatidae) assessed by molecular methods. *International Journal for Parasitology* 31(7) : 697-705.

Bernays E. A. 1998. The value of being a resource specialist: Behavioral support for a neural hypothesis. *The American Naturalist* 151(5) : 451-464.

Beverley-Burton M. & Klassen G. 1990. New approaches to the systematics of the ancyrocephalid monogenea from Nearctic freshwater fishes. *Journal of Parasitology* 76(1) : 1-21.

Boeger W. A. & Kritsky D. C. 1989. Phylogeny, coevolution, and revision of the Hexabothriidae Price, 1942 (Monogenea). *International Journal for Parasitology* 19 : 425-440.

Boeger W. A. & Kritsky D. C. 1993. Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology* 26(1) : 1-32.

Boeger W. A. & Kritsky D. C. 1997. Coevolution of the Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *International Journal for Parasitology* 27 : 1495-1511.

Boeger W. A. & Kritsky D. C. 2001. Phylogenetic relationships of the Monogenoidea. In *Interrelationships of the Platyhelminthes* (Littlewood D. T. J. & Bray R. A. Eds) pp. 92-102. Taylor & Francis, London.

Brooks D. R. 1979. Testing the context and extent of host-parasite coevolution. *Systematic Zoology* 28 : 299-307.

Brooks D. R. 1981. Hennig's parasitological method: A proposed solution. *Systematic Zoology* 30 : 229-249.

Brooks D. R. 1980a. Allopatric speciation and non-interactive parasite community structure. *Sytematic Zoology* 30 : 192-203.

Brooks D. R. 1980b. Brooks' response to Holmes and Price. *Systematic Zoology* 29(2) : 214-15.

Brooks D. R. 1985. Phylogenetics and the future of helminth systematics. *Journal of Parasitology* 71(6) : 719-27.

Brooks D. R. & Glen D. R. 1982. Pinworms and primates: a case study in coevolution. *Proceedings of the Helminthological Society of Washington* 49 : 76-85.

Brooks D. R. & McLennan D. A. 1991. *Phylogeny, Ecology, and Behavior. A Research Program in Comparative Biology*. The University of Chicago Press, London.

Brooks D. R. & McLennan D. A. 1993. *Parascript.* Smithsonian series in Comparative Evolutionary Biology, Washington and London.

Buchmann K. 1999. Immune mechanisms in fish against monogeneans – a model. *Folia Parasitologica* 46 : 1-9.

C

Caro A., Combes C. & Euzet L. 1997. What makes a fish a suitable host for Monogenea in the Mediterranean? *Journal of Helminthology* 71 : 203-210.

Cantatore P., Roberti M., Pesole G., Ludovico, A., Milella F., Gadaleta M. N. & Saccone C. 1994. Evolutionary analysis of cytochrome b sequences in some perciformes: evidence for a slower rate of evolution than in mammals. *Journal of Molecular Evolution* 39 : 589-597.

Charleston M. A. 1998. Jungles: a new solution to the host/parasite phylogeny reconciliation problem. *Mathematical Biosciences* 149:191-223.

Colwell R. K. 1986. Population structure and sexual selection for host fidelity in the speciation of hummingbird flower mites. In *Evolutionary Processes and Theory.* (Karlin S. & Nevo E. Eds), pp. 475-495. New York: Academic Press.

Cornell S. J., Desdevises Y. & Rigby M. C. 1999. Evolutionary Biology of Host-Parasite Relationships: Reality meets Models. *Trends in Ecology and Evolution.* 14(11) : 423-425.

Cunningham C. O. 1997. Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. *Journal of Parasitology* 83(2) : 215-19.

Cunningham C. O., McGillivray D. M. & MacKenzie K. 1995. Phylogenetic analysis of *Gyrodactylus salaris* Malmberg, 1957 based on the small subunit (18S) ribosomal gene. *Molecular and Biochemical Parasitology* 71 : 139-142.

Cunningham C. W., Omland K. E. & Oakley T. H. 1998. Reconstructing ancestral character states: a critical reappraisal. *Trends in Ecology and Evolution* 13(9) : 361-366.

D

Darlu P. & Tassy P. 1993. *Reconstruction phylogénétique. Concepts et méthodes.* Coll. Biologie théorique n°7. Paris: Masson.

De la Paz R. M. 1981. Note critique sur la nomenclature des *Diplodus* du groupe *sargus* (Pisces, Sparidae). *Bulletin du Muséum d'Histoire Naturelle, Paris* 4A(3) : 931-934.

De Meeûs T., Michalakis Y. & Renaud F. 1998. Santa Rosalia revisited: or why are there so many kind of parasites in "The Garden of Earthly Delights"? *Parasitology Today* 14 : 10-13.

Desdevises Y., Azouzi L., Legendre P. & Morand S. Partitioning the phylogenetically-structured environmental variation. *Journal of Evolutionary Biology*. Soumis.

Diniz-Filho J. A. F., de Sant'Ana C. E. R. & Bini L. M. 1998. An eigenvector method for estimating phylogenetic inertia. *Evolution* 52(5) : 1247-1262.

Downes B. J. 1990. Host-induced morphology in mites: implication for host-parasite coevolution. *Systematic Zoololgy* 39 : 162-168.

Dutilleul P., Stockwell J. D., Frigon D. & Legendre P. 2000. The Mantel-Pearson paradox: statistical considerations and ecological implications. *Journal of Agricultural, Biological and Environmental Statistics* 5 : 131-150.

E

Efron B. 1979. Bootstrap methods: another look at the jacknife. *Annals of Statistics* 7 : 1-26.

Euzet L. 1984. Diplectanidae (Monogenea) parasites de poissons des Iles Kerkennah (Tunisie). *Archives de l'Institut Pasteur de Tunis* 61(4) : 463-474.

Euzet L. & Combes C. 1980. Les problèmes de l'espèce chez les animaux parasites. In: Les problèmes de l'espèce dans le règne animal. Tome III. (Boquet C., Génermont J. & Lamotte M Eds). *Mémoires de la Société Zoologique Française* 40 : 239-285.

Euzet L. & Combes C. 1998. The selection of habitats among the Monogenea. *International Journal for Parasitology* 28 : 1645-1652.

Euzet L., Combes C. & Caro A. 1993. *A checklist of Monogenea of mediterranean fish.* Second International Symposium on Monogenea. Montpellier/Sète - 5-8 Juillet 1993.

Euzet L. & Oliver G. 1966. Diplectanidae (Monogenea) des Téléostéens de la Méditerranée occidentale. III. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Diplodus* (Sparidae). *Annales de Parasitologie Comparée* (Paris) 41(6) : 573-598.

Euzet L. & Oliver G. 1967. Diplectanidae (Monogenea) de Téléostéens de la Méditerranée occidentale. IV. Quelques *Lamellodiscus* Jonhston et Tiegs, 1922, parasites de poissons du genre *Pagellus* Cuvier, 1829 (Sparidae). *Annales de Parasitologie Comparée* (Paris) 42(4) : 407-425.

F

Farris J. S. 1970. Methods for computing Wagner trees. *Systematic Zoology* 19 : 83-92.

Farris J. S., Kallersjo M., Kluge A. G. & Bult C. 1994. Testing significance of incongruence. *Cladistics* 10 : 315-320.

Felsenstein J. 1985a. Phylogenies and the comparative method. *The American Naturalist* 125 : 1-15.

Felsenstein J. 1985b. Confidence limits on phylogenies : an approach using the bootstrap. *Evolution* 39(4) : 783-791.

Felsenstein J. 1988. Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetic* 22 : 521-565.

Fiedler K. 1991. Familie Sparidae. In *Lehbruch der Speziekken Zolologie. Tejl 2: Fishe* (Starck D. Ed). Gustav Fisher Verlag, Jena.

Fox L. R. & Morrow P. A. Specialization: species property or local phenomenom? *Science* 211 : 887-893.

Froese R. & Pauly D. 1995. *FishBase: a biological database on fish (version 1.2)*. Manila, Philippines: ICLARM.

Fry J. D. 1996. The evolution of host specialization: are trade-offs overrated? *The American Naturalist* 148 : S84-S107.

Futuyma D. J. & Moreno G. 1988. The evolution of ecological specialization. *Annual Review of Ecology and Systematic* 19 : 207-233.

G

Garland T., Jr, Harvey P. H. & Ives R. A. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology* 41 : 18-32.

Garrido-Ramos M. A., Jamilena M., Lozano R., Càrdenas S., Ruiz-Rejon C. & Ruiz-Rejon M. 1995. Phylogenetic relationship of the Sparidae family (Pisces, Perciformes) inferred from satellite-DNA. *Hereditas* 122 : 1-6.

Gower J. C. 1966.Some distant properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53 : 325-338.

Guégan, J.-F & Agnèse J.-F.. 1991. Parasite evolutionnary events inferred from host phylogeny: the case of *Labeo* species (Teleostei, Cyprinidae) and their dactylogirid parasites (Monogenea, Dactylogyridae). *Canadian Journal of Zoology* 69 : 595-603.

Gusev A. V. 1995. Some pathways and factors of monogenean microevolution. *Canadian Journal of Fisheries and Aquatic Sciences* 52 (Suppl. 1) : 52-56.

H

Hafner M. S. & Nadler S. A. 1990. Cospeciation in host-parasite assemblages: comparative analysis of rates of evolution and timing of cospeciation events. *Systematic Zoology* 39(3) : 192-204.

Hafner M. S. & Nadler S. A. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. *Nature* 332(6161) : 258-59.

Hafner M. S., Sudman P. D., Villablanca F. X., Spradling T. A., Demastes J. W. & Nadler S. A. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science* 265 : 1087-90.

Hanel R. & Sturmbauer C. 2000. Multiple recurrent evolution of trophic types in Northeastern Atlantic and Mediterranean Seabreams (Sparidae, Percoidei). *Journal of Molecular Evolution* 50 : 276-283.

Harvey P. H. & Pagel M. D. 1991. *The comparative method in evolutionnary biology.* Oxford University Press.

Hillis D. M. & Dixon M. T. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *Quaterly Review of Biology* 66 : 411-453.

Hoberg E. P. 1986. Evolution and historical biogeography of a parasite-host assemblage: Alcataenia sp. (Cyclophyllidea, Dilepidae) in Alcidae (Charadriiformes). *Canadian Journal of Zoology* 64 : 2576-2589.

Holmes J. C. 1973. Site selection by parasitic helminths: interspecific interactions, site segregation and the importance to development of helminth communities. *Canadian Journal of Zoology* 51 : 333-347.

Holmes J. C. & Price P. W. 1980. Parasite communities: the roles of phylogeny and ecology. *Systematic Zoology* 29 : 203-13.

Huelsenbeck J. P., Rannala B. & Yang Z. 1997. Statistical tests of host-parasite cospeciation. *Evolution* 51 : 410-419.

Huelsenbeck J. P., Rannala B. & Larget B. 2000. A Bayesian framework for the analysis of cospeciation. *Evolution* 54 : 352-364.

Humphery-Smith I. 1989. The evolution of phylogenetic specificity among parasitic organisms. *Parasitology Today* 5(12) : 385-87.

Huxley J. 1942. *Evolution: The modern synthesis*. George Allen & Unwin, London.

J

Jackson J. A., Tinsley, R. C. & Hinkel H. H. 1998. Mutual exclusion of congeneric monogenean species in a space-limited habitat. *Parasitology* (117) : 563-569.

Jaenike J. 1993. Rapid evolution of host specificity in a parasitic nematode. *Evolutionary Ecology* 7 : 103-108.

Janzen D. H. 1973. Comments on host-specificity of tropical herbivores and its relevance to species richness. In: *Taxonomy and Ecology* (Heywood V. H. Ed) pp. 201-211. Academic Press, New York.

Jovelin R. & Justine J.-L. 2001. Phylogenetic relationships within the Polyopisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. *International Journal for Parasitology* 31(4) : 393-401.

Justine J.-L. 1998. Non-monophyly of the monogeneans? *International Journal for Parasitology* 28(10) : 1653-1657

K

Kearn G. C. 1994. Evolutionary expansion of the Monogenea. *International Journal for Parasitology* 24(8) : 1227-1271.

Kennedy C. R. 1975. *Ecological Animal Parasitology*. Blackwell Scientific Publication, Oxford.

Kitahara M. & Fuji K. 1994. Biodiversity and community structure of temperate butterfly species within a gradient of human disturbance: an analysis based on the concept of generalist vs. specialist strategies. *Researches on Population Ecology* 36 : 187-199.

Klassen G. J. 1992. Coevolution: A history of the macroevolutionary approach to studying host parasite associations. *Journal of Parasitology* 78(4) : 573-587.

Klassen G. J. & Beverley-Burton M. 1987. Phylogenetic relationships of *Ligictaluridus* spp. (Monogenea: Ancyrocephalidae) and their Ictalurid (Siluriformes) hosts: and hypothesis. *Proceedings of the Helminthological Society of Washington* 54 : 84-90.

Klassen G. J. & Beverley-Burton M. 1988. North American Fresh Water Ancyrocephalids (Monogenea) with articulating haptoral bars: host-parasite coevolution. Systematic Zoology 37 : 179-189.

Kouider El Ouahed-Amine F. 1998. *Contribution à l'étude des Monogènes parasites de Poissons Sparidae (Téléostéens) du littoral algérois, approche taxonomique et écologique*. Thèse. Université des Sciences et de la Technologie Houari Boumediene. Alger.

L

Lanyon S. M. 1992. Interspecific brood parasitism in blackbirds (Icterinae): a phylogenetic perspective. *Science* 255 : 77-79.

Lapointe F.-J. 1998. How to validate phylogenetic trees? A stepwise procedure. In *Studies in Classification, Data Analysis, and Knowledge Organization: Data Science, Classification, and Related Methods* (C. Hayashi, N. Ohsumi, K. Yajima, Y. Tanaka, H. H. Bock and Y. Baba Eds) pp.71-88. Springer-Verlag Tokyo.

Le Brun N., Renaud F., Berrebi P. & Lambert A. 1992. Hybrid zones and host-parasite relationships: effect on the evolution of parasitic specificity. *Evolution* 46(1) : 56-61.

Legendre P. 2000. Comparison of permutation methods for the partial correlation and partial Mantel tests. *Journal of Statistical Computation and Simulation* 67 :37-73.

Legendre P., Desdevises Y. & Bazin E. A statistical test for host-parasite coevolution. *Systematic Biology*. Sous presse.

Legendre P., Galzin R. & Harmelin-Vivien M. L. 1997. Relating behavior to habitat: solutions to the fourth-corner problem. *Ecology* 78 : 547-562.

Legendre P. & Legendre L. 1998. *Numerical Ecology*. 2nd English Edition. Development in Environmental Modelling, 20. Elsevier.

Littlewood D. T. J., Rohde K. & Clough K. A. 1997. Parasite speciation within or between host species - phylogenetic evidence from site-specific polystome monogeneans. *International Journal for Parasitology*27(11) : 1289-1297.

Littlewood D. T. J., Rohde K. & Clough K. A. 1999a. The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. *Biological Journal of the Linnean Society* 66(1) : 75-114.

Littlewood D. T. J, Rohde K., Bray R. A. & Herniou E. A. 1999b. Phylogeny of the platyhelminthes and the evolution of parasitism. *Biological Journal of the Linnean Society* 68 : 257-287.

Ludwig H. W. 1982. Host specificity in anoplura and coevolution of anoplura and mammalia. *Mémoires du Muséum National d'Histoire Naturelle de Paris* 123 : 145-152.

Lymbery A. J. 1989. Host specificity, host range and host preference. *Parasitology Today* 5(9) : 298.

Lynch M. & Gabriel W. 1987. Environmental tolerance. *The American Naturalist* 129 : 283-303.

M

MacArthur R. H. & Levins R. 1964. Competition, habitat selection and character displacement in a patchy environment. *Proceedings of the National Academy of Sciences USA* 51 : 1207-1210.

MacDonald C. A. & Brooks D. R. 1989. Revision and phylogenetic analysis of the North American species of *Telorchis* Luehe, 1899 (Cercomeria: Trematoda: Digenea: Telorchiidae). *Canadian Journal of Zoology* 67 : 2301-2320.

McManus D. P. & J. Bowles J. 1996. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. *International Journal for Parasitology* 26(7) : 687-704.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27 : 209-220.

Matejusova I., Morand S. & Gelnar M. 2000. Nestedness in assemblages of gyrodactylids (Monogenea: Gyrodactylidea) parasitising two species of cyprinid - with reference to generalists and specialists. *International Journal for Parasitology* : 1153-1158.

Mo T. A. 1991a. Variations of opisthohaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in South-eastern Norway. *Systematic Parasitology* 20 : 1-9.

Mo T. A. 1991b. Variations of opisthohaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. *Systematic Parasitology* 20 : 11-19.

Mollaret I., Jamieson B. G. M. & Justine J.-L. 2000. Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *International Journal for Parasitology* 30(2) : 171-185.

Morand S. 1996. Biodiversity of parasites in relation with their life cycle. In: *The Genesis and Maintenance of Biological Diversity* (Hochberg M., Clobert J. & Barbault R. Eds), pp. 243-260. Oxford University Press.

Morand S., Page R. D. M., Hafner M. S. & Reed D. L. 2000. Comparative evidence of host-parasite coadaptation: body-size relationships in Pocket Gophers and their Chewing Lice. *Biological Journal of the Linnean Society* 70 : 239-249.

Morand S., Legendre P., Gardner S. L. & Hugot J.-P. 1996. Body size evolution of oxyurid (Nematoda) parasites: the role of hosts. *Oecologia* 107 : 274-82.

Morand S. & Sorci G. 1998. Determinants of life-history evolution in nematodes. *Parasitology Today* 14 : 193-196.

N

Nadler S. A., Hafner M. S., Hafner J. C. & Hafner D. J. 1990. Genetic differentiation among chewing louse population (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomydae). *Evolution* 44 : 942-951.

Neifar L. 1995. *Contribution à l'étude de la biodiversité des monogènes parasites de poissons du secteur nord-est de la Tunisie*. Mémoire de Diplôme d'Etudes Approfondies. Université de Tunis II.

Nelson G. & Platnick N. I. 1981. *Systematics and Biogeography: Cladistics and Vicariance*. Columbia University Press, New York.

Noble E. R., Noble G. A., Schad G. A. & MacInnes A. J. 1989. *Parasitology. The Biology of Animal Parasites*. 6th Edition. Lea & Febiger, Philadelphia.

Norton D. A. & Carpenter M. A. 1998. Mistletoe as parasites: host specificity and speciation. *Trends in Ecology and Evolution* 13(3) : 101-105.

O

Oliver G. 1968. Recherches sur les Diplectanidae (Monogenea) parasites de Téléostéens du Golfe du Lion. II. Lamellodiscinae nov. sub. fam. *Vie et Milieu* 19 : 95-138.

Oliver G. 1973. *Lamellodiscus obeliae* n. sp. une nouvelle espèce de Diplectanidae (Monogenea, Monopisthocotylea) parasite de *Pagellus centrodontus* (Delaroche, 1809) (Pisces, Sparidae). *Zeitschrift für Parasitenkunde* 41 : 103-108.

Oliver G. 1974. Nouveaux aspects du parasitisme des Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea) chez les Téléostéens Perciformes des côtes de France. *Comptes-Rendus de l'Académie des Sciences de Paris* 279 : 803-805.

Oliver G. 1987. *Les Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea, Dactylogyridea). Systématique. Biologie. Ontogénie. Écologie. Essai de phylogénèse*. Thèse d'état. Université des Sciences et Techniques du Languedoc.

Olson P. & Littlewood D. T. J. Phylogenetics of the Monogenea: evidence from a medley of molecules. *International Journal for Parasitology*. Sous presse.

P

Page R. D. M. 1993a. *COMPONENT 2.0. Tree comparison software for the use with Microsoft Windows*. The Natural History Museum, London.

Page R. D. M. 1993b. Genes, organisms, and areas: the problem of multiple lineages. *Systematic Biology* 42(1) : 77-84.

Page R. D. M. 1993c. Parasites, phylogeny and cospeciation. *International Journal for Parasitology* 23(4) : 499-506.

Page R. D. M. 1994a. Maps between trees and cladistic analysis of historical associations among genes, organisms, and areas. *Systematic Biology* 43(1) : 58-77.

Page R. D. M. 1994b. Parallel phylogenies: reconstructing the history of host-parasite assemblages. *Cladistics* 10 : 155-173.

Page R. D. M. & Charleston M. A. 1998. Trees within trees - phylogeny and historical associations. *Trends in Ecology and Evolution* 13(9) : 356-359.

Page R. D. M., Clayton D. H. & Paterson A. M. 1996. Lice and cospeciation: a reponse to Barker. *International Journal for Parasitology* 26(2) : 213-218.

Page R. D. M. & Hafner M. S. 1996. Molecular phylogenies of host-parasite cospeciation: gophers and lice as a model system. In *New Uses for New Phylogenies* (Harvey P. H., Brown A J. L., Maynard Smith J. & Nee S. Eds). Oxford University Press, Oxford.

Page R. D. M. & Holmes E. C. *Molecular Evolution: A Phylogenetic Approach*. Blackwell Science, Oxford.

Pagel M. D. & Harvey P. H. 1988. The taxon-level problem in the evolution of mammalian brain size: facts and artifacts. *The American Naturalist* 132 : 344-59.

Paterson A. M. & Banks J. B. 2001. Analytical approaches to measuring cospeciation of host and parasites: through a glass, darkly. *International Journal for Parasitology* 31 : 1012-1022.

Poulin R. 1992. Determinants of host specificity in parasites of freshwater fishes. *International Journal for Parasitology* 22(6) : 753-58.

Price P. W. 1980. *Evolutionary Biology of Parasites*. Princeton University Press, Princeton.

Purvis A. & Rambaut A. 1995. Comparative analysis by independent contrasts (CAIC): and Apple Macintosh application for analysis comparative data. *Computer Applied Biosciences* 11 : 247-251.

R

Reed D. L. & Hafner M. S. 1997. Host specificity of chewing lice on pocket gophers: a potential mechanism for cospeciation. *Journal of Mammalogy* 78(2) : 655-660.

Reina J., Martinez G., Amores A. & Alvarez M. C. 1994. Interspecific genetic differentiation in Western Mediterranean sparid fish. *Aquaculture* 125 : 47-57.

Rohde K. 1978. Latitudinal differences in host-specificity of marine Monogenea and Digenea. *Marine Biology* 47 : 125-134.

Rohde K. 1979. A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. *The American Naturalist.* 114(5) : 648-671.

Rohde K. 1982. *Ecology of Marine Parasites*. University of Queensland Press. St Lucia.

Rohde K. 1994. Niche restriction in parasites: proximate and ultimate causes. *Parasitology* 109 : S69-S84.

Ronquist F. 1995. Reconstructing the history of host-parasite associations using generalised parsimony. *Cladistics* 11 : 73-89.

Ronquist F. 1997. Phylogenetic approaches in coevolution and biogeography. *Zoologica Scripta* 26 : 313-322.

Rosenzweig M. L. 1987. Habitat selection as a source of biological diversity. *Evolutionary Ecology* 1 : 315-330.

Rosenzweig M. L. 1995. *Species diversity in space and time*. Cambridge University Press.

Roubal F. R. 1994. Attachment of eggs by *Lamellodiscus acanthopagri* (Monogenea, Diplectanidae) to the gills of *Acanthopagrus australis* (Pisces, Sparidae), with evidence for auto-infection and postsettlement migration. *Canadian Journal of Zoology* 72(1) : 87-95.

Roubal F. R., Quartararo N. & West A. 1996. Spatial and temporal variations in community of ectoparasites on young snapper, *Pagrus auratus* (Bloch and Schneider) (Sparidae), from the wild and captivity at Port-Hacking, Sydney, Australia. *Marine and Freshwater Research* 47(4) : 585-593.

Roughgarden J. 1972. Evolution of niche width. *The American Naturalist* 106 : 683-718.

Roy B. A. 2001. Patterns of association between crucifers and their flower-mimic pathogens: host jumps are more common than coevolution or cospeciation. *Evolution* 55(1) : 41-53.

S

Sanfilippo D. 1978. *Microhabitat des monogènes Dactylogyroidea parasites branchiaux de Téléostéens Mugilidae et Sparidae*. Thèse de Doctorat. Université des Sciences et Techniques du Languedoc.

Sasal, P., Y. Desdevises, and S. Morand. 1998. Host-specificity and species diversity in fish parasites: phylogenetic conservatism? *Ecography* 21 : 639-643.

Sasal P. & Morand S. 1998. Comparative analysis: a tool for studying Monogenean ecology and evolution. *International Journal for Parasitology* 28 : 1637-1644.

Sasal P., S. Morand & J.-F. Guégan. 1997. Determinants of parasite species richness in mediterranean marine fishes. *Marine Ecology Progress Series* 149 : 61-71.

Sasal P., Trouvé S., Müller-Graf C. & Morand S. 1999. Specificity and host predictability: a comparative analysis among monogenean parasites of fish. *Journal of Animal Ecology* 68 : 437-444.

Schmidt G. D. & L. S. Roberts. 1989. *Foundations of parasitology.* 4th ed. St.Louis/Toronto/Boston/Los Altos: Times Mirror/Mosby College Publishing.

Secord D & Kareiva P. 1996. Perils and pitfalls in the host specificity paradigm. *Bioscience* 46 : 448-453.

Simkova A., Desdevises Y., Gelnar M. & Morand S. 2000. Coexistence of nine gill ectoparasites (*Dactylogyrus* : Monogenea) parasitising the roach (*Rutilus rutilus* L.): history and present ecology. *International Journal for Parasitology* 30(10) : 1077-1088.

Simkova A., Desdevises Y., Gelnar M. & Morand S. 2001. Morphometric correlates of host specificity in *Dactylogyrus* species (Monogenea) parasites of European Cyprinid fish. *Parasitology* 123(2) : 169-177

Simpson G. G. 1953. *The major features of evolution.* Columbia University Press, New York.

Sinnappah N. D. 1998. *Phylogeography of Monogenean Polytomatidae: A molecular approach to infer the evolutionary history of this group of parasites.* Thèse de Doctorat. Université de Perpignan.

Sinnappah N. D., Lim L. S., Rohde K., Tinsley R., Combes C. & Verneau O. 2001. A paedomorphic parasite associated with a neotenic amphibian host: phylogenetic evidence suggests a revised systematic position for Sphyranuridae within Anuran and Turtle Polystomatoideans. *Molecular Phylogenetics and Evolution* 18(2) : 189-201.

Smiley J. 1978. Plant chemistry and the evolution of host specificity: new evidence from *Heliconius* and *Passiflora*. *Science* 201(25) : 745-747.

Snyder S.D. & J. Janovy, Jr. 1996. Behavioral basis of second intermediate host specificity among four species of *Haematoloechus* (Digenea: Haematoloechidae). *Journal of Parasitology* 82 : 94-99.

Solter L. F. & Maddox J. V. 1998. Physiological host specificity of microsporidia as an indicator of ecological host specificity. *Journal of Invertebrate Pathology* 71 : 207-216.

Song C. B., Near T. J. & Page L. M. 1998. Phylogenetic relationships among percid fishes as inferred from mitochondrial cytochrome b sequence data. *Molecular Phylogenetics and Evolution* 10 : 343-353.

Sorci G., Morand S. & Hugot J.-P. 1997. Host-parasite coevolution: comparative evidence for covariation of life history traits in primates and oxyurid parasites. *Proceedings of the Royal Society of London B.* 264 : 285-89.

Swofford D. L. 2001. *PAUP*: Phylogenetic analysis using parsimony and other methods. Version 4.0d8*. Champaign, Illinois: Illinois Natural History Survey.

Swofford D. L., Olsen G. J., Waddell P. J. & Hillis D. M. 1996. Phylogenetic inference. In *Molecular Systematics* (2nd Edition) (Hillis D. M., Moritz C. & Mable B. K. Eds). Sinauer Associates, Sunderland, MA.

T

Templeton, A. R. & Rothman E. D. 1974. Evolution on heterogenous environment. *The American Naturalist* 108 : 409-428.

Thompson J. N. 1994. *The coevolutionary process*. The University of Chicago Press. Chicago and London.

Timms R. & Read A. F. 1999. What makes a specialist special? *Trends in Ecology and Evolution* 14(9) : 333-334.

Tinsley R. C. & Jackson J. A. 1998. Correlation of parasite speciation and specificity with host evolutionary relationships. *International Journal for Parasitology* 28(1998) : 1573-1582.

Toft C. A. & Karter A. J. 1990. Parasite-host coevolution. *Trends in Ecology and Evolution* 5(10) : 326-29.

Tompkins D. M. & Clayton D. H. 1999. Host resource govern the specificity of swiftlet lice: size matters. *Journal of Animal Ecology* 68 : 489-500.

Tortonese E. 1975. *Fauna de Italia "Osteichthyes". Pessi Ossei.* Vol. XI. Calderini, Bologna.

V

Van Valen L. 1973. A new evolutionary law. *Evolutionary Theory* 1 : 1-30.

van Tienderen P. H. 1997. Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution* 51(5) : 1372-1380.

Verneau O, Renaud F. & Catzeflis F. M. 1997. Evolutionary relationships of sibling tapeworm species (Cestoda) parasitizing teleost fishes. *Molecular Biology and Evolution* 14 : 630-636.

W

Ward S. A. 1992. Assessing functional explanations of host specificity. *The American Naturalist* 139 : 883-891.

Westoby M., Leishmann M. R. & Lord J. M. 1995. On misinterpreting the 'phylogenetic correction'. *Journal of Ecology* 83 : 531-534.

Whitehead P. J. P., Bauchot M. L., Hureau J. C., Nielsen J. & Tortonese E. 1986. *Checklist of the fishes of the North-eastern Atlantic and of the Mediterranean*. Paris, UNESCO, Vol. III.

Wilson D. S. & Yoshimura J. 1994. On the coexistence of specialists and generalists. *The American Naturalist* 144(4) : 692-707.

Winemiller K. O. & Rose K. A. 1992. Patterns of life history diversification in North American Fishes: implications for population regulation. *Canadian Journal of Fisheries and Aquatic Sciences* 49 : 2196-2218.

Z

Zander C. D. 1998. Ecology of host parasite relationships in the Baltic Sea. *Naturwissenschaften* 85 : 426-436.

VI. ANNEXES

A. ParaFit: une nouvelle méthode pour l'étude de la coévolution hôte-parasite

LEGENDRE Pierre, Yves DESDEVISES and Eric BAZIN. ParaFit: a new statistical method to assess host-parasite coevolution. *Systematic Biology*. Sous presse.

Cet article présente une nouvelle méthode, ParaFit, pour étudier la coévolution hôte-parasite. Cette méthode compare les matrices de distance phylogénétique des hôtes et des parasites. Les matrices sont comparées par l'intermédiaire des associations hôte-parasite du système. La méthode peut considérer n'importe quel nombre de parasites par hôte ou d'hôtes par parasite. ParaFit permet de tester statistiquement par permutations la congruence entre les deux matrices de distance phylogénétique, ainsi que la contribution de chaque lien hôte-parasite individuel. L'erreur de type I, la puissance, ainsi que le comportement de la méthode sous diverses situations ont été évalués par une série de simulations. Enfin, un exemple d'utilisation de ParaFit sur des données réelles est présenté ; il s'agit de l'exemple classique de la coévolution entre les gaufres ("pocket gophers") et leurs poux ("chewing lice").

Participation du thésard :

- Recherche bibliographique
- Participation au design des simulations
- Analyse du cas test
- Rédaction (en particulier, des paragraphes de l'introduction et de la discussion)

A Statistical Test for Host-Parasite Coevolution

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Abstract. — A new method, ParaFit, has been developed to test the significance of a global hypothesis of coevolution between parasites and their hosts. Individual host-parasite association links can also be tested. The test statistics are functions of the host and parasite phylogenetic trees and of the set of host-parasite association links. Numerical simulations are used to show that the method has correct rate of type I error and good power except under extreme error conditions. An application to real data (pocket gophers and chewing lice) is presented.

Key words: Coevolution, Fourth-corner statistic, Host-parasite, Permutation test, Phylogenetic analysis, Power analysis, Statistical test, Simulations.

Running head: Host-parasite coevolution

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Parasites generally form tight ecological associations with their hosts. Biologists have assumed for a long time that the evolution of parasites was highly dependent upon that of their hosts (Barrett, 1986; Klassen, 1992). This has led to the establishment of several "rules", such as Farenholz's Rule (1913) that "parasite phylogeny mirrors host phylogeny", or Szidat's Rule (1940) that "primitive hosts harbour primitive parasites". A history (up to 1991) of host-parasite coevolution studies was written by Klassen (1992). The term "coevolution" was introduced by Ehrlich and Raven (1964) in a study on butterflies and their plant hosts. In this paper, coevolution is defined as the extent to which the host and parasite phylogenetic trees are congruent. Congruence is the degree to which parasites and their hosts occupy corresponding positions in the phylogenetic trees. Perfect congruence is a good indicator of host and parasite cospeciation; a total absence of congruence indicates random associations in their evolutionary history. This corresponds to the definition of Brooks (1979, 1985) and Klassen (1992), which refer to the macroevolutionary context.

Until the work of Brooks (1977, 1981) at the end of the 1970's, no rigorous analytical method had been developed to study host-parasite coevolution. Several methods have been developed to do so in subsequent years: Brooks Parsimony Analysis (BPA: Brooks and McLennan, 1991), Component analysis (Component: Page, 1993a), a method based on reconciled phylogenetic trees (TreeMap: Page, 1994), event-based methods (TreeFitter: Ronquist, 1995, 1997; Jungles: Charleston, 1998), and a maximum likelihood-based test (Huelsenbeck et al., 1997). This more rigorous framework led to the publication of several studies about host-parasite coevolution (e.g., Brooks and Glen, 1982; Hafner and Nadler, 1988, 1990; Klassen and Beverley-Burton, 1988; Demastes and Hafner, 1993; Page, 1993b; Paterson et al., 1993; Hafner et al., 1994; Page and Hafner, 1996; Boeger and Kritsky, 1997; Roy, 2001). This topic has gained considerable importance in evolutionary biology (Futuyma and Slatkin, 1983; Brooks and McLennan, 1991; Thompson, 1994; Page and Holmes, 1998).

The above-mentioned methods treat differently the different kinds of evolutionary events occurring in a host-parasite association (Ronquist, 1997, Page and Charleston, 1998). As a consequence, they can produce different results. The simultaneous evolution of hosts and parasites can exhibit four main different kinds of events (Ronquist, 1997; Charleston, 1998, Page and Charleston, 1998): cospeciation (simultaneous speciation of a

host and its parasite), duplication (independent parasite speciation), lineage sorting (disappearance of a parasite lineage on a host lineage), and host switching (colonization of a new host by a parasite). A drawback common to all the above-mentioned methods is that, as the numbers of hosts and parasites increase, optimal solutions become hard to find because the problem becomes highly computer-intensive. These methods are ideally designed for the one host-one parasite case. They aim at reconstructing a putative history of the host-parasite association, by adequately mixing the types of events and trying to minimize the overall cost of the estimated evolutionary scenario. They attempt to answer the following question: what is the most probable coevolutionary history of the host-parasite association, given the costs of the different events?

PRINCIPLE OF THE TEST

In the present paper, we want to know if the data agree with a model of coevolution of the hosts and parasites. The corresponding null hypothesis (H_0) is that the evolution of the two groups, as revealed by the two phylogenetic trees and the set of host-parasite association links, has been independent, which is the same as saying that one is random with respect to the other. Strict coevolution requires two conditions to be fulfilled:

1. Ideally, the "true phylogenies" of the hosts and parasites should be the same. In ectoparasites, for instance, the similarity may be due to the geographic separation of the host lineages, which leads to allopatric speciation of the hosts and parasites (e.g. Barker, 1994; Hafner et al., 1994). These phylogenies are only known by the estimates that have been worked out by researchers; these estimates may be imperfect.

2. The hosts and parasites located in corresponding positions of their respective trees must be associated (linked).

Ideally, there should be a one-to-one relationship between the hosts and parasites, but the existence of several parasites per host or several hosts per parasite does note rule out a strict pattern of coevolution generated, for example, by parasite intrahost speciation, or by host speciation not followed by parasite speciation (which is called 'parasite inertia' by Paterson and Banks, 2001). In all cases, each host-parasite link must be assessed separately

for its fit to the coevolution hypothesis, given the uncertainties of the estimates of the two phylogenetic trees and the H-P association matrix.

Coevolution can be tested by the method described in this paper, which focuses on the structure of the two trees and the matrix of host-parasite links. The global null hypothesis is that evolution of the hosts and parasites has been independent. One can also consider hypotheses about individual host-parasite links in order to estimate the contribution of each link to the overall relationship.

DESCRIPTION OF THE PARAFIT TEST STATISTICS

ParaFit statistic for the global test of host-parasite coevolution

Statistically assessing a hypothesis of host-parasite (H-P) coevolution requires combining three types of information that are jointly necessary to describe the situation. These elements are: the phylogeny of the parasites, that of the hosts, and the observed host-parasite associations (Fig. 1). A phylogeny can be described by a matrix of patristic distances among the species along the tree (Lapointe and Legendre, 1992). These in turn can be transformed into a matrix of principal coordinates (Gower, 1966; principal coordinate analysis is also described in textbooks of biological multivariate statistics, e.g., Legendre and Legendre, 1998) having n rows and at most $(n - 1)$ columns. If one is only interested to use the tree topology, without consideration of the differences in branch lengths, one may code the trees with branch lengths of 1 before computing the patristic distance matrix. Matrices **B** and **C** are obtained in that way; they respectively represent the phylogeny of the parasites and that of the hosts. Matrix **A** represents the host-parasite associations: it has the parasites in rows and the hosts in columns; it contains a "1" where a parasite was empirically found to be associated to a host and "0"s elsewhere.

If the reconstructed phylogeny for either the hosts or parasites, or both, is uncertain (poorly resolved phylogeny or uncertainty among several almost equivalent trees), one can, instead of patristic distances, use a matrix of phylogenetic distances computed directly from the raw data (morphology, DNA sequences, etc.). Matrix **B** or **C** can be derived from it using principal coordinate analysis. Since distances do not necessarily obey the four-point

condition (Buneman, 1974), they are more remote from the "true tree" than an estimated phylogeny would be and, thus, may be considered to represent a more noisy form of information, but this does not invalidate the use of the corresponding principal coordinates for testing a hypothesis of host-parasite coevolution.

Matrices **A**, **B**, and **C** can be combined in a meaningful way if they are positioned as in Fig. 2. Note that matrix **C** is transposed (the principal coordinates now form the rows of **C**) to insure that its columns correspond to the hosts, like the columns of **A**. Figure 2 suggests that the host-parasite association can be described by a matrix **D** which crosses **B** and **C** and depicts the H-P association between the two phylogenies. **D** can be obtained by the matrix operation

$$\mathbf{D} = \mathbf{C}\,\mathbf{A}'\,\mathbf{B} \qquad (1)$$

described by Legendre et al. (1997; see also Legendre and Legendre, 1998, Section 10.6) who coined the expression "fourth-corner statistics" to describe the individual values in matrix **D**, as well as some global statistic synthesizing the information in **D**. These authors have shown that when the variables in the columns of **B** and the rows of **C** are quantitative, the individual parameters in **D** are cross-products weighted by the presence-absence (1-0) values found in **A**.

There is no point in trying to interpret the individual parameter estimates found in **D** since they cross principal coordinates that are not meant to be individually interpretable in the present context. Instead, we will derive a global host-parasite statistic that will be used to test the hypothesis of coevolution. We will use the sum of squares of the values d_{ij} found in matrix **D** as the global statistic to test the hypothesis of coevolution:

$$ParaFitGlobal = trace = \sum (d_{ij}^2) \qquad (2)$$

This is analogous to a trace statistic computed on the matrix of sum of squares and cross products (SSCP) among either the rows or the columns of **D** — except for the centering, either by rows or by columns, that would be required to obtain a real SSCP matrix and its trace. We have, in the present case, no preference between the SSCP matrix among the rows of **D** (which would require centering by rows) or among the columns of **D** (which would require centering by columns); so we use no centering at all. For justification, we

will provide numerical simulations showing that the global *ParaFitGlobal* statistic has correct type I error.

ParaFit statistics for the tests of individual host-parasite association links

The procedure described in the next Section will allow us to test the individual links written in matrix **A**, which represent observed host-parasite associations. Two statistics have been developed to do that. They are both based upon the idea that the global statistic should decrease in value if we remove from **A** a link that represents an important contribution to the host-parasite relationship.

For the test, let us consider the host-parasite (H-P) link k from matrix **A**. The value "1" that represents this link in matrix **A** is replaced by a "0". We thus obtain a new matrix **A**(k). We now compute

$$trace(k) = \sum (d_{ij}^2) \qquad (3)$$

where the d_{ij}'s are now the values resulting from the product $\mathbf{D} = \mathbf{C}\,\mathbf{A}(k)'\,\mathbf{B}$. Using the *trace* statistic form Eq. 2, we obtain from the following equation a first test statistic for an individual link k:

$$ParaFitLink1(k) = trace - trace(k) \qquad (4)$$

This formula measures the contribution of link k to the global *trace* statistic.

The second statistic is constructed like a partial F-statistic (see for instance Sokal and Rohlf, 1995, Eq. 16.14) that would have lost its degrees of freedom in the numerator and denominator. This loss is of no consequence in permutation testing because the reference and permuted values of the statistic are affected in the same way by a constant multiplicative term, so that the ordering of the reference and permuted values (>, =, or <) is not changed by eliminating the degrees of freedom. The numerator of the statistic is (*trace* – *trace*(k)) from Eq. 4. The denominator is analogous to a residual sum of squares. To construct it, we need a measure of the maximum trace value that can occur in **D**. The maximum possible value occurs when the hosts and parasite phylogenetic trees are fully congruent; this is a relevant reference situation to test a hypothesis of coevolution. In that case, it can be shown that the trace of **D** is equal to the sum of squares of the eigenvalues of

the principal coordinates found in matrix **B** or matrix **C**. In principal coordinate analysis, the eigenvalues measure the variances of the principal coordinates (Gower, 1966; Legendre and Legendre, 1998). In most empirical situations, the estimates of the two phylogenetic trees will not be exactly alike, so that we will actually use:

TraceMax = max(sum of squared eigenvalues of **B**, sum of squared eigenvalues of **C**)(5)

as our estimate of maximum trace value. The second test statistic that we are proposing for an individual link *k* is thus:

$$ParaFitLink2(k) = (trace - trace(k))/(TraceMax - trace) \quad (6)$$

This statistic cannot be used when the host and parasite phylogenetic trees are identical because, in that situation, the denominator of Eq. 6 is 0. This situation may happen in simulation work but should rarely occur when analyzing empirical data.

On occasions, "*trace(k)*" may happen to be slightly larger than "*trace*". This is of no consequence since it indicates a situation where the H-P relationship is stronger without link *k* than with it. In other words, link *k* does not increase the global H-P relationship and, thus, is not indicative of host-parasite coevolution. The test of significance (next Section) will never find such a link significant; this is the correct answer.

TESTING PROCEDURES

Novel statistics, such as described in Eqs. 2, 4 and 6, may be tested for significance using the method of permutations, also called randomization, which is now widely used in biological work. The method is described in several texts such as Sokal and Rohlf (1995), Manly (1997), and Legendre and Legendre (1998).

Global test of host-parasite coevolution

Consider matrices **A**, **B,** and **C** described above. The two phylogenetic trees are given as fixed because they have been formed through evolutionary time; we are not directly interested in testing their similarity. The random component is clearly the set of association links found in matrix **A**, which, under the null hypothesis, may change through ecological time. Siddall (1996) provides other arguments in favor of randomizing the host-parasite

associations. The test will involve random permutations of the hosts associated with each parasite. The reason is that the parasites parasitize the hosts, not the opposite. So, if there is no coevolutionary H-P association, each parasite species should parasitize hosts selected at random on the host phylogenetic tree. This is the null hypothesis of the test (H_0). The alternative hypothesis (H_1) is that the positions of the individual H-P links are not random but associate corresponding branches of the two evolutionary trees; in that sense, they are critical to the overall H-P association. The testing procedure is the following:

1. Compute matrix **D** using Eq. 1. Compute statistic *ParaFitGlobal* using Eq. 2, which provides the reference value ($ParaFitGlobal_{ref}$) for the test. Save this value as $trace_{ref}$ in view of the tests of individual host-parasite links, described below.

2. To obtain a realization of the null hypothesis, permute at random the values within each row of matrix **A**, and this independently from row to row. Recompute matrix **D** using Eq. 1, and statistic *ParaFitGlobal* using Eq. 2. This provides a value (*ParaFitGlobal**) of the statistic under permutation. Save each value *trace** = *ParaFitGlobal** in view of the tests of individual host-parasite links (below).

3. Repeat step 2 a large number of times to obtain an estimate of the distribution of the statistic under permutation. Add the reference value $ParaFitGlobal_{ref}$ to the distribution, following Hope (1968).

4. Calculate the one-tailed probability (*P*-value) of the data under the null hypothesis as the proportion of values in the *ParaFitGlobal** distribution that are larger than or equal to $ParaFitGlobal_{ref}$. The test indicates that the data are unlikely to correspond to the null hypothesis if $ParaFitGlobal_{ref}$ is larger than or equal to most (say, 95% for $\alpha = 0.05$) of the *ParaFitGlobal** values obtained under permutation.

Tests of individual host-parasite association links

Tests of individual host-parasite association links can also be computed to determine the probability that individual H-P links conform to the null hypothesis. This is done as follows:

1. Compute *TraceMax* from matrices **B** and **C** using Eq. 5.

2. Choose a H-P link *k* and remove it from matrix **A**.

3. Compute matrix **D** for the modified matrix **A** using Eq. 1. Compute *trace(k)* using Eq. 3. Calculate the values of the two reference statistics for the tests: $ParaFitLink1(k)_{ref}$ (Eq. 4) and $ParaFitLink2(k)_{ref}$ (Eq. 6), using the value $trace_{ref}$ saved from the global test of coevolution.

4. Permute at random the values within each row of matrix **A**, independently from row to row, using the same sequence of random permutations as were used during the global test of host-parasite coevolution (above). Recompute matrix **D** using Eq. 1. and *trace(k)** using Eq. 3, followed by the values of the two statistics under permutation: *ParaFitLink1(k)** (Eq. 4) and *ParaFitLink2(k)** (Eq. 6), using the first value *trace** saved from the global test of coevolution.

5. Repeat step 2 a large number of times to obtain an estimate of the distribution of the two statistics under permutation. Add the reference values $ParaFitLink1(k)_{ref}$ and $ParaFitLink2(k)_{ref}$ to the distributions, following Hope (1968).

6. Calculate the one-tailed probabilities (*P*-values) of the data under the null hypothesis as the proportions of values in the *ParaFitLink1(k)** and *ParaFitLink2(k)** distributions that are larger than or equal to $ParaFitLink1(k)_{ref}$ or $ParaFitLink2(k)_{ref}$ respectively. The tests indicate that link *k* is unlikely to be random with respect to the coevolutionary structure if $ParaFitLink1(k)_{ref}$ or $ParaFitLink2(k)_{ref}$ are larger than or equal to most (say, 95% for α = 0.05) of the *ParaFitLink1(k)** and *ParaFitLink2(k)** values obtained under permutation.

NUMERICAL SIMULATIONS

Type I error

Simulations have been performed to check the type I error and power of the test of host-parasite coevolution. Type I error occurs when the null hypothesis is rejected while the data conform to H_0. To be valid, a test of significance should have a rate of rejection of the null hypothesis no larger than the nominal (α) significance level of the test when H_0 is true (Edgington, 1995).

To study type I error, the simulated data used random phylogenetic trees for the hosts and parasites, and a matrix **A** containing links sampled at random (without replication) from among all possible host-parasite links. Unrooted random additive (i.e., phylogenetic) trees were generated following the method of Pruzansky et al. (1982). The two random trees were transformed into matrices **B** (for the parasites) and **C** (for the hosts) using principal coordinate analysis. The statistics for the global test and for the individual H-P links were calculated and tested using random permutations.

A simulation study can never explore all parameter combinations. The simulation effort reported in this paper for type I error involves the following parameter combinations which were thought to represent commonly encountered situations in host-parasite studies:

- 10 hosts, 10 parasites. Number of random H-P links: {5, 10, 15, 20, 25}.
- 10 hosts, 15 parasites. Number of random H-P links: {5, 10, 15, 20, 25}.
- 15 hosts, 10 parasites. Number of random H-P links: {5, 10, 15, 20, 25}.

For each combination of parameters, 10000 random simulations were produced. 99 random permutations were used in each test of significance. The statistic of interest is the rate of rejection of the null hypothesis, called "Type I error rate", which was computed for various significance levels $\alpha = \{0.01, 0.02, 0.03, 0.04, 0.05\}$, together with 95% confidence intervals based upon the results of the 10000 independent simulations. A test of statistical significance is valid if the rejection rate is not larger than the significance level α, for any value of α, when the null hypothesis is true (Edgington, 1995). So, one hopes that the rate of rejection of the null hypothesis will be approximately equal to α whose value should lie within the 95% confidence interval of the rejection rate.

Power

A test of significance should be able to reject the null hypothesis in most instances when H_0 is false. The ability to reject H_0 in these circumstances is referred to as the power of the test. In the present study, power is defined as the rate of rejection of the null hypothesis when H_0 is false by construct. Power was studied using the same type of

simulations as described above, except that this time the alternative hypothesis (H_1) was made to be true. Three types of simulations were done:

1. A random additive tree was generated, as described above, for each simulation, but here the same tree was used for the parasites (matrix **B**) and the hosts (matrix **C**); this is a condition for coevolution. A H-P link was created between each host and the parasite found in the corresponding position on the tree. Following that, a certain percentage of randomly-located links were *added* to matrix **A** (without replication of existing links), using the same procedure as in the type I error study. The simulation parameters were the following:

• 10 hosts, 10 parasites, 10 basic H-P links. Number of random H-P links *added* to the basic links: {0%, 10%, 20%, ..., 100%}, or {0, 1, 2, ..., 10} supplementary random links, for a total of {10, 11, 12, ..., 20} links.

2. In the second series of power simulations, a random additive tree was generated for each simulation, and the same tree was used for the parasites (matrix **B**) and hosts (matrix **C**). H-P links were created between each host and the parasite in the corresponding position on the common tree. Following that, a certain percentage of randomly-located links were *removed* from the list and *replaced* (without replication of existing links) by randomly-located links. The simulation parameters were the following:

• 10 hosts, 10 parasites, 10 basic H-P links. Number of random H-P links *replacing* basic links: {0%, 10%, 20%, ..., 100%} or {0, 1, 2, ..., 10} links.

3. In the third type of power simulations, the host and parasite phylogenetic trees were made to have a common portion while the remainder of each tree was random. The number of coevolving species was the main parameter in these simulations. (In the previous type of simulations, the trees were the same, but some of the links were random. In the present simulations, the two trees were only partly similar; coevolutionary links were created only in the similar portions of the trees while the remaining links were random.) The simulation parameters were the following:

• 10 hosts, 10 parasites, 10 H-P links. Number of species that shared the same tree (coevolving species): {0, 1, 2, 3, 4, 5, 6, 7, 8, 9}; hence the number of independent species (not coevolving) was {10, 9, 8, 7, 6, 5, 4, 3, 2, 1}.

In all power simulations, there were 10000 independent simulations per combination of parameters, and 99 permutations per test.

Power was computed for various significance levels α = {0.01, 0.02, 0.03, 0.04, 0.05}. The 95% confidence intervals were not plotted because they would have made the graphs difficult to read. We want to identify the simulated situations where power is low, medium, or high; this will provide guidance for the interpretation of real-case studies. We also want to see if one of the statistics (*ParaFitLink1* and *ParaFitLink2*) created for the tests of individual host-parasite links has higher power than the other, at least in some situations.

All the simulations described above were repeated using random rooted ultrametric trees instead of unrooted additive trees. The generation of random ultrametric trees for n species involved two steps, described by Lapointe and Legendre (1991):

1. Random node positions were obtained by drawing (n – 1) numbers at random from a uniform (0, 1] distribution and placing these values in the subdiagonal of a (n x n) matrix. The remainder of the matrix was filled using the procedure FillMat of Lapointe and Legendre (1991).

2. The species (1 to n) were attributed at random to the leaves of the tree.

This obtains a random ultrametric tree, which is a special type of phylogenetic tree. The simulation results were very similar to those obtained using random unrooted additive trees. Only the latter are discussed in detail below.

Simulation Results

Global test of host-parasite coevolution

Type I error was correct in all cases, so we can conclude that the global test is valid. Figure 3a shows the results for random and independent phylogenies for the hosts and the parasites, for equal numbers of hosts (10) and parasites (10) and various numbers (5 to 25) of random H-P links; the significance level used in the permutation tests (α = 0.01, 0.02, 0.03, 0.04, 0.05) was always within the 95% confidence interval of the type I error rate. The

simulations for type I error were repeated using unequal numbers of hosts and parasites (10, 15 and 15, 10), with results similar to those presented in Fig. 3a: the significance levels of the permutation tests were always within the confidence intervals of the type I error rates computed from the 10000 simulations. Similar results were obtained again when the two phylogenies were the same, the H-P links being positioned at random.

The power study is based upon simulations in which the null hypothesis is false by construct; we are thus certain that the data used in these simulations represent simulated cases of coevolution. When the data exactly conformed the hypothesis of coevolution, power was maximum, the null hypothesis being rejected in nearly all cases (Figs. 3b and 3c, number of random links or supplementary random links = 0). Power decreased as the number of supplementary random links added to the coevolutionary structure increased (Fig. 3b). For $\alpha = 0.05$, power was about 55% when there were as many supplementary random links (10) as coevolutionary links (10).

Power also decreased as coevolutionary links were replaced by random links (Fig. 3c). When all coevolutionary links were replaced by random links (10 random links in Fig. 3c), the rejection level, which measures type I error in that case, was equal to the α significance level of the test. This is not a surprising result; it simply indicates that the simulation method used to generate replacement links was correct.

When the host and parasite phylogenetic trees contained a common portion, the remainder of each tree being random, power was affected by the proportion of coevolving species (Fig. 3d): with no coevolving species (left of the graph), we are in a situation where H_0 is true, and the simulation results are identical to those of Fig. 3a. Power increased with the number of coevolving species, to reach a maximum when all species were coevolving; the rejection rate was then 100% or very nearly so, as in Figs. 3b and 3c (left). As the number of coevolving species increased, the global test was more likely to identify as significant the coevolutionary relationship present in portions of the trees, but power was low when there were less than 70% coevolving species. The effect on the global test is the same as when the host and parasite trees were the same but some of the coevolutionary links were replaced by random links (Fig. 3c, abscissa reversed). We conclude that

coevolution may not be detected by the global test when a good portion of the hosts and parasites are not coevolving.

The third series of power simulations was repeated with larger numbers of hosts and parasites and more links. Power of the global test increased with host and parasite sample sizes, for given proportions of coevolutionary links.

Tests of individual host-parasite association links

In tests of individual host-parasite association links, type I error was always correct for both statistics, *ParaFitLink1* and *ParaFitLink2*, using equal (10, 10) or unequal (15, 10 or 10, 15) numbers of hosts and parasites. So the two statistics provide valid tests of significance. As an example, Figs. 4a and 4b present the type I error rates obtained for the first H-P link of each simulation, in a 10000-simulation run involving 10 hosts and 10 parasites.

For pure coevolutionary structures, the test of an individual link using statistic *ParaFitLink1* had good power, but not as good as the global test. Compare Fig. 3b to Fig. 5a (left, where the number of supplementary links is 0); Fig. 3c to Fig. 6a (left, where the number of random links is 0); and Fig. 3d to Fig. 7a (right, where all 10 species were coevolving). Tests using *ParaFitLink2* also have fairly good power, but this cannot be shown for pure coevolutionary structures for reasons given above. Power of individual tests increases with the number of hosts and parasites for given proportions of coevolutionary links (simulation results not shown in detail).

In the presence of supplementary random links (supplementary to a saturated coevolutionary model containing a coevolutionary links for every H-P pair), statistic *ParaFitLink1* generally had greater power than *ParaFitLink2* for detecting the links that significantly contributed to host-parasite coevolution (Fig. 5). As noted above, statistic *ParaFitLink2* cannot be computed for a perfect coevolutionary structure in the absence of random links; this is why no rejection rate is reported, in Fig. 5b, for no supplementary link.

Power of the test of individual coevolutionary H-P links decreased as the proportion of supplementary random links increased. For *ParaFitLink1* for instance (Fig. 5a), the

presence of as many supplementary links (10) as there were coevolutionary links (10) reduced power by about half, compared to the simulations without supplementary random links. With *ParaFitLink1*, the probability of correctly detecting a coevolutionary link (Fig. 5a) was 1.5 to 2.5 times larger than that of wrongly declaring a random link significant (Fig. 5c). The difference is not as great for statistic *ParaFitLink2* (compare Figs. 5b and 5d). So, the *ParaFitLink1* statistic is preferable in this situation. (In Fig. 5d, a single supplementary link added to a perfect coevolutionary structure cannot be tested because the test of significance requires that the added link be removed, which brings us back to the perfect coevolutionary case where the *ParaFitLink2* statistic cannot be computed.) Figs. 5c and 5d also show that in the presence of coevolutionary links, type I error for the tests of the random links was greater than α; as a consequence, test results in this situation have to be interpreted in a conservative manner.

When coevolutionary links were replaced by random links, the coevolutionary model was no longer saturated, meaning that it did not contain a coevolutionary link for each H-P pair. In that case, statistic *ParaFitLink2* had greater power than *ParaFitLink1* for detecting the links that significantly contributed to host-parasite coevolution (Figs. 6a, 6b). With both statistics, the probability of correctly detecting a coevolutionary link (Figs. 6a, 6b) was 1.5 to 2 times larger than that of wrongly declaring a random link significant (Figs. 6c, 6d) in the presence of 20% random links. Note that the graphs in Figs. 6c and 6d converge towards the alpha significance level when all 10 coevolutionary links have been replaced by random links. Figs. 6c and 6d also show that in the presence of coevolutionary links, the type I error on the random links was greater than a; as a consequence, test results in this situation have to be interpreted in a conservative manner.

When the host and parasite phylogenetic trees contained a common portion, the remainder of each tree being random, power and type I error were affected by the proportion of coevolving species (Fig. 7). Power to detect significant coevolutionary links increased with the proportion of coevolving species (Fig. 7a, b) in the same manner as the global test of significance (Fig. 3d). On the other hand, type I error for the test of the random links increased well above the a significance level when the number of coevolutionary species reached about one half the total number of species; the effect on

statistic *ParaFitLink1* was less important than on statistic *ParaFitLink2* (Fig. 7c, d), so that *ParaFitLink1* seems preferable. The probability of correctly detecting a coevolutionary link was larger than that of wrongly declaring a random link significant by about the same factor with both statistics (compare Figs. 7a and 7c on the one hand, and Figs. 7b and 7d on the other), so that the two statistics are equivalent from that point of view. Considering the smaller degree of inflation of type I error displayed by *ParaFitLink1*, compared to *ParaFitLink2*, statistic *ParaFitLink1* seems preferable in this situation. In any case, test results in this situation have to be interpreted with caution.

Further simulations conducted with larger numbers of hosts and parasites and more links showed that power of the *ParaFitLink1* and *ParaFitLink2* tests increased with host and parasite sample sizes, for given proportions of coevolutionary links.

Interpretation of the Test Results

1. The null hypothesis of the global test of significance for host-parasite coevolution is that the evolution of the two groups, as revealed by the two phylogenetic trees and the set of host-parasite association links, has been independent. Test results are interpreted as follows:

• When the global test is significant, it has detected a significant host-parasite association, with a probability of type I error equal to α.

• When the global test is not significant, it may mean either that there is no host-parasite association, or that the host-parasite association is masked by supplementary random H-P links (see the results of the first series of power simulations in which randomly-located links were added to matrix **A**), or that the structure is a mixed one with part of the two trees coevolving while other portions are not coevolving (see the results of the second and third series of power simulations). The test has good power only when most of the host and parasite species are coevolving and there are not too many random links. Tests of individual H-P links are still possible but results must be interpreted in a conservative way, see below.

2. In the tests of individual host-parasite association links, the null hypothesis is that the link under test is random. Results of tests of individual links are interpreted as follows:

• When the global and the test of an individual link are both significant, the test has detected a significant host-parasite link, with a probability of type I error equal to α.

• When the global test is significant and the test of an individual link is not, the data do not support the hypothesis that the link represents a coevolutionary link. Since the test of individual links has less power than the global test, the test of a host-parasite link based upon a small number of hosts, parasites, and coevolutionary links may turn out not to be significant due to lack of power.

• When the global test is not significant but the test of an individual link is significant, this indicates that we are dealing with a mixed structure containing perhaps a coevolutionary structure with some random links added (as in the first series of power simulations), or a coevolutionary and a random portion (as in the second and third series of power simulations). Only the links that are found to be very highly significant should be considered, in order to compensate for the fact that the tests of individual links have inflated type I error in this situation.

• When neither the global test nor the test of an individual link are significant, the link is unlikely to represent a coevolutionary host-parasite association.

The *ParaFitLink1* statistic generally behaved better in simulations and should be preferred to *ParaFitLink2*.

Application of the Test: Gophers and Lice

We tested our method using phylogenetic trees for pocket gophers and their chewing lice (Hafner and Nadler, 1988, 1990; Hafner et al., 1994) reconstructed from the mtDNA cytochrome-oxidase I sequences used in Hafner et al. (1994). This data set has become a test case for coevolutionary studies, and has been re-examined several times using new methods (e.g. Ronquist, 1995; Page, 1996; Charleston, 1998). We used Hasegawa-Kishino-Yano (HKY85) corrected distances because of an observed heterogeneity of nucleotide frequencies and the occurrence of a transition bias. Trees were reconstructed using the

neighbor-joining (NJ) method (Saitou and Nei, 1987) available in the program PAUP* (Swofford, 2001). Neighbor-joining was used to quickly obtain an estimate of the phylogeny of the hosts and parasites; this should not be taken as an endorsement of NJ as the best method for exploring tree space. The trees that we obtained differ slightly from those published by Hafner et al. (1994) because we used distances corrected under a different evolutionary model. This produced a topology slightly different from that published in the Hafner et al. (1994) paper. The exact tree topology is of little importance for illustrating our method in the present study.

Coevolution was first tested using the global ParaFit statistic (H_0: evolution of the hosts and parasites has occurred independently). The result indicates that the null hypothesis must be rejected (permutational $P = 0.001$ after 999 permutations). This gives support to the alternative hypothesis (H_1) of coevolution, revealed by the similarity of the two phylogenetic trees and the matrix of host-parasite association links. This result is in agreement with previous studies on this host-parasite model: they suggest an important level of cospeciation between pocket gophers and their chewing lice. When we assessed the significance of each host-parasite association using the *ParaFitLink1* statistic (tested at $\alpha = 0.05$), seven of the 17 H-P links were not significant (Fig. 8).

The significant links display extensive coevolution between the hosts and parasites. The discrepancies between the two phylogenies are associated with the non-significant H-P links. If we remove them from Fig. 8, and remove also the species that have no significant H-P link, we end up with two identical phylogenetic trees displaying perfect coevolution for a subset of the gophers and lice (Fig. 9).

The ParaFit method does not intend to infer an evolutionary scenario explaining the observed historical association between gophers and their lice, contrary to the TreeMap method for example. It allowed us, however, to identify (a) the hosts and parasites that are likely to have undergone cospeciation, and (b) the species that have most likely been subjected to host-switching or sorting events (parasite extinction, or primary absence on daugther host lineage).

DISCUSSION

We have described a new method designed to answer the following biological question: are the host and parasite phylogenetic trees congruent? In other words, do the parasites tend to use hosts that occupy corresponding positions in the phylogenetic tree? The ParaFit method allows a statistical test of this particular global hypothesis of coevolution to be done, *and also* a test of significance of each host-parasite link contributing to the relationship, leading to the identification of the species involved in cospeciation. Through this process, the incongruent host-parasite links are also identified; these links are often worth examining. Previously described methods either did not provide statistical tests (such as BPA), or tested only a global fit (such as TreeMap), or else tested the contributions of different kinds of events to the overall relationship (such as TreeFitter). ParaFit permits to deal with any kind of host-parasite association in a reasonable amount of computing time.

Johnson et al. (2001) have recently proposed a method, involving several calculation steps, whose objective is very similar to that of ParaFit: it was meant to identify the incongruences in the list of host-parasite links, in order to produce a joint scenario of the cospeciation and incongruent events. Noteworthy is the fact that they used cospeciation as the null model for statistical testing; the test admittedly overestimated the number of congruent (i.e., cospeciation) events. In ParaFit on the contrary, the null model is that of independence of the host-parasite associations. In hypothesis testing, it is better to identify cospeciation events by rejecting a null hypothesis with a known (and small) type I error, than by failing to reject a null hypothesis of cospeciation with an unknown (and usually large, especially with small sample size) type II error. The Johnson et al. method certainly takes much longer to compute than ParaFit, and it would be hard to compute it using moderate to large data sets.

Another advantage of ParaFit is that if, for some reason, one or the other phylogenetic tree is not available, one can use phylogenetic distances instead of trees; this is a nice feature since distances can be directly computed from raw data (morphology, sequences, etc.), without having to reconstruct a tree. The distance matrix is transformed into a rectangular matrix by principal coordinate analysis before being used in the ParaFit

program. This can be interesting in the case of poorly resolved phylogenies or multiple trees, which often present a problem in this kind of studies, or else if one does not want or need to estimate the phylogeny. On the other hand, when phylogenetic trees are available, they can be expressed as distance matrices by calculating patristic distances among the species (which are the leaves of the tree). The patristic distance matrices, transformed by principal coordinate analysis, are then used in the ParaFit tests.

The statistics *ParaFitLink1* and *ParaFitLink2* both have their usefulness. *ParaFitLink1* has greater power for correctly detecting coevolutionary links in saturated coevolutionary models in which additional random links are present, whereas *ParaFitLink2* has greater power for correctly detecting coevolutionary links in unsaturated coevolutionary models in which only a fraction of the links are coevolutionary, the other links being random. *ParaFitLink2* cannot be used in perfect coevolutionary situations because its denominator is then zero.

A FORTRAN program (PARAFIT: source code, compiled versions for Macintosh and DOS, and program documentation) to carry out the host-parasite coevolution test described in this paper is available on the WWWeb site <http://www.fas.umontreal.ca/biol/legendre/> as well as the WWWeb site of the Society for Systematic Biologists <http://www.systbiol.org>. The user's manual contains matrices **A**, **B**, and **C** used to compute the gopher-lice example discussed in this paper, as well as the patristic distance matrices that led to **B** and **C**.

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REFERENCES

BARKER S. C. 1994. Phylogeny and classification, origins, and evolution of host associations of lice. Int. J. Parasitol., 24:1285-1291.

BARRETT, J. A. 1986. Host-parasite interactions and systematics. Pages 1-17 *in* Coevolution and systematics (A. R. Stone and D. L. Hawksworth, eds.). Oxford: Clarendon Press.

BOEGER, W. A., AND D. C. KRITSKY. 1997. Coevolution of the monogenoidea (platyhelminthes) based on a revised hypothesis of parasite phylogeny. Int. J. Parasitol., 27:1495-1511.

BROOKS, D. R. 1977. Evolutionary history of some plagiorchioid trematodes of anurans. Syst. Zool., 26:277-279.

BROOKS, D. R. 1979. Testing the context and extent of host-parasite coevolution. Syst. Zool., 28:299-307.

BROOKS, D. R. 1981. Hennig's parasitological method: a proposed solution. Syst. Zool., 30:229-249.

BROOKS, D. R. 1985. Historical ecology: a new approach to studying the evolution of ecological associations. Ann. Mo. Bot. Gard., 72:660-680.

BROOKS, D. R. AND GLEN, D. R. 1982. Pinworms and primates: a case study in coevolution. Proc. Helminthol. Soc. Wash., 49:76-85.

BROOKS, D. R., AND D. A. MCLENNAN. 1991. Phylogeny, ecology, and behavior. a research program in comparative biology. Chicago: University of Chicago Press.

BUNEMAN, P. 1974. A note on the metric properties of trees. J. Comb. Theory B, 17:48-50.

CHARLESTON, M. A. 1998. Jungles: A new solution to the host/parasite phylogeny reconciliation problem. Math. Biosci., 149:191-223.

DEMASTES, J. W., AND M. S. HAFNER. 1993. Cospeciation of pocket gophers (*Geomys*) and their chewing lice (*Geomydoecus*). J. Mammal., 74:521-530.

EDGINGTON, E. S. 1995. Randomization tests. Third edition. New York: Marcel Dekker.

EHRLICH, P. R., AND P. H. RAVEN. 1964. Butterflies and plants: a study in coevolution. Evolution, 18:586-608.

FARENHOLZ, H. 1913. Ectoparasiten und Abstammungslehre. Zool. Anz., 41:371-374.

FUTUYMA, D. J., AND M. SLATKIN. 1983. Coevolution. Sunderland, Massachusetts: Sinauer Associates.

GOWER, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika, 53:325-338.

HAFNER, M. S., AND S. A. NADLER. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. Nature, 332:258-259.

HAFNER, M. S., AND S. A. NADLER. 1990. Cospeciation in host-parasite assemblages: comparative analysis of rates of evolution and timing of cospeciation events. Syst. Zool., 39:192-204.

HAFNER, M. S, P. D. SUDMAN, F. X. VILLABLANCA, T. A. SPRADLING, J. W. DEMASTES, AND S. A. NADLER. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science, 265:1087-1090.

HUELSENBECK J. P., B. RANNALA, AND Z. YANG. 1997. Statistical tests of host-parasite cospeciation. Evolution, 51:410-419.

HOPE, A. C. A. 1968. A simplified Monte Carlo test procedure. J. Roy. Stat. Soc. B, 50:35-45.

JOHNSON, K. P., D. M. DROWN, AND D. H. CLAYTON. 2001. A data based parsimony method of cophylogenetic analysis. Zool. Scr., 30:79-87.

KLASSEN, G. J. 1992. Coevolution: a history of the macroevolutionary approach to studying host-parasite associations. J. Parasitol., 78:573-587.

KLASSEN, G. J., AND M. BEVERLEY-BURTON. 1988. North american fresh water ancyrocephalids (Monogenea) with articulating haptoral bars: host-parasite coevolution. Syst. Zool., 37:179-189.

LAPOINTE, F.-J., AND P. LEGENDRE. 1991. The generation of random ultrametric matrices representing dendrograms. J. Classif., 8:177-200.

LAPOINTE, F.-J., AND P. LEGENDRE. 1992. A statistical framework to test the consensus among additive trees (cladograms). Syst. Biol., 41:158-171.

LEGENDRE, P., R. GALZIN, AND M. L. HARMELIN-VIVIEN. 1997. Relating behavior to habitat: solutions to the fourth-corner problem. Ecology, 78:547-562.

LEGENDRE, P., AND L. LEGENDRE. 1998. Numerical ecology. Second English edition. Amsterdam: Elsevier Science BV.

MANLY, B. J. F. 1997. Randomization, bootstrap and Monte Carlo methods in biology. Second edition. London: Chapman and Hall.

PAGE, R. D. M. 1993a. COMPONENT 2.0. Tree comparison software for the use with Microsoft Windows. London: The Natural History Museum.

PAGE, R. D. M. 1993b. Parasites, phylogeny and cospeciation. Int. J. Parasitol., 23:499-506.

PAGE, R. D. M. 1994. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics, 10:155-173.

PAGE, R. D. M. 1996. Temporal congruence revisited: comparison of mitochondrial DNA sequence divergence in cospeciating pocket gophers and their chewing lice. Syst. Biol., 45:151-167.

PAGE, R. D. M., AND M. A. CHARLESTON. 1998. Trees within trees – Phylogeny and historical associations. Trends Ecol. Evol., 13:356-359.

PAGE, R. D. M., AND M. S. HAFNER. 1996. Molecular phylogenies and host-parasite cospeciation: gophers and lice as a model system. Pages 255-270 *in* New uses for new phylogenies (P. H. Harvey, A. J. Leigh Brown, J. Maynard Smith, and S. Nee, eds.). New York: Oxford University Press.

PAGE, R. D. M., AND E. H. HOLMES. 1998. Molecular evolution: a phylogenetic approach. Oxford: Blackwell Science.

PATERSON, A. M., AND J. B. BANKS. 2001. Analytical approaches to measuring cospeciation of host and parasites: through a glass, darkly. Int. J. Parasitol., 31:1012-1022.

PATERSON, A. M., R. D. GRAY, AND G. P. WALLIS. 1993. Parasites, petrels and penguins: does louse presence reflect seabird phylogeny? Int. J. Parasitol., 23:515-526.

PRUZANSKY, S., A. TVERSKY, AND J. D. CARROLL. 1982. Spatial versus tree representations of proximity data. Psychometrika, 47:3-19.

RONQUIST, F. 1995. Reconstructing the history of host-parasite associations using generalised parsimony. Cladistics, 11:73-89.

RONQUIST, F. 1997. Phylogenetic approaches in coevolution and biogeography. Zool. Scr., 26:313-322.

ROY, B. A. 2001. Patterns of association between crucifers and their flower-mimic pathogens: host jumps are more common than coevolution or cospeciation. Evolution, 55:41-53.

THOMPSON, J. N. 1994. The coevolutionary process. Chicago: University of Chicago Press.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4:406-425.

SIDALL, M. E. 1996. Phylogenetic covariance probability: confidence and historical associations. Syst. Biol., 45:48-66.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry — The principles and practice of statistics in biological research. Third Edition. New York: W. H. Freeman.

SWOFFORD, D. L. 2001. PAUP*. Phylogenetic analysis asing aarsimony (*and other methods). Version 4b8. Sunderland, Massachusetts: Sinauer Associates.

SZIDAT, L. 1940. Beiträge zum Aubfau eines natürlichen Systems der Trematoden. I. Die Entwicklung von Echinocercaria choanophila U. Szidat zu Cathaemasia hians und die Ableitung der Fasciolidae von den Echinostomidae. Z. Parasitenk., 11:239-283.

FIGURE CAPTIONS

FIGURE 1. The three elements of the host-parasite coevolution problem can be translated into rectangular data matrices **A**, **B** and **C**. See text.

FIGURE 2. Given the information in matrices **A**, **B,** and **C**, the problem is to estimate the parameters in the fourth-corner matrix **D** that crosses the principal coordinates of the hosts with those of the parasites.

FIGURE 3. Global test of host-parasite coevolution (*ParaFitGlobal* statistic): (a) Type I error rates and 95% confidence intervals, at $\alpha = 0.01$ to 0.05, of the test for 5 to 25 host-parasite association links (abscissa). The phylogenies were generated independently for the hosts and the parasites. The confidence intervals are based upon 10000 replicate simulations for each combination of parameters. (b) Power of the global test of host-parasite coevolution in simulations in which a number of supplementary random host-parasite links (abscissa) were added to the coevolutionary links. (c) Same, for simulations in which some (abscissa) of the coevolutionary links were removed and replaced by random host-parasite links. (d) Same, for simulations in which the phylogenetic trees of the hosts and parasites contained a common portion, the remainder of each tree being random. Abscissa: number of coevolving species. There were 10 hosts and 10 parasites in all these simulations.

FIGURE 4. Type I error rates and 95% confidence intervals, at $\alpha = 0.01$ to 0.05, of the test of individual host-parasite association for the first H-P coevolutionary link generated during each simulation, for 5 to 25 host-parasite association links. (a) *ParaFitLink1* statistic. (b) *ParaFitLink2* statistic. There were 10 hosts and 10 parasites in these simulations; similar results were obtained using 10 hosts and 15 parasites, or 15 hosts and 10 parasites.

FIGURE 5. Power of the test of individual host-parasite association in simulations in which randomly-located links were *added* to matrix **A**. There were 10 hosts and 10 parasites in these simulations. There were also 10 coevolutionary links, and 0 to 10 supplementary random links. (a) Simulation results for one of the coevolutionary H-P links, *ParaFitLink1*

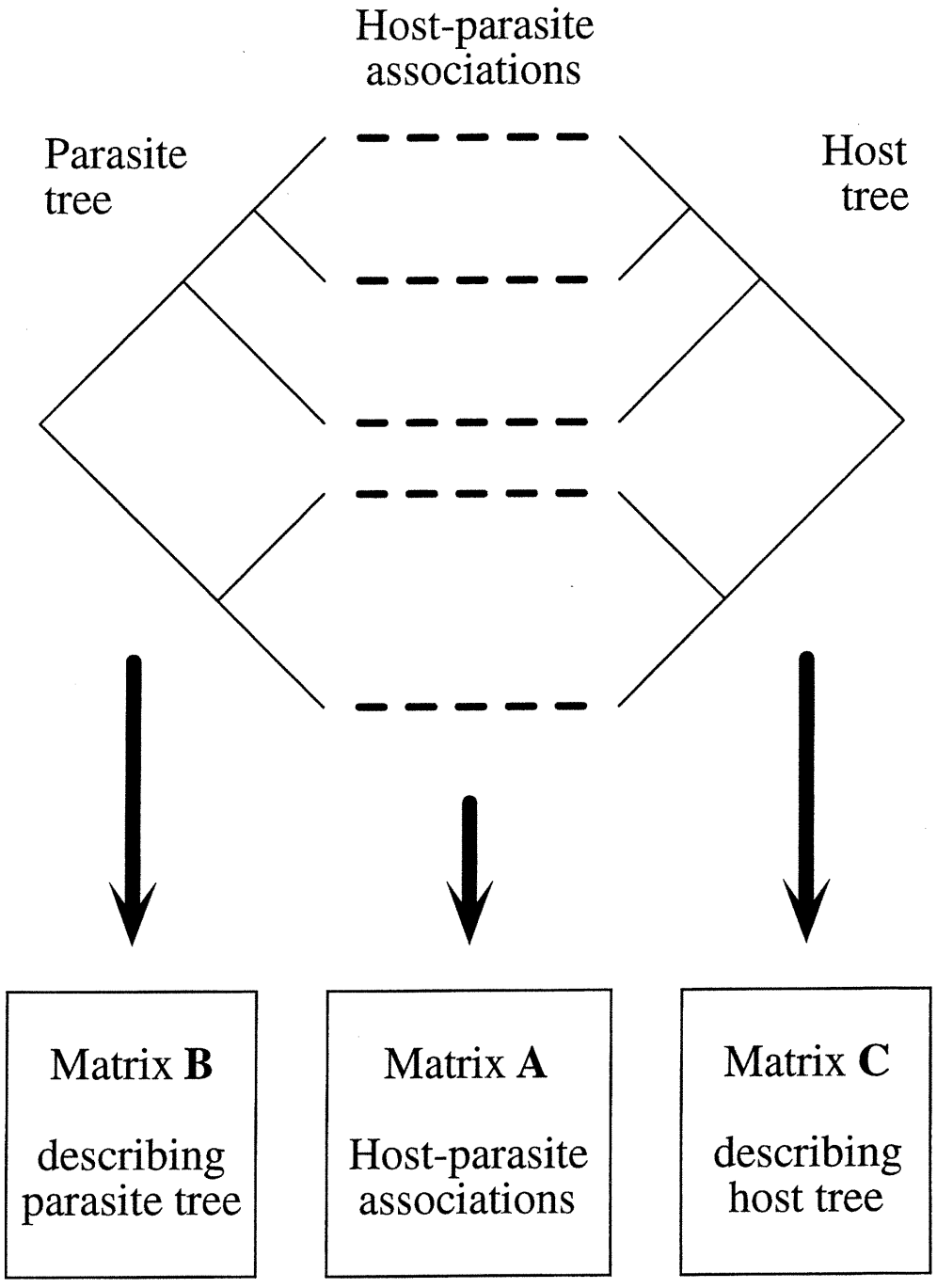
statistic; (b) *ParaFitLink2* statistic. (c) Simulation results for one of the supplementary random H-P links, *ParaFitLink1* statistic; (d) *ParaFitLink2* statistic.

FIGURE 6. Power of the test of individual host-parasite association in simulations in which a certain percentage of randomly-located links were *removed* from matrix **A** and *replaced* by randomly-located links. There were 10 hosts and 10 parasites in these simulations. There were also 10 coevolutionary links; 0 to 10 of them were replaced by random links. (a) Simulation results for one of the coevolutionary H-P links, *ParaFitLink1* statistic; (b) *ParaFitLink2* statistic. (c) Simulation results for a random H-P link replacing a coevolutionary link, *ParaFitLink1* statistic; (d) *ParaFitLink2* statistic.

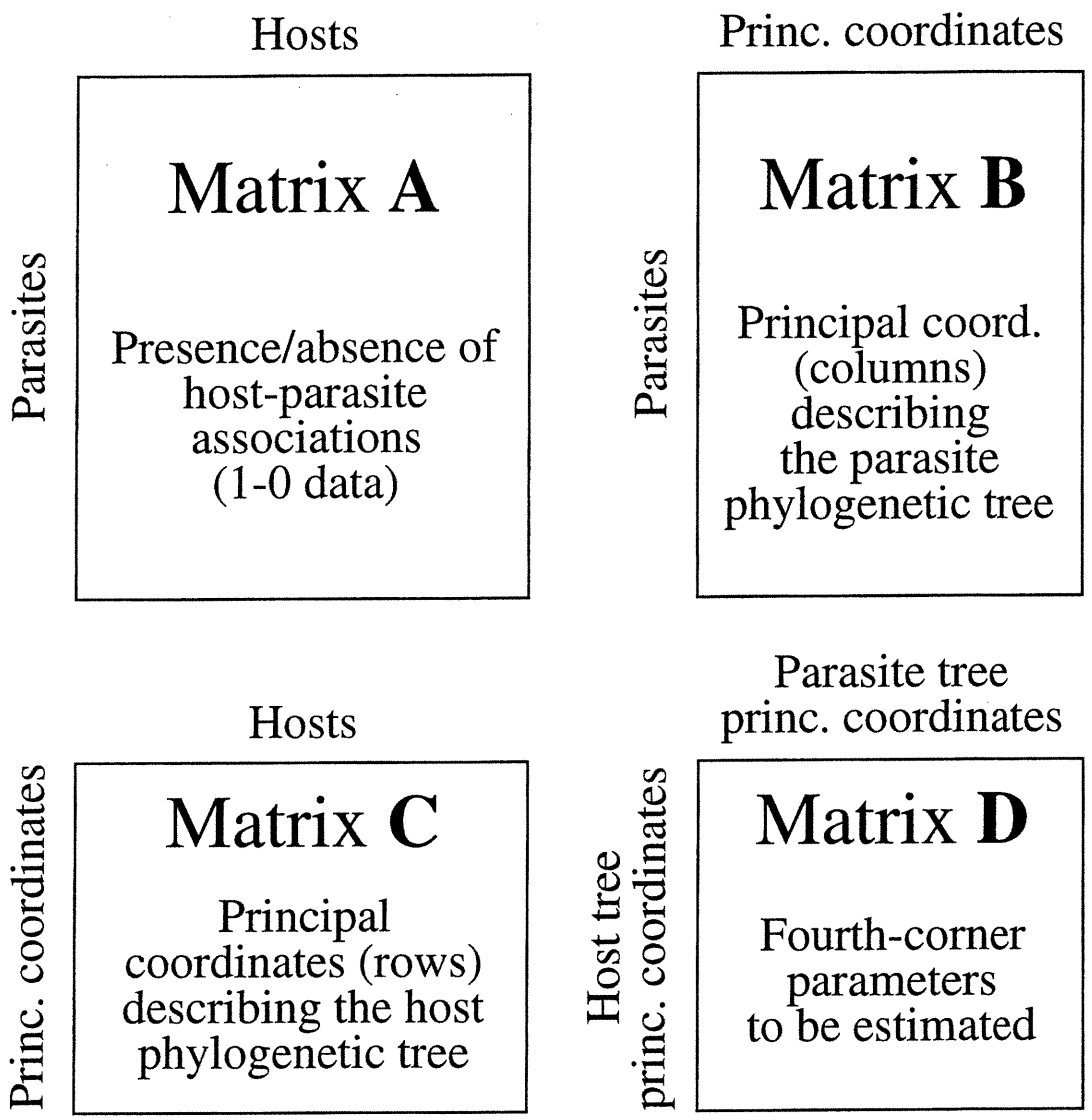
FIGURE 7. Power of the test of individual host-parasite association in simulations in which the phylogenetic tree of the hosts and that of the parasites were partly similar and partly random. There were 10 hosts, 10 parasites, and 10 links in this particular set of simulations. Abscissa: number of species of hosts and parasites (among 10) that shared the same tree and were linked. (a) Simulation results for one of the coevolutionary H-P link, *ParaFitLink1* statistic; (b) *ParaFitLink2* statistic. (c) Simulation results for one of the random H-P links, *ParaFitLink1* statistic; (d) *ParaFitLink2* statistic.

FIGURE 8. Pocket gophers and chewing lice phylogenetic trees and host-parasite links. Significant H-P links are represented by full lines, non-significant links by dashed lines.

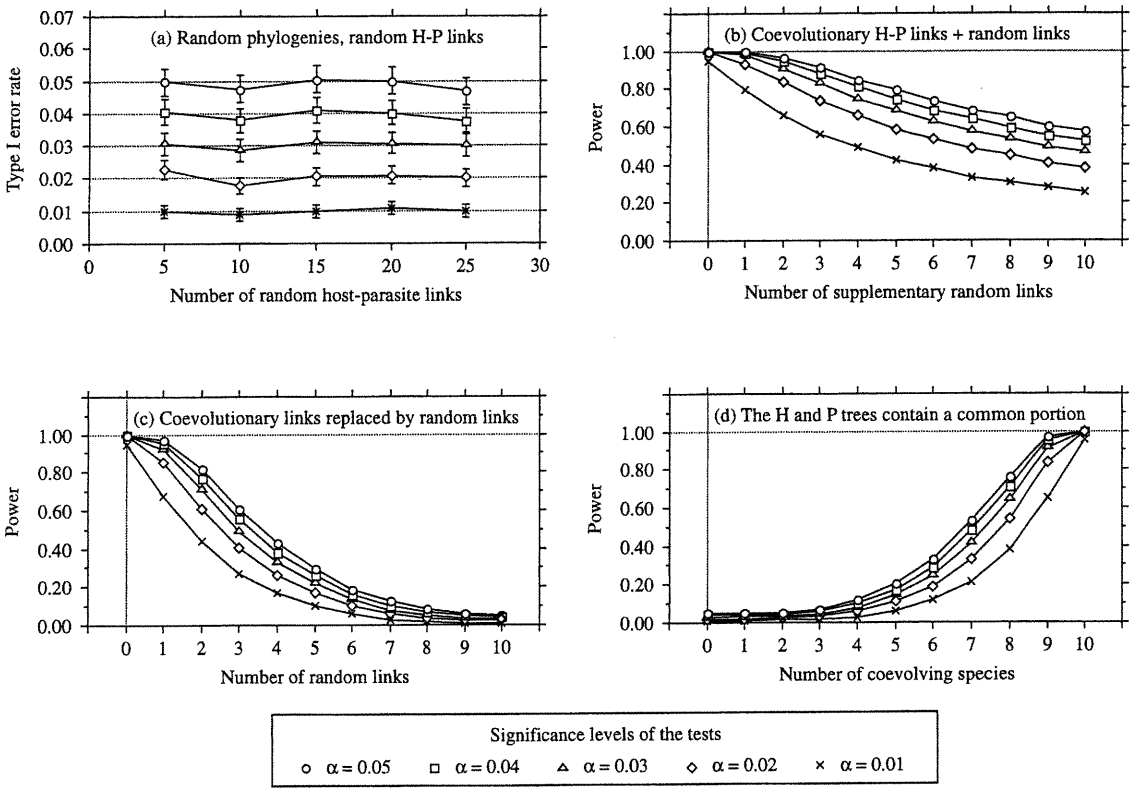
FIGURE 9. Pruned trees: the trees are now identical and display perfect coevolution for a subset of the animals.



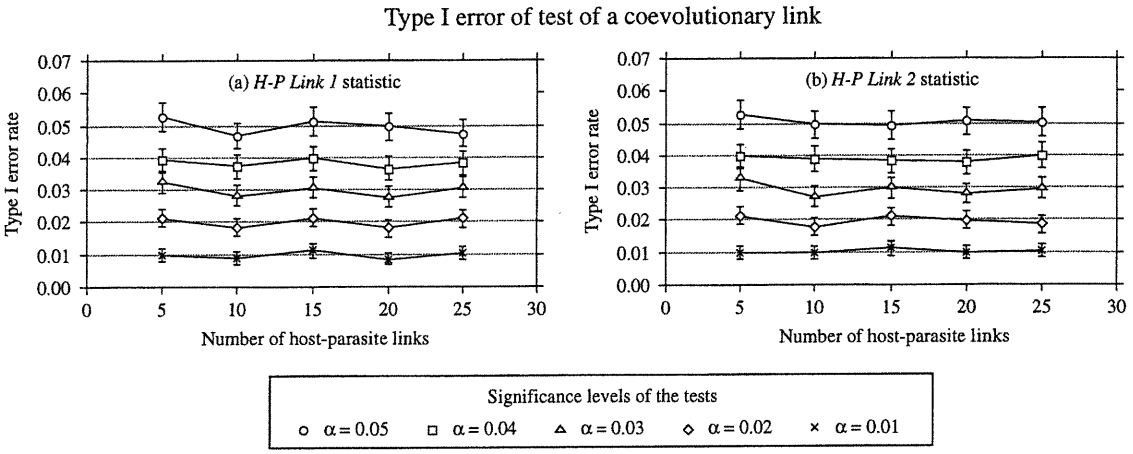
Legendre et al., Fig. 1



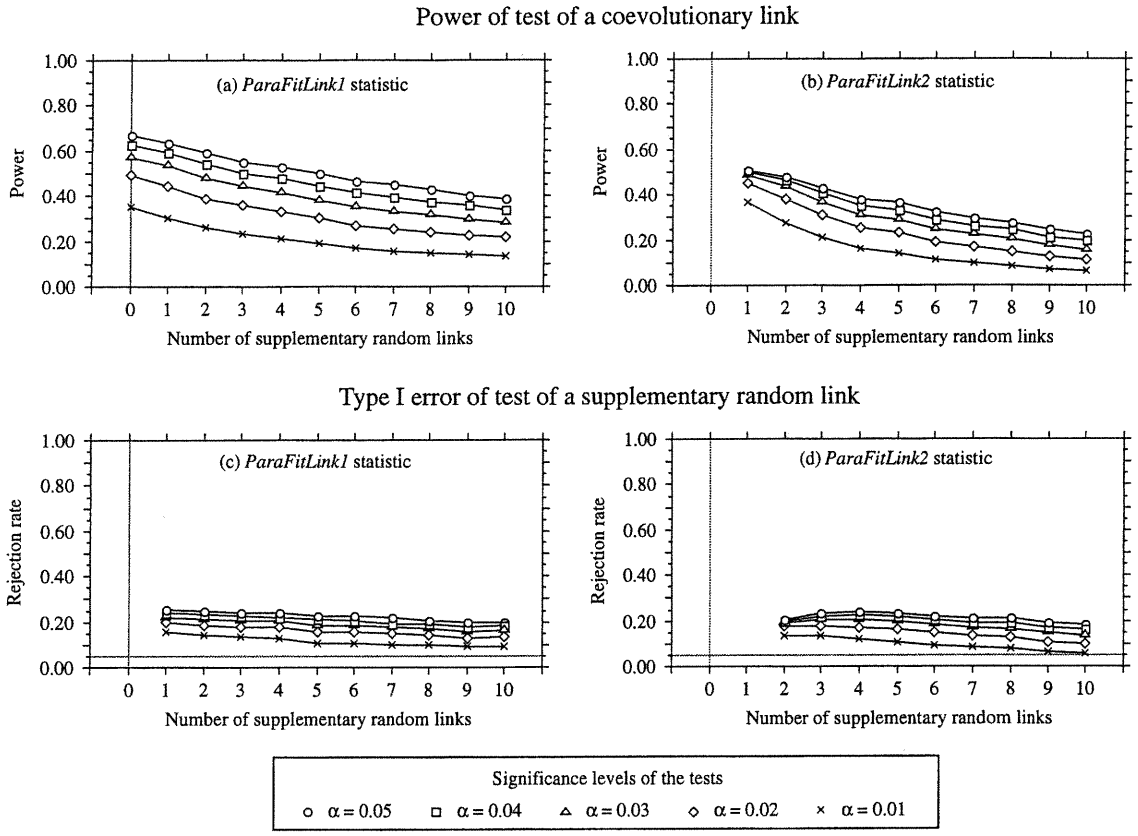
Legendre et al., Fig. 2



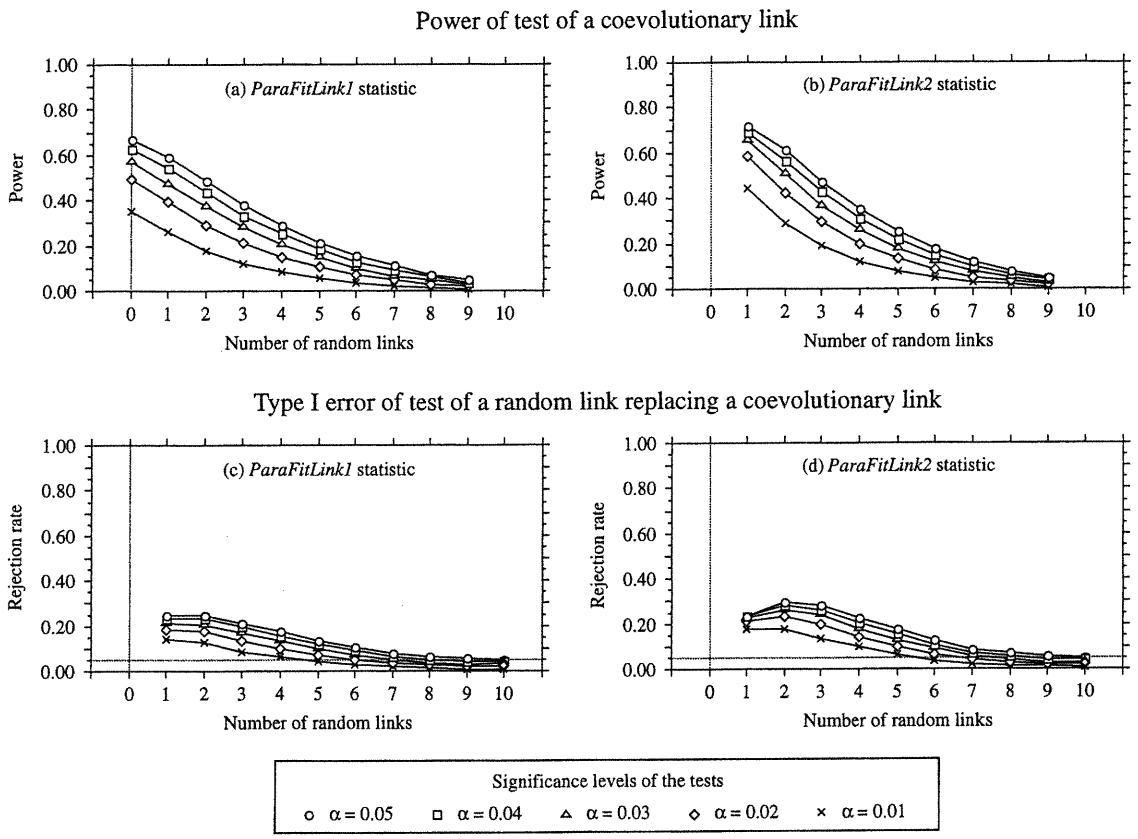
Legendre et al., Fig. 3



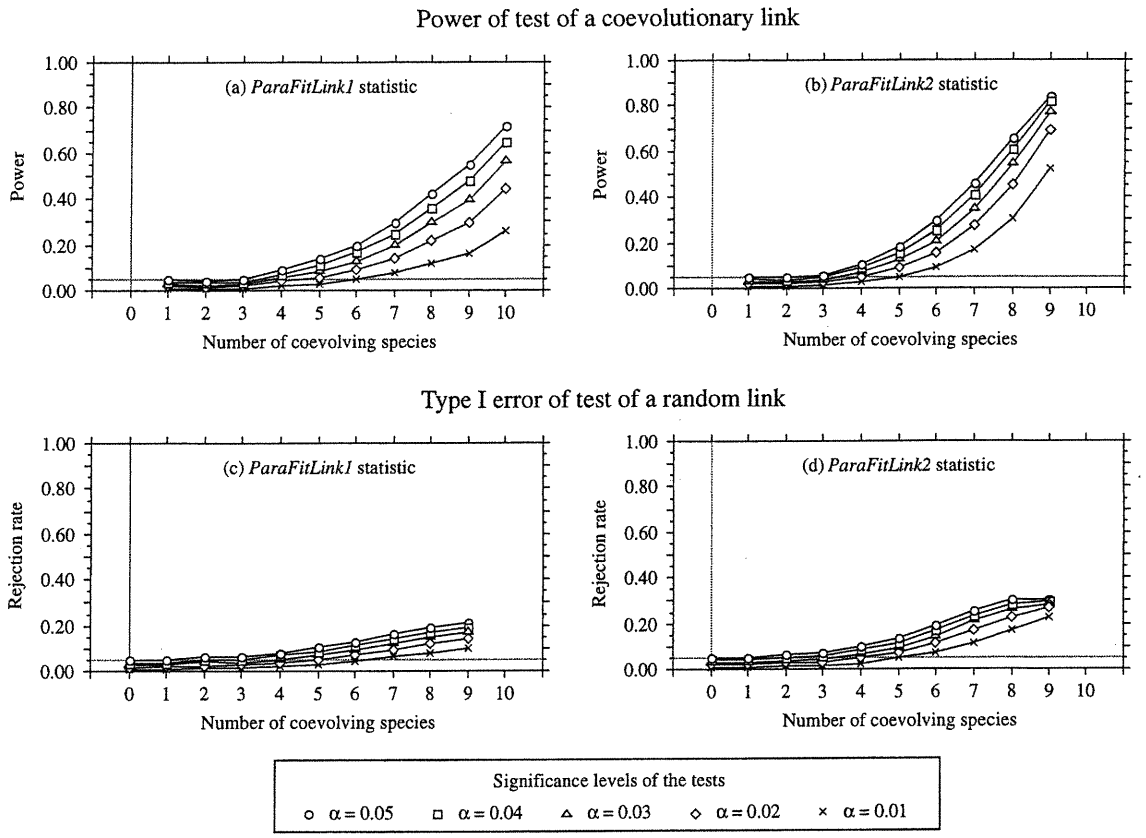
Legendre et al., Fig. 4



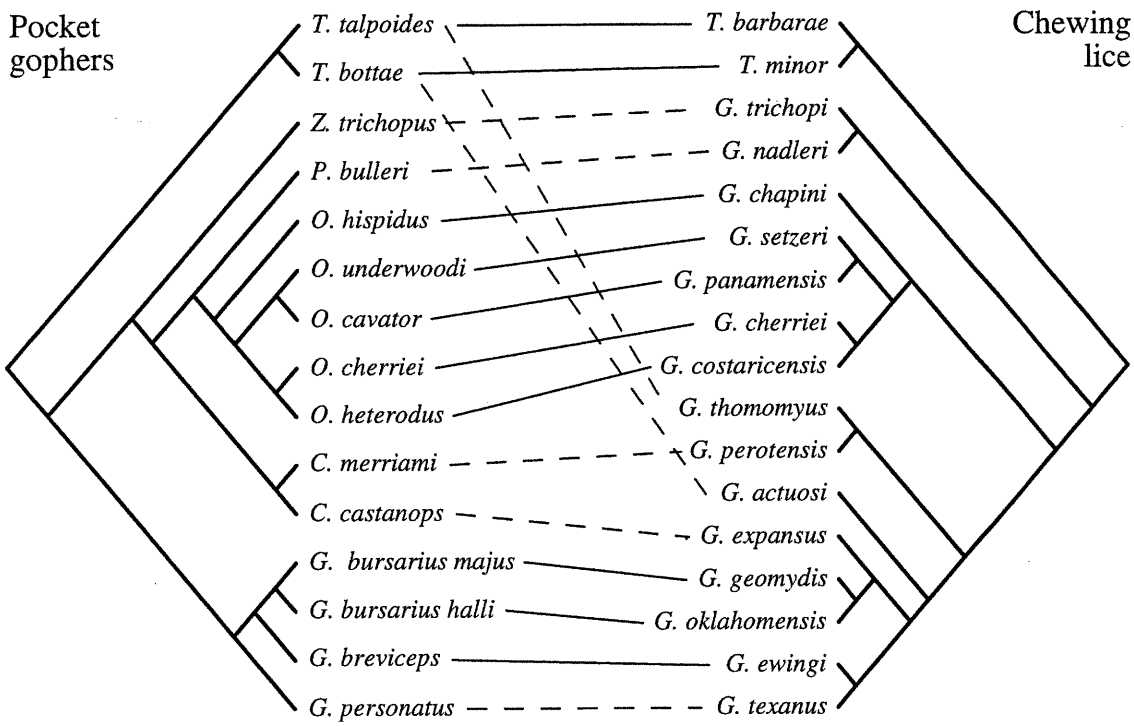
Legendre et al., Fig. 5



Legendre et al., Fig. 6



Legendre et al., Fig. 7



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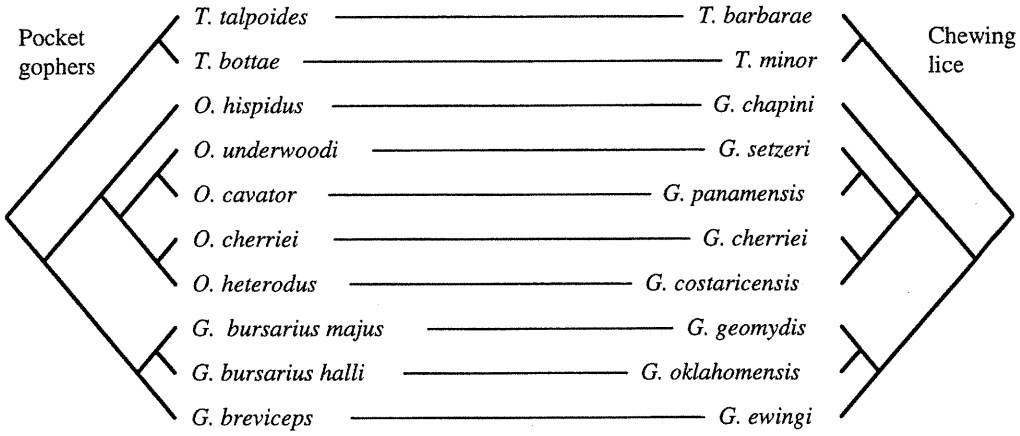

Legendre et al. Fig.9

B. UNE MÉTHODE POUR LE PARTITIONNEMENT DE LA VARIATION PHYLOGÉNÉTIQUEMENT STRUCTURÉE

DESDEVISES Yves, Lamia AZOUZI, Pierre LEGENDRE and Serge MORAND. Partitioning the phylogenetically-structured environmental variation. Soumis.

Cet article présente une méthode destinée à partitionner la variation d'un trait phénotypique soumis à des influences environnementale (i.e., écologique) et phylogénétique. Il est ainsi possible d'estimer les effets respectifs de ces deux facteurs, ainsi que leur influence commune, sur la variable réponse. Cela permet de quantifier le "phylogenetic niche conservatism" sensu Harvey & Pagel (1991). La méthode consiste à exprimer l'inertie phylogénétique à l'aide d'une analyse en coordonnées principales, puis à effectuer le partitionnement par une série de régressions partielles sur les coordonnées principales et les variables environnementales considérées. Un exemple d'utilisation de la méthode est présenté : l'étude de la relation entre la masse corporelle et la densité chez les mammifères.

Participation du thésard :

- Recherche bibliographique
- Mise au point de la méthode
- Analyse des données
- Rédaction

PARTITIONING THE PHYLOGENETICALLY-STRUCTURED ENVIRONMENTAL VARIATION

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Running head: PHYLOGENETIC VARIANCE PARTITION

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ABSTRACT

Comparative analysis methods remove all variation linked to phylogeny before attempting to correlate the remaining variation to present-day conditions (i.e., ecology and/or environment). However, a portion of the phylogenetic variation of a trait may be related to ecology; this portion is called phylogenetic niche conservatism. We propose a method of variation partitioning which allows users to quantify this portion of the variation; we call it the phylogenetically-structured environmental variation. This method is applied to published data to study the link between body mass and population density in 79 species of mammals, in a phylogenetic framework. The results suggest that an important part of the variation of mammals body mass is related to the common influence of phylogeny and population density.

Key-Words: comparative analysis, phylogenetically-structured environmental variation, phylogenetic correction, phylogenetic niche conservatism, variation partitioning, equivalent energetic rule

THE PROBLEM

Comparative analysis has become widely used in evolutionary biology and ecology during the past 20 years. It consists in comparing two or more traits across species, or a trait and an environmental variable, while taking phylogenetic autocorrelation into account (Harvey & Pagel, 1991). Several methods have been proposed for this approach (e.g., Felsenstein, 1985; Grafen, 1989, Diniz-Filho et al., 1998). A few years ago, Westoby et al., (1995a) initiated a controversy (Ackerly & Donoghue, 1995; Fitter, 1995; Harvey et al., 1995a,b; Rees, 1995; Westoby et al., 1995b,c; see Ricklefs, 1996) about what they called "phylogenetic correction", which is the control for phylogeny in comparative analysis. They argued that comparative methods partition the explained variation of ecological data in such a way that they "...allocate the maximum possible variation in a trait to phylogeny, considering only the residual as potentially attributable to ecology" (p. 531). Indeed, these methods first remove the phylogenetic component from the variables before estimating the influence of present-day ecological factors. This is justified by the principle of parsimony, also called Ockham's razor: one should call upon the most obvious and simple explanation (here, the phylogeny) of the observed correlation between variables, before invoking more complex explanations.

As Westoby et al. (1995a) correctly pointed out, the phylogenetic portion of the total variance may contain a phylogenetic effect related to ecology, which Harvey & Pagel (1991) called "phylogenetic niche conservatism". This includes the shared attributes that related species have acquired because they tended to occupy similar niches during evolutionary history. Westoby et al. (1995a) proposed to partition the variance of the data into three portions (Figure 1 from Westoby et al., 1995a, p. 531, equivalent to fractions [a], [b] and [c] in Fig. 1 of the present paper): a part strictly due to ecology ([a]), a part strictly due to phylogeny ([c]), and a part due to the common influence of these two factors ([b]), which we call "phylogenetically-structured environmental variation", an expression equivalent to "phylogenetic niche conservatism". However, no method has been proposed to-date to calculate this variation partitioning in the phylogenetic context. The purpose of this paper is to propose such a method.

We propose to partition the variation in not three but four components (Fig. 1), adding the unexplained part of the variation [d]. Note that it is not our intention to defend a position against the principle of parsimony. We simply want to show how variation can be partitioned to quantify the portion of the variation which can be explained jointly by both causes.

THE PARTITIONING METHOD

Variation partitioning has been proposed a few years ago for multivariate ecological data showing spatial variation (Borcard et al., 1992; Borcard & Legendre, 1994). The spatial variation can be expressed as a trend-surface equation, or some other statistical model, based upon the geographic coordinates of the sampling sites; see Legendre & Legendre (1998, Section 13.5). Borcard & Legendre (2002) have shown how this procedure can be generalized to model spatial structures at all the spatial scales that can be perceived by the sampling design. The variation of species assemblages can be decomposed into a fraction [a] which is explained by environment variables but is not spatially structured; a fraction [b] which is the spatially-structured component of environmental variation; a fraction [c] which is not explained by the environmental variables used in the model but is spatially structured; and an unexplained component [d].

The decomposition of phylogenetic variation can be seen as a problem of the same nature. Remains the technical problem of representing the phylogeny in the analysis. When using the method of independent contrasts (Felsenstein, 1985), phylogenetic relationships are subtracted from the variables under study instead of being expressed on their own. One possible solution would be to express the phylogeny as a distance matrix which would be included in a multiple regression equation computed on distance matrices (Legendre et al., 1994), using either the original data (obtained for instance by alignment of sequences) or a derived phylogenetic tree expressed in the form of a patristic distance matrix. This would require to also express all the other variables as distance matrices, which leads to work on distances instead of the raw data. Dutilleul et al. (2000, Table 2) have shown, however, that the correlation between two data vectors $\mathbf{x}_1$ and $\mathbf{x}_2$ is higher than the Mantel correlation statistic between two distance matrices $\mathbf{D}_1$ and $\mathbf{D}_2$ derived from $\mathbf{x}_1$ and $\mathbf{x}_2$. Legendre (2000)

has also shown that the power of the *t*-test of the Pearson correlation coefficient between $\mathbf{x}_1$ and $\mathbf{x}_2$ is higher than that of the Mantel test between $\mathbf{D}_1$ and $\mathbf{D}_2$.

It is also possible to use the autoregressive method (Cheverud & Dow, 1985; Cheverud et al., 1985) to estimate the level of phylogenetic inertia, partitioning the total variation of a quantitative trait into a specific component and a phylogenetic component, i.e., the phylogenetic inertia. Another approach was proposed by Diniz-Filho et al. (1998): it consists in expressing the phylogeny in the form of principal coordinates via a principal coordinate analysis (PCoA: Gower, 1966) computed from the phylogenetic distance matrix. Using numerical simulations, these authors have shown that this method provides a better estimate of phylogenetic inertia than the autoregressive method when phylogenetic autocorrelation is low.

Any method for assessing phylogenetic inertia can be used in the approach that we are proposing here; the important point is to obtain an estimate of the unique effect of phylogeny on the variation of the trait(s) under study. We will detail our method by using principal coordinates to estimate phylogenetic inertia, but it should be kept in mind that the principle would remain the same with any method. Our aim is not to propose a new method of comparative analysis but rather a new way of using existing approaches to obtain supplementary information.

Contrary to Diniz-Filho et al. (1998) who only used the first few principal coordinates selected by reference to a broken-stick model (Barton & David, 1956; Frontier, 1976), we are proposing to use all the principal coordinates, extracted from the distance matrix, that are significantly related to the dependent(s) variable(s). There is no particular reason why the broken-stick model would preferably select principal coordinates that are of importance for the explanation of the dependent variable. Since the phylogenetic distance matrix may not always be Euclidean, negative eigenvalues may occur. When this is the case, it is possible to apply correction methods, described in Gower & Legendre (1986) and Legendre & Legendre (1998, Section 9.2.4) to render all eigenvalues positive. Again, this phylogenetic distance matrix can be calculated either from the raw data (e.g., sequence alignments), which avoids the reconstruction of a tree, or from a patristic distance matrix representing a phylogenetic tree; no negative eigenvalues should appear in the latter case.

Our method for partitioning the variation of a trait is the following. Y is the dependent variable representing the trait under study, X_E represents the matrix of ecological explanatory variable(s), and PCs stands for the matrix of principal coordinates representing the phylogeny.

1. Compute a regression of Y on X_E. This is a multiple regression if matrix X_E contains several variables. A forward, backward, or stepwise variable selection procedure can be used to reduce the number of explanatory variables in matrix X_E, retaining only the environmental variables that significantly contribute to the model. The coefficient of (multiple) determination of the regression, R^2, is equal to fraction [a+b] of the decomposition.
2. Compute a multiple regression of Y on all PCs. The coefficient R^2 is equal to fraction [b+c] of the decomposition. Only the principal coordinates significantly contributing to modelling Y are retained to represent the phylogeny in the remainder of the analysis. No stepwise selection procedure is needed to choose them since they are orthogonal to one another and, thus, linearly independent.
3. Compute a multiple regression of Y on both X_E and the PCs. The coefficient R^2 is equal to fraction [a+b+c] of the decomposition.
4. The individual values of [a], [b], and [c] can be obtained by subtraction from the previous results: [a] = R^2(step 3) – R^2(step 2); [b] = R^2(step 1) + R^2(step 2) – R^2(step 3); [c] = R^2(step 3) – R^2(step 1). Fraction [b] is the phylogenetically-structured environmental variation, corresponding to the phylogenetic niche conservatism of Harvey & Pagel (1991).
5. Find [d] =1 – [a+b+c].

It is possible to obtain the fitted values corresponding to fractions [a] and [c]. For that, it is necessary to add two steps to the procedure:

6. Compute a partial regression of Y on X_E, using the PCs as matrix of covariables. The fitted values of the regression, which can be computed from the partial regression equation, correspond to and materialize fraction [a]. Fraction [a] can be tested for significance.

7. Compute a partial regression of Y on PCs, using X_E as matrix of covariables. The fitted values of the regression, which can be computed from the partial regression equation, correspond to and materialize fraction [c]. Fraction [c] can be tested for significance.

Fraction [b], which corresponds to the phylogenetically-structured environmental variation, can only be obtained by subtraction. This makes it impossible to compute fitted values for this fraction or to test it for significance. It is possible to obtain fractions [a], [b], [c], and [d] from steps 3, 6 and 7 only, but if the fitted values are not needed, it is easier to use steps 1, 2 and 3 since multiple regression is less computation-intensive than partial regression.

This method can easily be adapted to analyse a multivariate table Y of dependent variables using canonical analysis, instead of multiple regression, decomposing the variation of several traits, considered simultaneously, with respect to environmental and phylogenetic components. For example, one could wish to study the environmental and phylogenetic structure of several morphological traits taken simultaneously. This extension of variation partitioning has been described by Borcard et al. (1992), Borcard & Legendre (1994), and Legendre & Legendre (1998).

EXAMPLE USING REAL DATA

The partitioning method was applied to the study of the link between body mass and population density in 79 species of mammals (Morand & Harvey, 2000; the data can be found at URL: http://www.pubs.roysoc.ac.uk). Body mass has been shown to be inversely related to population density (Damuth, 1981, 1986; Silva & Downing, 1995). The negative relationship between body mass and population density has been discussed extensively in the ecological literature (Damuth, 1981, 1993; Lawton, 1990; Nee et al., 1991; Silva & Downing, 1995; Morand & Poulin, 1998) as well as its consequences in terms of the amount of energy used (Damuth, 1981). Damuth (1981) emphasized the fact that the exponent linking body mass to population density should be equal to –0.75 because the energy used by an individual is positively linked to its body mass with an exponent of +0.75. The energy used by a local population of a species in a community should then be independent of body mass; this is the so-called "energetic equivalent" rule. Criticisms

against the energetic equivalent rule come from two sides: estimation of the exponent, and possible confounding phylogenetic effects.

These two life-history traits, body mass and population density, are strongly constrained by phylogeny (Morand & Poulin, 1998). We will estimate here what proportion of the body mass variable is explained by population density alone, phylogeny alone, and jointly by population density and phylogeny (which corresponds to phylogenetic niche conservatism). We will also quantify the unexplained portion of the variation. We used the data compiled by Morand & Poulin (1998; see also Morand & Harvey, 2000, for sources of references).

The data for mammal body masses and population densities were linearized using a natural logarithmic transformation (ln). From now on, ln(body mass) and ln(density) will simply be referred to as mass and density. Departure from normality did not have to be assessed because all tests of significance were carried out through permutational procedures (999 permutations).

The regression of population density on body mass, which provided fraction [a+b] (Fig. 1), was highly significant ($P = 0.001$). Population density, which contains an embedded portion of phylogenetic structure, explained 65 % of the body mass variation ($R^2 = 0.65$).

A patristic distance matrix was derived from the phylogenetic tree (Fig. 2) by considering each branch length to be equal to one unit. A principal coordinate analysis (PCoA) was then performed on this matrix using THE R PACKAGE V.4.0 (Casgrain, P. & Legendre, P. 2000. The R Package for Multivariate and Spatial Analysis, version 4.0 d3 - User's Manual. Département de sciences biologiques, Université de Montréal. Freeware available at URL http://www.fas.umontreal.ca/BIOL/legendre/). Each principal coordinate (eigenvector, called PC hereafter) represented an amount of phylogenetic variance proportional to the associated eigenvalue. The principal coordinates analysis generated n–1 PCs for n species; the PCs were listed in decreasing order of variance, from PC1 to PC78. A multiple regression was then computed between mass and all PCs to assess their global and individual significance in the explanation of body mass variation. The high number (n-1) of independent variables presented a problem, however: the coefficient of multiple determination (R^2) of the multiple regression was equal to one, and no test of significance

by permutation could be performed when all 78 PCs were considered together. To go around this difficulty, we used the property that principal coordinates are uncorrelated to one another. As a consequence, the same standard regression coefficients are obtained if Y is regressed on the principal coordinates taken one by one, or in small groups, or all together. So, we regressed body mass on the PCs taken in small groups; the PCs that did not significantly contribute to explaining the variation of body mass were identified and discarded. This procedure is justified by the fact that our objective is not to make statements about the PCs remaining in the model, but simply to discard as many non-significant PCs as possible. The multiple regression was carried out using the program PERMUTE 3.4 written by P. Casgrain (freeware available at URL http://www.fas.umontreal.ca/BIOL/Casgrain/en/labo/permute; see Legendre et al., 1994). This program uses a permutation procedure to test the R^2 and the regression coefficients for significance. Eight PCs which were significant were retained into the model: PC1, PC2, PC5, PC7, PC8, PC19, PC21 and PC56. The sum of their eigenvalues represented 35 % of the total phylogenetic variance of the patristic distance matrix. They explained together 84 % of the body mass variation in the 79 species of mammals ($R^2 = 0.84$, $P = 0.010$).

Fraction [a+b+c] was found by regressing body mass on population density and the eight significant PCs together. It was equal to 89% ($R^2 = 0.89$, $P = 0.001$). Fractions [a], [b], [c] and [d] were obtained by subtraction:

[a] = [a+b+c] – [b+c] = 0.89 – 0.84 = 0.05, thus 5 %

[b] = [a+b] – [a] = 0.65 – 0.05 = 0.60, thus 60 %

[c] = [b+c] – [b] = 0.84 – 0.60 = 0.24, thus 24 %

[d] = 1 – [a+b+c] = 1 – 0.89 = 0.11, thus 11 %

To obtain the fitted values corresponding to fractions [a] and [c], two partial regression equations were computed:

• For fraction [a]: population density was regressed on all significant PCs and the residuals were computed. The partial regression was obtained by regressing body mass on these residuals; the fitted values were computed from the regression equation. The same value for [a] as above was found ($R^2 = 0.05$). The slope of the relationship between population density (controlled for phylogeny) and mass is –0.49. The value of this slope is noticeably smaller than –0.75; this finding challenges the equivalent energetic rule.

• The fitted values for fraction [c] were found in a similar way: each individual PC was regressed on density and the residuals of these eight simple linear regressions were computed. Body mass was then regressed on the eight vectors of residuals, using multiple linear regression, and the coefficient of multiple determination (R^2) was computed; this coefficient was equal to [c]. The fitted values can be computed from the regression equation. The same value for [c] as above was found ($R^2 = 0.24$).

The calculation procedure may appear tedious, especially if a large number of PCs are used. The program CANOCO (ter Braak & Smilauer, 1998) simplifies the manipulation. We repeated the calculations using the program CANOCO. The calculations were simpler and the results were the same. The dependent variable (body mass) and the independent variable (population density) were represented as simple vectors, whereas the phylogeny was represented as a matrix containing the eight significant PCs. In the partial linear regression analyses needed to compute fractions [a] and [c], we only had to specify one of the two sets of independent variables, in turn, as the matrix of covariables of the analysis. The permutation tests for significance computed by CANOCO in partial regression do take the number of covariables into account and can thus be reported. The permutational P-value for fraction [a] was 0.040 and that for fraction [c] was 0.009.

DISCUSSION

Phylogenetic niche conservatism as well as purely phylogenetic constraints (*sensu* Westoby et al., 1995a) can cause related species to be similar in many traits. The expression "related to phylogeny" encompasses many potential causes of similarity among species, including phylogenetic niche conservatism and phylogenetic constraints (see Harvey & Pagel, 1991). It is a purely statistical statement. If species are used as independent data when testing the correlation between traits of interest, the phylogenetic dependence between them leads to pseudoreplication (Hurlbert, 1984). If one wants to know if trait X explains trait Y, then *all* phylogenetic dependence between them must be removed, using an appropriate comparative method, to make the objects independent of one another in the analysis, thus avoiding pseudoreplication.

Classical comparative methods (e.g., Cheverud et al., 1985; Felsenstein, 1985; Grafen, 1989; Garland et al., 1992; Gittleman & Kot, 1990) eliminate all variation linked to phylogeny, which is a sound procedure when one wants to *test* the correlation between traits and to establish a causative relationship between them, based on strong biological hypotheses. This is because most testing procedures require the independence of data. In a simple cross-species comparison, there is no elimination of any variation due to phylogeny, thus no control for this potential confounding variable. This technique can be used if one wants to describe the pattern of covariation among several traits and then answer the question: *Is trait X associated to trait Y?* It is also a good procedure when one wants to predict the value of a trait from other trait(s), then answer the question: *What is the predictive value of Y for a given X?* Comparative methods aim at answering the question: *Does trait X explain trait Y?* This supposes controlling the effects of confounding variables. In the present case, the confounding variable is the phylogeny. The method proposed here allows one to answer the question: *What is the nature of the variation of Y explained by X?*

Our method is not proposed to defend a position against the principle of parsimony. Clearly, one should attribute the maximum amount of variation to a single well-established cause (phylogeny) rather than invoke multiple independent causes (e.g., adaptations). Our proposal allows comparative biologists to quantify Harvey & Pagel's (1991) concept of phylogenetic niche conservatism for the data at hand. The need to interpret more finely the

results of comparative analyses has been pointed out by Westoby et al. (1995b) who argued that current phylogenetic correction methods (i.e., comparative methods) were not able to answer questions calling for a detailed partitioning of the phylogenetic variation.

Our results show that in some cases, as in the example presented above, an important fraction of the variation of an ecological variable can be related to phylogenetic niche conservatism, and therefore the phylogenetic relationships between the variables are not fully related to genetic constraints. We can now quantify the relative portions of those influences and obtain a more precise explanation of the variation of the trait under study.

LITERATURE CITED

Ackerly, D. D. & Donoghue, M. J. 1995. Phylogeny and ecology reconsidered. *J. Ecol.* 83: 730-732.

Barton, D. E. & David, F. N. 1956. Some notes on ordered random intervals. *J. Roy. Statist. Soc. Ser. B* 18: 79-94.

Borcard, D., Legendre, P. & Drapeau, P. 1992. Partialling out the spatial component of ecological variation. *Ecology* 73: 1045-1055.

Borcard, D. & Legendre, P. 1994. Environmental control and spatial structure in ecological communities: an example using oribatid mites (Acari, Oribatei). *Environ. Ecol. Stat.* 1: 37-53.

Borcard, D. & Legendre, P. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecol. Model.*, in press.

Catzeflis, F., Hänni, C., Sourrouille, P. & Douzery, E. 1995 Molecular systematics of Hystricognath Rodents: the contribution of Sciurognath Mitochondrial 12S rRNA sequences. *Mol. Phyl. Evol.* 4: 357-360.

Cheverud, J. M. & Dow, M. 1985. An autocorrelation analysis of genetic variation due to lineal fission in social groups of Rhesus Macaques. *Am. J. Physic. Anthropol.* 67: 113-121.

Cheverud, J. M., Dow, M. & Leutenegger, W. 1985. The quantitative assessment of Phylogenetic constraints in comparative analyses: sexual dimorphism in body weight among primates. *Evolution* 39: 1335-1351.

Cooper, A. & Fortey, R. 1998. Evolutionary explosions and the phylogenetic fuse. *TREE* 13: 151-156.

Damuth, J. 1981 Population density and body size in mammals. *Nature* 290: 699-700.

Damuth, J. 1987. Interspecific allometry of population density in mammals and other animals: the independence of body mass and population energy-use. *Biol. J. Linn. Soc.* 31: 193-246.

Damuth, J. 1993. Cope's rule, the island rule and the scaling of mammalian population density. *Nature* 365: 748-750.

Diniz-Filho, J. A. F., de Sant'Ana, C. E. R. & Bini, L. M. 1998. An eigenvector method for estimating phylogenetic inertia. *Evolution* 52: 1247-1262

Dutilleul, P., Stockwell, J. D., Frigon, D. & Legendre, P. 2000. The Mantel-Pearson paradox: statistical considerations and ecological implications. *J. Agr. Biol. Environ. Stat.* 5: 131-150.

Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125: 1-15.

Fitter, A. H. 1995. Interpreting quantitative and qualitative characteristics in comparative analyses. *J. Ecol.* 83: 730.

Frontier, S. 1976. Étude de la décroissance des valeurs propres dans une analyse en composantes principales: comparaison avec le modèle du bâton brisé. *J. Exp. Mar. Biol. Ecol.* 25: 67-75.

Garland, T. Jr., Harvey, P. H. & Ives, A. R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* 41: 18-32.

Gittleman, J. L., & Kot, M. 1990. Adaptation : statistics and a null model for estimating phylogenetic effects. *Syst. Biol.* 39: 227-241.

Gower, J. C. 1966.Some distant properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 325-338.

Grafen, A. 1989. The phylogenetic regression. *Proc. Roy. Soc. Lond. B* 205: 581-598.

Harvey, P. H. & Pagel, M. D. 1991. *The comparative method in evolutionary biology.* Oxford University Press, Oxford.

Harvey, P. H., Read, A. F. & Nee, S. 1995. Why ecologists need to be phylogenetically challenged. *J. Ecol.* 83: 535-536.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54: 187-211.

Lawton, J. H. 1990. Species richness and population dynamics of animal assemblages. Patterns in body size : abundance space. *Philos. Trans. R. Soc. Lond. B* 330: 283-291.

Legendre, P. 2000. Comparison of permutation methods for the partial correlation and partial Mantel tests. *J. Stat. Comput. Sim.* 67: 37-73.

Legendre, P., Lapointe, F.-J. & Casgrain, P. 1994. Modeling brain evolution from behavior: a permutational regression approach. *Evolution* 48: 1487-99.

Legendre, P., & Legendre, L. 1998. *Numerical Ecology. 2nd English Edition.* Elsevier Science BV, Amsterdam.

Morand, S. & Poulin, R. 1998. Density, body mass and parasite species richness of terrestrial mammals. *Evol. Ecol.* 12: 717-727.

Morand, S. & Harvey, P. H. 2000 Mammalian metabolism, longevity and parasite species richness. *Proc. Roy. Soc. Lond. B* 267: 1999-2003.

Nee, S., Read, A. F., Greenwood, J. J. D. & Harvey, P. H. 1991 The relationship between abundance and body size in British birds. *Nature* 351: 312-313.

Rees, M. 1995. EC-PC comparative analyses. *J. Ecol.* 83: 891-893.

Ricklefs, R. E. 1996. Phylogeny and Ecology. *TREE* 11: 229-230.

Silva, M. & Downing, J. A. 1995. The allometric scaling of density and body mass; a nonlinear realtionship for terrestrial mammals. *Am. Nat.* 145: 704-727.

ter Braak, C. J. F. & Smilauer, P. 1998. *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4).* Microcomputer Power, Ithaca, New York.

Westoby, M., Leishmann, M. R. & Lord, J. M. 1995a. On misinterpreting the 'phylogenetic correction'. *J. Ecol.* 83: 531-534.

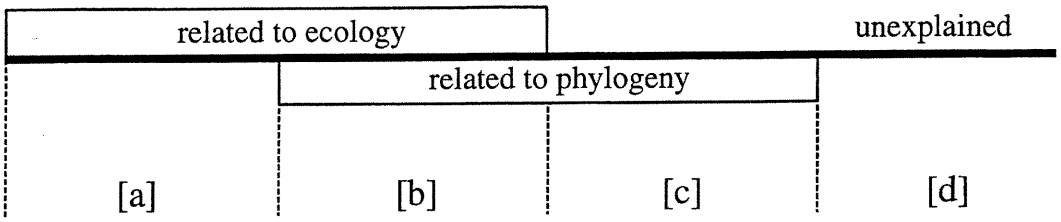
Westoby, M., Leishmann, M. R. & Lord, J. M. 1995b. Further remarks on 'phylogenetic correction'. *J. Ecol.* 83: 727-730.

Westoby, M., Leishmann, M. R. & Lord, J. M. 1995c. Issues of interpretation after relating comparative datasets to phylogeny. *J. Ecol.* 83: 892-893.

FIGURE LEGENDS

Fig. 1: Partitioning the variation of a dependent variable (horizontal line) among ecological and phylogenetic components.

Fig. 2: Phylogeny of the 79 species of mammals used in the example. This phylogeny was derived from several sources (see Catzeflis et al., 1995; Cooper & Fortey, 1998; Morand & Poulin, 1998; Morand & Harvey, 2000) and published in Morand & Poulin (1998).



Desdevises et al., Fig. 1

Desdevises et al., Fig. 2