Université de Montréal

SYSTÉMATIQUE DU GENRE *ESSIGELLA* (HEMIPTERA : STERNORRHYNCHA) AU MOYEN DE DONNÉES MOLÉCULAIRES

Par

Thomas Théry

Centre sur la Biodiversité
Institut de Recherche en Biologie Végétale
Département de Sciences Biologiques
Faculté des Arts et Sciences

Thèse présentée
en vue de l'obtention du grade de Philosophiæ Doctor
en Sciences Biologiques

Janvier, 2018

Résumé

Contexte : La spécificité à une plante hôte est un phénomène courant chez les insectes phytophages et notamment chez les pucerons. L'identité de l'hôte est par conséquent souvent utilisée dans l'identification des espèces, pour son côté pratique mais aussi pour des groupes chez lesquels peu de caractères morphologiques sont fiables. Ce problème est notamment important dans le cas des espèces cryptiques qui, par définition, ne présentent pas de différences morphologiques avec les espèces apparentées. Cependant, les populations d'une même espèce d'insecte vivant sur différentes plantes hôtes peuvent parfois montrer des différences phénotypiques induites par celles-ci. Par conséquent, bien que l'association avec une plante hôte puisse être un caractère diagnostique pratique, il peut aussi être trompeur dans la reconnaissance et la délimitation des espèces. L'utilisation de l'association avec un hôte comme caractère diagnostique est plus fiable quand les espèces en question sont bien décrites et délimitées en utilisant d'autres critères diagnostiques tels que des séquences ADN. *Essigella* (Insecta, Hemiptera, Sternorrhyncha, Aphididae, Lachninae) est un genre de pucerons regroupant treize espèces, dont deux avec deux sous-espèces chacune. Elles se nourrissent de phloème sur les aiguilles des pins (*Pinus*) et des douglas (*Pseudotsuga*). La plupart des espèces d'*Essigella* sont monophages et se développent sur une seule ou quelques espèces d'hôtes apparentés. Deux espèces d'*Essigella*, *E. californica* et *E. pini*, sont toutefois oligophages et sont trouvées sur plusieurs espèces de pins. Les espèces d'*Essigella* sont naturellement néarctiques. Une espèce, *E. californica*, a toutefois été accidentellement introduite dans plusieurs pays autour du monde, devenant dans certains cas, un important ravageur dans les plantations de pins. *Essigella*, *Cinara*, *Eulachnus* et *Pseudessigella* appartiennent à la tribu des Eulachnini, bien que les relations phylogénétiques entre ces genres n'aient pas encore été clarifiées. Les espèces d'*Essigella* présentent une forte variabilité morphologique intra- et interspécifique. De plus, il existe peu ou pas de caractères diagnostiques fiables, rendant la taxonomie de ce genre difficile. Plusieurs espèces ont été circonscrites par de subtiles variations morphologiques seulement détectables avec des mesures morphométriques et des analyses multivariées. Aussi, l'identification des espèces d'*Essigella* est traditionnellement basée sur la combinaison de la morphométrie et de l'identité de la plante hôte. Parce que la systématique d'*Essigella* n'a pas

été testée par des moyens moléculaires, il est possible que certaines espèces proches morphologiquement et vivant sur des plantes hôtes apparentées correspondent en fait à des populations d'une même espèce. En même temps, il est également possible que différentes populations d'une présumée espèce puissent correspondre à différentes espèces cryptiques.

Cette thèse présente une révision de la systématique d'*Essigella* en utilisant des données moléculaires et plus particulièrement les séquences ADN de quatre gènes : *ATP6*, *COI*, *EF-1α* et *Gnd*.

Méthodes : J'ai estimé une phylogénie d'*Essigella* en utilisant le maximum de vraisemblance et l'inférence bayésienne avec les séquences ADN de quatre gènes : *ATP6* et *COI* (mitochondriaux), *EF-1α* (nucléaire), et *Gnd* (de l'endosymbiote obligatoire *Buchnera aphidicola*). Des espèces représentatives des trois autres genres d'eulachnines ont été utilisées comme groupes externes. J'ai employé cinq méthodes de délimitation d'espèces dans le but de tester la taxonomie d'*Essigella*. Celles-ci furent la méthode traditionnelle du barcode utilisant *COI* avec un seuil de 2%, la méthode de l'« Automatic Barcode Gap Discovery » (ABGD), celle du « General Mixed Yule Coalescent » (GMYC), celle du « Bayesian Poisson Tree Process » (bPTP), ainsi que celle du « Refined Single Linkage » (RESL) via la base de données BOLD (Barcode of Life Data Systems). J'ai aussi comparé les séquences d'*ATP6*, *COI*, *EF-1α* et *Gnd* de populations exotiques d'*E. californica* avec celles de populations nord-américaines pour confirmer qu'elles appartenaient bien à cette espèce.

Résultats : La phylogénie d'*Essigella* a montré que *Pseudessigella* était le groupe frère d'*Essigella* et qu'*Eulachnus* était le groupe frère du groupe *Essigella* + *Pseudessigella*. Cette phylogénie, combinée avec deux méthodes de délimitation d'espèces, ABGD et le barcode utilisant *COI*, a confirmé que toutes les espèces connues d'*Essigella* étaient valides et que par conséquent, les variations morphologiques observées étaient réellement spécifiques et non liées à la plante hôte. Aussi, les spécificités respectives de chaque espèce d'*Essigella* avec sa plante hôte ont été confirmées. Les analyses ont aussi révélé que les taxons connus comme *E. californica* et *E. pini* incluaient en réalité respectivement quatre et deux espèces, et que la base de données de BOLD renfermait les séquences de trois autres espèces non répertoriées. Dans le cas d'*E. pini*, l'espèce cryptique additionnelle correspond à son synonyme, *E. patchae*, qui ainsi a été rétablie. Dans le cas d'*E. californica*, les trois espèces nouvelles ne correspondent à aucun synonyme connu de l'espèce. Par conséquent, *E. californica* sensu lato inclut au moins quatre

espèces distinctes, *E. californica* sensu stricto et trois autres décrites comme nouvelles : *Essigella domenechi*, *E. gagnonae* et *E. sorenseni*. À cause de la forte proximité morphologique entre les quatre espèces, et pour la première fois dans la taxonomie des pucerons, les différences nucléotidiques de séquences ADN (*ATP6*, *COI* et *Gnd*) sont utilisées comme caractères dans les diagnoses respectives de trois espèces nouvelles. Malgré la découverte de ces trois espèces, j'ai confirmé que seule *E. californica* sensu stricto avait été introduite en dehors de l'Amérique du Nord, et que les introductions se sont produites indépendemment et au moins quatre fois.

Conclusion : Cette thèse a en grande partie confirmé la délimitation spécifique d'*Essigella* basée sur la morphométrie et la spécificité étroite de la plupart des espèces avec une plante hôte. Cependant, elle a aussi mis au jour l'existence de plusieurs espèces cryptiques et suggéré que de telles autres espèces pouvaient exister au sein d'*Essigella*, leur confirmation nécessitant un travail supplémentaire et un matériel additionnel substantiel. Cette étude souligne aussi que l'utilisation des données ADN comme caractères diagnostiques est essentielle dans la délimitation mais aussi dans la reconnaissance des espèces cryptiques.

Mots-clés : Eulachnini, plante hôte, espèce cryptique, phylogénie, délimitation d'espèces, taxonomie, spéciation, barcode, populations.

Abstract

Background: Host plant specificity is a common phenomenon in phytophagous insects and notably in aphids. Host identity is therefore often used in species recognition, for its practicality but also for groups for which few morphological characters are reliable. This issue is notably important in the case of cryptic species that, by definition, exhibit no morphological difference with related species. However, populations of one same insect species living on different host plants can sometimes show host-mediated phenotypic differences. As a consequence, although host plant association can be a practical diagnostic character, it can also be misleading when recognizing and circumscribing species. The use of host association as a diagnostic character is most reliable when the species in question are well described and delimited using other diagnostic criteria such as DNA sequences. *Essigella* (Insecta, Hemiptera, Sternorrhyncha, Aphididae, Lachninae) is an aphid genus encompassing thirteen species, two of which have two subspecies each. They are phloem-feeders on the needles of true pines (*Pinus*) and Douglas firs (*Pseudotsuga*). Most *Essigella* species are monophagous and develop on a single or a few closely-related host species. Two species of *Essigella*, *E. californica* and *E. pini*, are oligophagous, however, and found on several pine species. *Essigella* species are naturally Nearctic. One species, *E. californica*, has however been accidentally introduced in several countries around the world, becoming in some instances an important pest in pine plantations. *Essigella*, *Cinara*, *Eulachnus* and *Pseudessigella* comprise the tribe Eulachnini, although the phylogenetic relationships between these genera have not yet been clarified. *Essigella* species exhibit high intra- and interspecific morphological variability. Moreover, there exists few or no diagnostic reliable characters, making the taxonomy of the genus difficult. Several species were circumscribed by subtle morphological variations only detectable with morphometric measurements and multivariate analyses. Thus, identification of *Essigella* species has traditionally been based on a combination of morphometry and host plant identity. Because *Essigella* systematics has not been tested with molecular means, it is likely that some morphologically close species living on related host plants actually correspond to populations of a single species. At the same time, it is also possible that different populations of one apparent species may actually correspond to different cryptic species. This thesis presents a revision of

Essigella systematics using molecular data and more particularly DNA sequences of four genes: *ATP6*, *COI*, *EF-1α* and *Gnd*.

Methods: I estimated a phylogeny of *Essigella* using maximum likelihood and Bayesian inference using DNA sequences of four genes: *ATP6* and *COI* (mitochondrial), *EF-1α* (nuclear), and *Gnd* (from the obligate endosymbiont *Buchnera aphidicola*). Representative species of the three other eulachnine genera were used as outgroups. I employed five species delimitation methods in order to test the species taxonomy of *Essigella*: the traditional 2% *COI* barcode threshold, the Automatic Barcode Gap Discovery (ABGD), the General Mixed Yule Coalescent (GMYC), the Bayesian Poisson Tree Process (bPTP), and the Refined Single Linkage (RESL) via the BOLD (Barcode of Life Data Systems) database. I also compared *ATP6*, *COI*, *EF-1α* and *Gnd* sequences from exotic *E. californica* populations with sequences of North American ones to confirm they actually belongs to that species.

Results: The *Essigella* phylogeny showed that *Pseudessigella* is the sister-group of *Essigella* and that *Eulachnus* is the sister group of *Essigella* + *Pseudessigella*. That phylogeny, combined with two species delimitation methods, ABGD and *COI* barcoding, confirmed that all known *Essigella* species were valid and, as a result, morphological variations were truly specific and not linked to the host plant. Thus, respective specificity between *Essigella* species and their host plant were confirmed. The analyses also revealed that the taxa known as *E. californica* and *E. pini* actually include four and two cryptic species, respectively, and that the BOLD database contained sequences of three other unknown species. In the case of *E. pini*, the additional cryptic species corresponds to its synonym, *E. patchae*, which I thus re-established. In the case of *E. californica*, the three new species do not correspond to any known synonym of the species. Thus *E. californica* sensu lato includes at least four distinct species, *E. californica* sensu stricto and three others described as new: *Essigella domenechi*, *E. gagnonae* and *E. sorenseni*. Because of the strong morphological proximity between the four species, and for the first time in aphid taxonomy, nucleotide differences in DNA sequences (*ATP6*, *COI* and *Gnd*), are used in the respective diagnoses of the three new species. Despite discovery of these three species, I confirmed that only *E. californica* sensu stricto was introduced outside North America, and that the introductions occurred independently and at least four times.

Conclusion: This thesis largely confirmed the morphometrics-based species delimitation of *Essigella* and the narrow host plant specificity of most of the species. However, it also

uncovered the existence of several cryptic species and suggested that other such species may exist within *Essigella*, confirmation requiring further work and substantial additional material. This study also highlights that the use of DNA data as diagnostic characters is essential in delimiting but also in recognizing cryptic species.

Keywords: Eulachnini, host plant, cryptic species, phylogeny, species delimitation, taxonomy, speciation, barcoding, populations.

Table des matières

Résumé i

Abstract iv

Table des matières vii

Liste des tableaux xi

Liste des figures xii

Liste des abréviations xiii

Remerciements xvi

1. Introduction 1

1.1 Concepts et délimitation d'espèces 2
1.2 Spéciation et diversité chez les insectes phytophages 4
1.3 Les pucerons 5
1.4 Le genre *Essigella* Del Guercio, 1909 6
1.4.1 Historique et systématique du groupe 6
1.4.2 Biologie et écologie 7
1.4.3 Répartition 8
1.4.4 La notion d'espèce chez *Essigella* 9
1.5 Problématique générale de la thèse et objectifs 10

2. *EF-1α* DNA sequences indicate multiple origins of introduced populations of *Essigella californica* (Hemiptera: Aphididae) 13

2.1 Résumé / Abstract 15
2.2 Introduction 16
2.3 Materials and Methods 18
2.3.1 Taxon sampling 18
2.3.2 DNA Extraction, Amplification, and Sequencing 19
2.3.3 Data analyses 19
2.4 Results 21
2.4.1 Mitochondrial and *Buchnera* genes 21

2.4.2 Comparison of *EF-1α* Sequences 21
2.5 Discussion 23
2.5.1 *COI*, *ATP6*, and *Gnd* 23
2.5.2 Heterozygosity of *EF-1α* Sequences 24
2.5.3 Retracing the Routes of Introduction 25
2.6 Acknowledgments 26
3. Phylogenetic analysis and species delimitation in the pine needle-feeding aphid genus *Essigella* (Hemiptera, Sternorrhyncha, Aphididae) 27
3.1 Résumé / Abstract 28
3.2 Introduction 30
3.3 Materials and Methods 33
3.3.1 Taxon sampling 33
3.3.2 DNA Extraction, Amplification, and Sequencing 35
3.3.3 Phylogenetic analyses 35
3.3.4 Species delimitation 37
3.4 Results 39
3.4.1 DNA Extraction, Amplification, and Sequencing 39
3.4.2 Phylogenetic analyses 39
3.4.3 *EF-1α* sequences 41
3.4.4 Species delimitation 41
3.4.4.1 *COI* barcoding 41
3.4.4.2 Automatic Barcode Gap Discovery (ABGD) 41
3.4.4.3 General Mixed Yule Coalescent (GMYC) and Bayesian Poisson Tree Process (bPTP) 44
3.4.4.4 Refined Single Linkage (RESL) analysis 44
3.5 Discussion 45
3.5.1 Species delimitation methods issues 45
3.5.2 Within Eulachnini 46
3.5.3 Within *Essigella* 47
3.5.4 *EF-1α* sequences 50

3.5.5 The host plant issue 50
3.6 Acknowledgments 52
4. Molecular species diagnoses in *Essigella* Del Guercio, 1909 (Sternorrhyncha : Aphididae : Lachninae), with the description of three new species 54
4.1 Résumé / Abstract 55
4.2 Introduction 55
4.3 Materials and Methods 57
4.3.1 Abbreviations used 57
4.3.2 Taxon sampling 57
4.3.3 Preparation, measurements and pictures 57
4.3.4 Molecular data 59
4.4 Taxonomy 59
4.5 Discussion 72
4.5.1 *Essigella californica* 72
4.5.2 *Essigella patchae* and *E. pini* 73
4.6 Acknowledgments 74
5. Conclusion 75
5.1 *Essigella* au sein des Eulachnini 75
5.2 Monophylie et structuration interne du genre *Essigella* 76
5.3 La délimitation d'espèces au sein d'*Essigella* 76
5.4 *Essigella* et ses plantes hôtes 78
5.5 *Essigella californica* en dehors du continent nord-américain 78
5.6 Perspectives 78
Bibliographie i
Annexe 1. Morphologie d'*Essigella californica* i
Annexe 2. *COI* distance results ii
Annexe 3. OTUs and MOTUs according to morphology, 2% threshold *COI* and ABGD iv
Annexe 4. MOTUs according to GMYC v
Annexe 5. MOTUs according to bPTP vi

Annexe 6. NJ tree with *Essigella* BOLD sequences vii

Annexe 7. Results of the NJ tree ix

Annexe 8. Results of the BOLD identification tool xi

Annexe 9. Details of measurements xii

Annexe 10. Measurements used in descriptions xvii

Liste des tableaux

2.1. Collecting data of specimens and Genbank accession numbers of DNA sequences……20

2.2. Primers and PCR protocols……..……22

2.3. Heterozygosity patterns and locations……..…24

3.1. Current classification of the genus *Essigella* (Sorensen, 1994)……....34

3.2. Collecting data of specimens and Genbank accession numbers of DNA sequences……36

3.3. Primers and PCR protocols……..38

4.1. Diagnostic nucleotides differences between *E. domenechi* n. sp. and *E. californica*, *E. gagnonae* n. sp., *E. hoerneri* and *E. sorenseni* n. sp. for *ATP6*, *COI* and *Gnd*……60

4.2. Diagnostic nucleotides differences between *E. gagnonae* n. sp. and *E. californica*, *E. domenechi* n. sp., *E. hoerneri* and *E. sorenseni* n. sp. for *ATP6*, *COI* and *Gnd*……..63

4.3. Diagnostic nucleotides differences between *E. sorenseni* n. sp. and *E. californica*, *E. domenechi* n. sp., *E. gagnonae* n. sp. and *E. hoerneri* for *ATP6*, *COI* and *Gnd*……...65

Liste des figures

3.1. *Essigella hoerneri* on *Pinus monophylla* (left) and *Essigella* sp. on *Pinus ponderosa* (right)…….……32

3.2. Phylogenetic tree (ML and BI) of *Essigella* species using concatenate *ATP6*, *COI*, *EF-1α* and *Gnd*. *Pinus* species of the subgenus *Strobus* appear in hot colors, those of the subgenus *Pinus* in cold colors. Colour nuances represent infragroups within each subgenus. Non identified *Pinus* and other Pinaceae are in black. Values indicate ML bootstrap % values followed by Bayesian posterior probabilities % values……..42

3.3. *Essigella* specific differences in intron region 4 of *EF-1α*…….....43

4.1. Habitus of viviparous apterae: a. *Essigella pini*; b. *E. patchae*; c. *E. californica*; d. *E. domenechi* n. sp.; e. *E. gagnonae* n. sp.; f. *E. sorenseni* n. sp. …….....68

4.2. Morphological structures in *Essigella pini* and in *E. patchae*: a. URS in *E. pini* (slide-mounted specimen); b. URS in *E. patchae* (slide-mounted specimen); c. URS in *E. pini* (SEM); d. URS in *E. patchae* (SEM); e. genital plate in *E. pini* (SEM); f. genital plate in *E. patchae* (SEM); g. details in genital plate in *E. pini* (SEM); h. details in genital plate in *E. patchae* (SEM)……..……...69

Liste des abréviations

ABGD : automatic barcode gap discovery

ADN / DNA : acide désoxyribonucléique / deoxyribonucleic acid

AFLP : amplified fragment length polymorphism

ATP6 : *ATP synthase A chain subunit 6*

BC : British Columbia

BEAST : Bayesian evolutionary analysis sampling trees

BI : Bayesian inference

BIN : Barcode index number

BOLD (Systems) : Barcode of life data (Systems)

Bp : base pair

bPTP : Bayesian Poisson tree process

CA : California

CIPRES : cyberinfrastructure for phylogenetic research

COI : *cytochrome oxidase subunit 1*

CTT : collection privée de Thomas Théry

EF-1α : *elongation factor-1α*

EMEC : Essig Museum of Entomology, University of California, Berkeley, CA, USA

ESD : secondary electron detector

GMYC : general mixed Yule coalescent

Gnd : *gluconate 6 phosphate dehydrogenase*

i. e. : *id est*

kV : kiloVolt

K2P : Kimura 2 parameter

MCL : Markov clustering

MCMC : Markov Chain Monte Carlo

MD : Maryland

ML : maximum likelihood

MN : Minnesota

NC : North Carolina

NJ : Neighbor Joining

NSW : New South Wales

NV : Nevada

OAX : Oaxaca

OR : Oregon

p : distance sequence

PCR : polymerase chain reaction

pp : *posterior probability*

QC : Québec

QMOR : Collection Entomologique Ouellet-Robert

RFLP : restriction fragment length polymorphism

SA : South Australia

SEM : scanning electron microscope

UMSP : University of Minnesota Insect Collection, St Paul, MN, USA

USDA : U. S. Department of Agriculture, Beltsville, MD, USA

USNM : U. S. National Museum of Natural History, Beltsville, MD, USA

UTO / OTU : unité taxonomique opérationnelle / operational taxonomic unit

UTOM / MOTU : unité taxonomique opérationnelle moléculaire / operational taxonomic unit

V : Victoria

Je dédie cette thèse à ma famille et plus particulièrement à mes parents et mes grands-pères. Je dédie également cette thèse à mon ami Julien Raslan-Loubatié ainsi qu'à mon ami et mentor François Secchi, qui, partis trop tôt, n'auront pas vu l'aboutissement de ce travail.

… Alors qu'il s'en allait, John Sorensen se tourna vers moi et me lança avec un regard malicieux : « Good Luck ! ». Quelques mois plus tard, à Montréal, je compris alors ce qu'il avait voulu dire…

Remerciements

Ma reconnaissance va en premier lieu à mes parents pour la confiance qu'ils ont toujours eue en moi et pour m'avoir toujours soutenu dans mes choix.

Je remercie mon directeur de recherche, Colin Favret, pour m'avoir accepté en tant qu'étudiant, pour son soutien et les enseignements qu'il m'a prodigués.

Je remercie également John et Kathleen Sorensen pour leur chaleureux accueil en Californie, leur aide et leurs conseils sur le terrain ; Emmanuelle Jousselin pour m'avoir accueilli au sein de son laboratoire au CBGP de Montferrier sur Lez (France) ; les différents coauteurs des travaux présentés dans cette thèse : Eckehard G. Brockerhoff, Angus J. Carnegie, Rui Chen, Stephen R. Elms, Maurice Hullé, Richard Glatz, Mariusz Kanturski, Jaime Ortego, Ge-Xia Qiao et Évelyne Turpeau.

Je tiens également à remercier le Centre de la Science de la Biodiversité du Québec (CSBQ), l'Institut de Recherche en Biologie Végétale (IRBV) ainsi que la Société Entomologique du Canada (SEC) pour les différentes bourses qui m'ont été allouées pour participer à plusieurs conférences.

Enfin ma gratitude va à toutes celles et tous ceux qui d'une manière ou d'une autre sont intervenus durant ces 5 années de doctorat que cela fut pour un conseil, une aide matérielle ou tout simplement par leur présence : Khalil Abas, Amilkar Almanza, Raymond Archambault, Jocelyne Ayotte, Gonzalo Bilbao, Anaïs Boa, Michel Boulanger, Nicolas Boivin, Luc Brouillet, Anne Bruneau, Thérèse Cabana, Alexandre Caron, Gheylen Daghfous, Boris Domenech, Anne-Marie Gagnié, Édeline Gagnon, Mathilde Gaudreau, Pedro Grillo, Mohamed Hijri, Simon Joly, Alexandra Kack, Stéphane Le Tirant, Mirabelle Miron, Étienne Normandin, Étienne Richard-Dionne, Carole Sinou, Royce Steeves, Franck Stefani, Yves Terrat, Alexis Trépanier…

1. Introduction

La grande diversité du monde vivant provient majoritairement des interactions et des mécanismes de coévolution qui existent entre les organismes (Althoff *et al.*, 2014 ; Hembry *et al.*, 2014 ; Althoff, 2016). Elle prend notamment sa source dans les adaptations et spécialisations qui apparaissent et évoluent au sein d'associations comme celles qui existent entre les parasites et leurs hôtes, les insectes phytophages et les plantes dont ils se nourrissent, ou encore au sein d'associations mutualistes (Peccoud *et al.*, 2010 ; Yoder & Nuismer, 2010 ; Cruaud *et al.*, 2012 ; Wilson *et al.*, 2012 ; Joy, 2013 ; Thompson *et al.*, 2013). Avec le temps, les adaptations et spécialisations se font plus étroites entre l'organisme et son hôte, ce qui conduit inéluctablement à l'apparition de barrières génétiques entre les populations. Ce processus de sélection basé sur des divergences écologiques amène la ségrégation totale des populations d'une espèce et à l'apparition de nouvelles. Il correspond à un processus de spéciation écologique (Rundle & Nosil, 2005 ; Nosil, 2012). La forte spécialisation d'une espèce avec sa plante hôte est souvent utilisée comme un critère important pour l'identification chez certains groupes d'insectes phytophages (Lesage, 1995 ; Reid & Beatson, 2015 ; Blackman & Eastop, 2017). Celle-ci apparaît d'autant plus déterminante et pratique pour des taxons chez lesquels les caractères morphologiques pertinents sont peu nombreux ou difficilement accessibles. Toutefois, considérer la spécificité d'un insecte avec sa plante hôte comme un caractère infaillible n'est pas toujours vrai. En effet, une espèce d'insecte phytophage peut renfermer des populations présentant des différences morphologiques induites par les plantes sur lesquelles elles se développent (Favret & Voegtlin, 2004a ; Jorge *et al.*, 2011 ; Paris *et al.*, 2016). En outre, des populations considérées comme des espèces à part entières et supposées hôtes-dépendantes se révèlent parfois appartenir à la même espèce (Cocuzza *et al.*, 2007). Dans certains cas, les populations présentent déjà une spécialisation à une plante hôte particulière (Carletto *et al.*, 2009 ; Downey *et al.*, 2011). À l'opposé, des espèces considérées comme polyphages peuvent en réalité correspondre à plusieurs espèces cryptiques spécialisées sur un hôte (Hebert *et al.*, 2004). Dans de tels cas, et dans un contexte évolutif, la délimitation d'une espèce peut alors s'avérer difficile voire inexacte. Dans cette thèse, j'aborderai le problème de l'espèce chez *Essigella*, un genre de pucerons néarctiques, dont les espèces sont associées aux arbres des genres *Pinus* et *Pseudotsuga* et chez qui la délimitation des espèces demeure problématique.

1.1 Concepts et délimitation d'espèces

Bien que l'espèce soit l'unité de base en biologie (de Queiroz, 2005a, c, 2007 ; Hohenegger, 2014) sa définition ainsi que sa délimitation ont toujours constitué un problème récurrent (Dobzhansky, 1976 ; de Queiroz, 1998, 2007 ; Hohenegger, 2014). Plusieurs définitions se sont succédé et pas moins d'une trentaine de concepts ont été recensés (Mayden, 1997 ; Lherminié et Solignac, 2005 ; de Queiroz, 2005a ; Hohenegger, 2014). Je ne reviendrai pas sur chacune de ces définitions, ceci ayant déjà été fait maintes fois par de nombreux auteurs (voir de Queiroz, 1998 ; Lherminier & Solignac, 2005 ; Hohenegger, 2014). Je ne m'intéresserai qu'aux concepts qui sont en rapport direct avec le travail présenté ici. Une des définitions de l'espèce les plus populaires est sans doute celle de Mayr (1942), ou définition biologique de l'espèce : « les espèces sont des groupes de populations naturelles réellement ou potentiellement interfécondes et reproductivement isolées d'autres groupes semblables » (Mayr, 1942 ; de Queiroz, 1998 ; Lherminier & Solignac, 2005). Toutefois, les critères d'interfécondité utilisés pour son application sont souvent inaccessibles. Par exemple, les études taxonomiques sont basées principalement sur des spécimens morts, parfois seuls représentant de leur groupe. Il est alors impossible de tester leur interfécondité avec des groupes proches. Ce problème se pose également dans le cas des taxons fossiles ou dans celui de groupes qui se reproduisent de façon asexuée. Aussi, ce sont surtout des concepts ayant recours à des ressemblances morphologiques qui sont utilisés. C'est notamment le cas du concept morphologique de l'espèce qui se sert de caractères morphologiques diagnostiques et qui correspond au concept le plus couramment employé (Lherminier & Solignac, 2005). L'utilisation d'un spécimen type, considéré à tort comme morphologiquement représentatif de tous les individus de son espèce, symbolise l'usage à l'extrême des caractères morphologiques. Le concept phénétique de l'espèce est quant à lui un concept où une ressemblance plus globale est utilisée pour classer de manière arbitraire des individus (Sokal & Crovello, 1970 ; Lherminier & Solignac, 2005). Il est employé par commodité, le plus souvent dans l'optique d'études phylogénétiques. Dans ce cas, l'espèce est présomptive et l'on parle d'unité taxonomique opérationnelle (UTO ou *OTU* en anglais) (Doyen & Slobodchikoff, 1974 ; Ratnasingham & Hebert, 2013). Bien que les caractères utilisés pour séparer ces unités soient le plus souvent morphologiques, ils peuvent aussi être moléculaires (Ratnasingham & Hebert, 2013). Certains auteurs les nomment alors des unités taxonomiques

opérationnelles moléculaires (UTOM ou *MOTU* en anglais) (Floyd *et al.*, 2002; Vogler & Monaghan, 2007). Toutes les définitions ou concepts d'espèce possèdent leurs propres caractéristiques et sont souvent incompatibles (de Queiroz, 2005a, c, 2007). De plus, ils peuvent correspondre à la fois au concept de l'espèce mais aussi à sa délimitation qui sont pourtant deux notions totalement distinctes (de Queiroz, 2005a, c, 2007).

Ces deux dernières décennies ont vu l'apparition de nouvelles réflexions pour tenter de résoudre le problème de l'espèce, notamment celle de Pigliucci avec le *family resemblance concept* ou *cluster concept* (2003) reprenant en fait un concept préexistant (Wittgenstein, 1953) et l'appliquant au problème de l'espèce, et celle de Queiroz avec le *general lineage concept* (1998), requalifié en *unified species concept* (de Queiroz 2005a, b, c, 2007). Dans son concept, Pigliucci représente l'espèce comme un regroupement (*cluster*) de caractéristiques. Tous les individus d'une même espèce, semblant liés par un élément commun, ne sont en fait que reliés par des similitudes qui se chevauchent (Pigliucci, 2003). Pour de Queiroz, au contraire, toutes les définitions de l'espèce (tout du moins, pour les concepts modernes) partagent un trait commun, l'idée qu'une espèce correspond à une lignée d'organismes qui évolue indépendamment des autres au cours du temps (de Queiroz, 2005c, 2007). Plus précisément, ce concept représente l'évolution d'une métapopulation indépendamment des autres lignées, cette caractéristique devenant la seule propriété pour définir ce qu'est une espèce (de Queiroz, 2005c, 2007). Aussi, les propriétés ou caractéristiques utilisées dans les autres concepts, comme l'isolement écologique, l'isolement reproductif, la monophylie ou encore les différences morphologiques, deviennent secondaires et servent non plus à définir l'espèce en tant que telle mais à la délimiter (de Queiroz, 2005c, 2007). Ces caractéristiques représentent des processus ou des évènements qui apparaissent à différents moments au cours de l'évolution d'une population en cours de spéciation (de Queiroz, 2005c, 2007). Malheureusement, ces nouvelles tentatives n'ont pas clos l'éternel débat sur le problème de l'espèce (Richards, 2010 ; Ereshefsky, 2011 ; Hausdorf, 2011 ; Hohenegger, 2014 ; Kull, 2016 ; Freudenstein *et al.*, 2017 ; Pušić, 2017). Toutefois, la réflexion portée par de Queiroz a contribué à la création de nouvelles méthodes pour la délimitation des espèces, et plus particulièrement dans un cadre de reconstructions phylogénétiques (Carstens *et al.*, 2013). Ces méthodes emploient notamment la théorie de la coalescence (Pons *et al.*, 2006 ; Fujita *et al.*, 2012 ; Carsten *et al.*, 2013) qui permet d'estimer le moment où deux lignées distinctes se sont séparées, couplée à des analyses qui appliquent un ou plusieurs seuils au

résultat obtenu. Ce seuil permet de décider si la population a suffisamment divergé des autres qui lui sont proches pour être considérée ou non comme une espèce à part entière. Cette notion de seuil rappelle ainsi la méthode du *barcoding* (Hebert *et al.*, 2003a, b). Celle-ci utilise un fragment de 658 paires de bases du gène mitochondrial codant pour la *Cytochrome c oxidase subunit I* (*COI*) (Hebert *et al.*, 2003a, b). Deux individus pourront être considérés comme appartenant à deux espèces distinctes si le taux de divergence entre leur séquence de *COI* respective dépasse un seuil préalablement déterminé. Cette méthode permet non seulement de séparer et de délimiter des espèces, mais aussi d'identifier un organisme par comparaison avec des séquences de *COI* déjà connues. Dans le cas du barcoding utilisant *COI*, le seuil est habituellement fixé à 2% (Hebert *et al.*, 2003b). Toutefois, il est apparu que celui-ci pouvait varier en fonction des taxons (Davison *et al.*, 2009 ; Rakauskas *et al.*, 2011 ; Madeira *et al.*, 2017 ; Hu *et al.*, 2017).

1.2 Spéciation et diversité chez les insectes phytophages

Les relations plantes-insectes seraient à l'origine d'une partie importante de la diversité du monde vivant (Turcotte *et al.*, 2014 ; Althoff, 2016), le changement d'hôte étant l'un des principaux mécanismes à l'origine de la spéciation des insectes phytophages (Berlocher *et al.*, 2002 ; Peccoud *et al.*, 2010 ; Jousselin *et al.*, 2013). La coévolution d'un insecte phytophage avec sa plante hôte, c'est-à-dire le processus de sélection réciproque qui existe entre les deux organismes (de Vienne *et al.*, 2013 ; Hembry *et al.*, 2014), amène à une forte spécialisation de l'insecte avec la plante sur laquelle il se développe et se reproduit, et conduit à un phénomène de spéciation (Althoff, 2016). Ce processus de spéciation peut suivre deux mécanismes distincts : un changement d'hôte suivi d'une poursuite phylogénétique (Mitter & Brook, 1983 ; Althoff *et al.*, 2014) ou la cospéciation (de Vienne *et al.*, 2013 ; Althoff *et al.*, 2014). Dans les deux cas, l'évolution des deux lignées et la cladogénèse de leurs espèces respectives apparaissent comme parallèles. Toutefois, dans le cas de la poursuite phylogénétique, les cladogénèses ne sont pas simultanées, celle des insectes se réalisant postérieurement à celle des plantes. Les nouveaux hôtes colonisés sont la plupart du temps apparentés à la plante hôte d'origine (Mitter & Brooks, 1983 ; Percy *et al.*, 2004). Parfois, d'autres groupes végétaux sont colonisés quand les barrières chimiques, qui empêchaient jusque-là leur consommation, sont

surmontées. De nouvelles espèces végétales se trouvent ainsi disponibles favorisant la radiation de nouvelles espèces d'insectes (Ehrlich & Raven, 1964 ; Thompson, 1989). Ce mécanisme de poursuite phylogénétique a été particulièrement bien étudié pour le groupe des psylles (Hemiptera : Psylloidea) des Îles Canaries vivant sur des légumineuses endémiques de ces territoires (Percy, 2003 ; Percy *et al.*, 2004). Au contraire de la poursuite phylogénétique, la cospéciation implique des cladogénèses synchrones entre les deux groupes (Percy *et al.*, 2004). Le phénomène de cospéciation est donc beaucoup plus rare (Althoff *et al.*, 2014 ; Kergoat *et al.*, 2017). Les exemples parmi les mieux connus et les plus étudiés sont notamment celui de l'association des lépidoptères des genres *Tegeticula* Zeller et *Parategeticula* Davis (Prodoxidae) avec les plantes des genres *Yucca* Linné et *Hesperoyucca* (Engelm.) Baker (Asparagaceae) (Pellmyr, 2003), ainsi que celui des hyménoptères Agaonides avec les arbres du genre *Ficus* Linné (Moraceae) (Ramírez, 1974 ; Herre *et al.*, 1996 ; Machado *et al.*, 2001 ; Rønsted *et al.*, 2005 ; Cruaud *et al.*, 2012 ; Althoff *et al.*, 2014).

1.3 Les pucerons

Les pucerons (Hemiptera : Aphididae) regroupent environ 5000 espèces d'insectes phytophages (Favret, 2017), de petite taille, se nourrissant à partir du phloème des plantes qu'ils colonisent (Dixon, 1998). Ils sont largement étudiés pour leur implication dans les domaines de l'agriculture et de l'horticulture. Nombreuses sont en effet les espèces responsables de dommages agricoles (Blackman & Eastop, 2000), qu'elles soient polyphages comme *Aphis fabae* Scopoli, 1763, le puceron noir de la fève (Dixon, 1998), *Aphis gossypii* Glover, 1877, le puceron du coton (Ebert & Cartwright, 1997), *Myzus persicae* (Sulzer, 1776) le puceron vert du pêcher (Blackman & Eastop, 2000), ou plus spécialisées comme *Essigella californica* (Essig, 1909) sur les pins (Wharton & Kriticos, 2004 ; Kimber et *al.*, 2010, 2013). Certaines espèces sont également vectrices de pathogènes (Blackman & Eastop, 2000). Au-delà de leurs relations avec les végétaux, les pucerons font l'objet de recherches pour les associations symbiotiques qu'ils entretiennent avec des micro-organismes bactériens. Ils hébergent dans leurs tissus des bactéries qui leurs apportent des nutriments indispensables à leur développement et leur survie. Certaines associations sont exclusives comme avec *Buchnera aphidicola* Munson *et al.*, 1991 (Hook & Griffiths, 1980 ; Febvay *et al.*, 1999 ; Rabatel *et al.*, 2013). L'insecte ne pouvant vivre

sans cette bactérie et celle-ci ne pouvant se développer dans un autre milieu, ces deux organismes ont coévolué l'un avec l'autre. Aussi, le génome de *Buchnera aphidicola* a-t-il été utilisé dans la reconstruction phylogénétique et dans l'étude de la spéciation de divers groupes de pucerons (Moran *et al.*, 1993, 1999 ; Lozier *et al.*, 2007 ; Jousselin *et al.*, 2009 ; Chen *et al.*, 2013 ; Nováková *et al.*, 2013 ; Chen *et al.*, 2015). Il a notamment été employé dans l'étude de la phylogénie des Lachninae (Chen *et al.*, 2017) et dans l'étude de la spéciation et la délimitation d'espèces chez le genre *Cinara* (Aphididae : Lachninae : Eulachnini) (Jousselin *et al.*, 2013).

1.4 Le genre *Essigella* Del Guercio, 1909

1.4.1 Historique et systématique du groupe

Essigella est un genre de pucerons de petite taille (de 1,2 à 2,6 mm), de forme allongée, présentant une coloration allant du vert-jaunâtre au brun-orangé (Sorensen, 1994 ; Blackman & Eastop, 2017). Le genre fut créé en 1909 par Del Guercio pour isoler l'espèce *Lachnus californicus* Essig, 1909 des autres genres de Lachninae et notamment du genre *Eulachnus* Del Guercio. Par la suite, et ce jusqu'en 1994 et la révision du genre par Sorensen, vingt-deux autres espèces furent décrites (table 3.1) (Wilson, 1919 ; Gillette & Palmer, 1924 ; Hottes 1957, 1958 ; Sorensen, 1988). Le genre compte actuellement treize espèces reconnues comme valides dont deux avec deux sous-espèces chacune (Sorensen, 1994) (table 3.1). *Essigella* appartient à la sous-famille des Lachninae (Hemiptera : Aphididae) et fait partie de la tribu des Eulachnni qui regroupe les genres *Cinara* Curtis, 1835 (incluant *Schizolachnus* Mordvilko, 1909), *Eulachnus* Del Guercio, 1909 et *Pseudessigella* Hille Ris Lambers, 1966 (Chen *et al.*, 2016). Tandis que les espèces du genre *Cinara* (excluant le sous-genre *Schizolachnus*) se développent sur l'écorce des Pinaceae, les espèces des genres *Essigella*, *Eulachnus*, *Pseudessigella* et *Cinara* (du sous-genre *Schizolachnus*), se nourrissent au dépend des aiguilles de ces arbres. *Essigella* est très proche morphologiquement des genres *Eulachnus* et *Pseudessigella* et s'en sépare par les caractères suivants : antennes de 5 articles (6 chez *Eulachnus*), griffes des pattes incisées (simples chez *Eulachnus* et *Pseudessigella*), partie tergale des segments abdominaux 2 à 7 sclérotinisés chez les adultes (membraneux chez *Eulachnus* et *Pseudessigella*) (Sorensen, 1994), tête et pronotum entièrement fusionnés (non fusionnés chez *Eulachnus*) (Kanturski *et al.*,

2017a). Le genre se caractérise également par un mesonotum et un metanotum fusionnés dorsalement, par son premier tergite abdominal libre (sauf chez *E. essigi*), par les tergites abdominaux II à VII fusionnés et le huitième tergite abdominal libre (Sorensen, 1994) (annexe 1).

Si *Essigella* se distingue bien des autres genres d'Eulachnini, la séparation des espèces se révèle particulièrement délicate (Sorensen, 1994). La grande variabilité morphologique intra- et interspécifique rend en effet très difficile la délimitation de caractères stables et systématiquement informatifs (Sorensen, 1994). Ainsi, la validité des différentes espèces a été remise en question par Sorensen qui entreprit la révision du genre en employant des mesures morphométriques et des analyses multivariées (Sorensen, 1994).

1.4.2 Biologie et écologie

Malgré la révision de Sorensen et l'intérêt croissant envers l'*E. californica* ces dernières années, la biologie et l'écologie des autres espèces d'*Essigella* restent très mal connues, la plupart des informations étant souvent inférées à partir de nos connaissances d'*E. californica*. Le genre se rencontre principalement sur les arbres du genre *Pinus*, mais aussi sur *Pseudotsuga* (*E. wilsoni*), rarement sur *Picea* (*E. alyeska*) (Sorensen, 1994). Selon Sorensen (1994), la plupart des espèces d'*Essigella* seraient spécifiques à une espèce ou à un groupe d'espèces de pin phylogénétiquement proches. Parmi elles, seules *E. californica* et *E. pini* montreraient un plus grand éventail dans le choix des espèces consommées (Sorensen, 1994). Le cycle vital est monoécique, c'est-à-dire se déroulant sur une seule espèce végétale ou sur des espèces végétales apparentées. Ce cycle est théoriquement holocyclique (présence d'une génération sexuée et de plusieurs générations asexuées) bien qu'il soit le plus souvent anholocyclique (générations asexuées uniquement) dans des climats non continentaux (Sorensen, 1994). Il est connu pour être holocyclique chez *E. californica* en altitude en Amérique du Nord et anholocyclique partout ailleurs (Sorensen, 1994). Ainsi, les insectes passent l'hiver sous la forme de femelles vivipares aptères ou bien, si les hivers sont plus rigoureux ou en haute montagne, sous la forme d'œufs (Dixon, 1998).

Sauf pour ce qui est relatif à leur association avec leurs plantes hôtes, peu de choses ont été publiées concernant les relations d'*Essigella* vis-à-vis d'autres organismes. *Essigella* constitue

la proie de nombreux invertébrés généralistes, comme les araignées ou les pseudoscorpions, ou d'insectes classiquement aphidivores tels que les coléoptères Coccinellidae, les diptères Syrphidae et les névroptères Chrysopidae (Carver & Kent, 2000 ; Triapitsyn *et al.*, 2015). *Essigella californica* est parasitée par l'hyménoptère braconide *Diaeretus essigellae* Starý & Zuparko, 2002 (Kimber *et al.*, 2010 ; Triapitsyn *et al.*, 2015) et par le champignon entomopathogène *Entomophthora planchoniana* (Turpeau & Remaudières, 1990). *Essigella* peut aussi entretenir des relations mutualistes. Comme la plupart des pucerons, ses espèces sont parfois visitées par les fourmis (Sorensen, 1994), mais très peu de données ont été publiées à ce sujet. Les pucerons se nourrissent de sève élaborée riche en glucides mais pauvre en acides aminés (Febvay *et al.*, 1999 ; Sabri *et al.*, 2011). Comme les autres genres de pucerons, *Essigella* abrite la bactérie symbiotique *Buchnera aphidicola* (Nováková *et al.*, 2013), un endosymbionte primaire (obligatoire) qui leurs permet de parer à ce manque et contribue à leur développement (Hook & Griffiths, 1980 ; Munson *et al.*, 1991 ; Febvay *et al.*, 1999 ; Rabatel *et al.*, 2013). D'autres endosymbiotes, facultatifs (secondaires), comme *Serratia symbiotica* (Sabri *et al.*, 2011), sont connus pour vivre chez les pucerons mais leur présence chez *Essigella* n'a pas encore été démontrée.

1.4.3 Répartition

Les espèces d'*Essigella* sont toutes néarctiques, majoritairement réparties dans l'ouest du continent nord-américain, du sud de l'Alaska au Mexique. *Essigella californica* présente la distribution la plus étendue puisqu'elle est présente du sud de la Colombie Britannique au nord, au sud du Mexique au sud, et jusqu'au Nebraska à l'est. Elle est également citée de Floride, ce qui pourrait indiquer son existence dans les Caraïbes et tout le Mexique (Sorensen, 1994). *Essigella pini* et *E. alyeska* présentent quant à elles des distributions différentes. *Essigella pini*, occupe la façade est du continent, du Québec et du Maine au nord, jusqu'en Floride au sud, atteignant l'est de l'Oklahoma à l'ouest (Sorensen, 1994). *Essigella alyeska* semble posséder une répartition boréale. Elle n'est connue que de quelques localités de l'Alaska, de l'Ontario et du Québec (Sorensen, 1994). *Essigella californica* est à ce jour la seule espèce du genre à avoir été introduite en dehors de l'Amérique du Nord. Cette espèce semble avoir été introduite en même temps que certains pins sur lesquels elle vit et dont plusieurs sont largement utilisés en

sylviculture (Wharton *et al.*, 2004). Les premiers signalements ont eu lieu en France à partir de 1989 (Turpeau & Remaudière, 1990). Par la suite, sa présence a été décelée dans les territoires suivants : Espagne (Seco Fernandez & Mier Durante, 1992), Australie (Carver & Kent, 2000), Madère (Aguiar & Ilharco, 2001), Nouvelle-Zélande (Carver & Kent, 2000 ; Teulon *et al.*, 2003), Brésil (Carvalho & Lazzari, 2000), Italie (Barbagallo *et al.*, 2005), Tunisie (Boukhris-Bouhachem *et al.*, 2007 ; Blackman & Eastop, 2017), Malte (Mifsud *et al.*, 2009), Argentine (Ortego & Mier Durante, 2012) et en Grande-Bretagne (Reid *et al.*, 2015). L'espèce semblerait être également présente en Chine continentale (Rui Chen *com. pers.*) sans toutefois que cela ait été confirmé. Jusqu'à encore récemment, le genre *Essigella* n'était pas connu pour être responsable de dégâts importants sur le continent nord-américain ni d'être vecteur de pathogène (Carver & Kent, 2000). Les principaux dégâts enregistrés correspondaient à des problèmes esthétiques sur des formes ornementales de *Pinus radiata* (Burke, 1937 ; Ohmart, 1981) et à des pullulations dans des plantations de « sapins de Noël » dans le sud-est des USA (Sorensen, 1994). Les premiers dégâts hors Amérique furent signalés en France à partir de 1989 avec des jaunissements de branches et des phénomènes de défoliation notamment sur *Pinus radiata* (Turpeau & Remaudière, 1990). Dès lors, des problèmes similaires furent recensés dans d'autres territoires où *E. californica* avait été signalée. En Australie, la situation, devenue préoccupante, entraîne chaque année de lourdes pertes estimées à plusieurs millions de dollars (May & Carlyle, 2003 ; Wharton & Kriticos, 2004 ; Kimber *et al.*, 2010, 2013). *Essigella californica* est depuis considérée comme une peste dans ce pays (Wharton & Kriticos, 2004 ; Kimber *et al.*, 2010, 2013).

1.4.4 La notion d'espèce chez *Essigella*

Les problématiques de la notion d'espèce et surtout de sa délimitation (de Queiroz, 1998, 2007 ; Wiens, 2007) sont particulièrement marquées au sein du genre *Essigella*. Ainsi, la notion d'espèce au sein de ce genre a évolué au cours du temps en lien avec l'évolution des connaissances et des techniques. Plusieurs conceptions se sont alors succédé. Les premiers auteurs qui ont travaillé sur *Essigella* (Essig, Wilson, Hottes, Gillette & Palmer) avaient une notion morphologique de l'espèce. Hottes (1957, 1958) semble avoir toutefois suivi ce principe de manière abusive puisqu'il aurait attribué à de simples variations individuelles, mais aussi à

des artéfacts de préparations, une valeur de caractère spécifique (Sorensen, 1994). Pour s'affranchir de la forte variabilité morphologique propre à *Essigella*, Sorensen proposa une nouvelle approche utilisant des analyses multivariées basées sur des caractères morphométriques. Suite à ces analyses, il obtint quinze groupes, treize qu'il considéra comme spécifiques et deux comme subspécifiques selon leur proximité phylogénétique, la nature de la plante hôte ainsi que leur répartition géographique. Il rassembla ces différents taxons en 3 sous-genres : *Archeoessigella*, *Lambersella* et *Essigella* s. str. (tableau 3.1). Selon Sorensen, l'évolution et la spécialisation des espèces du genre *Essigella* suivrait en partie un modèle de suivi des ressources (« *resource tracking model* »; Brooks, 1981). Ce modèle se rapproche de celui de changement d'hôte avec poursuite phylogénétique (Mitter & Brooks, 1983). La notion d'espèce suivie par Sorensen est une notion phénétique (Sorensen, 1983, 1994) et les espèces discriminées correspondent à des unités taxonomiques opérationnelles (UTO).

1.5 Problématique générale de la thèse et objectifs

Bien que la révision de Sorensen corresponde à un travail rigoureux, plusieurs éléments pourraient remettre en question une partie de ses résultats. Plus particulièrement, plusieurs espèces ainsi que les sous-genres qu'il a décrits pourraient être invalidés. Premièrement, plusieurs espèces d'*Essigella* sont très proches morphologiquement, si proches que leur identification requiert la prise de mesures et l'utilisation de calculs complexes. Celles-ci vivent de plus sur des espèces de pins fortement apparentées. C'est notamment le cas des espèces des complexes *E. fusca* et *E. knowltoni* (Sorensen, 1994). Plusieurs études ont montré qu'une plante pouvait influer sur la morphologie des insectes sur laquelle ils vivent (Favret & Voegtlin, 2004a ; Paris *et al.*, 2016). Aussi, les différences observées par Sorensen pourraient ne représenter que de la variabilité intraspécifique due à la nature de la plante hôte. Deuxièmement, parmi toutes les espèces recensées, seules deux apparaissent comme oligophages : *E. californica* et *E. pini*. Dans le cas d'*E. californica*, Sorensen avait conclu à l'existence d'une seule et unique espèce malgré le nombre important de plante hôtes connues et la discrimination dans ses analyses de certaines populations hôte-dépendantes. Cette variation avait été considérée comme intraspécifique par l'auteur (Sorensen, 1983). Toutefois, son étude ayant été effectuée dans un cadre phénétique (Sorensen, 1983, 1994), Sorensen n'excluait pas que des analyses moléculaires

pussent révéler quelques taxons cryptiques au sein de cette espèce. Cette contradiction entre la description d'espèces distinctes au sein des complexes *E. fusca* et *E. knowltoni* mais pas au sein de l'espèce *E. californica* pose problème. Troisièmement, tous les sous-genres de Sorensen ne sont pas basés sur des synapomorphies. En particulier, *Archeoessigella* n'est pas, selon les termes de Sorensen (1983) cladistiquement monophylétique (« *not cladistically monophyletic* ») et est basé seulement sur des symplésiomorphies (Sorensen, 1983). En résumé, ces différentes données invitent à une remise en question de la nature de certains taxons et par conséquent de leur validité.

Aussi, la problématique de cette thèse est de savoir si la systématique des espèces d'*Essigella* proposée par Sorensen est valide. Plus particulièrement, il s'agit de tester si les variations morphologiques observées entre les différents groupes que Sorensen a considérés comme des espèces sont vraiment de nature spécifique ou au contraire ne correspondent qu'à de la variabilité intraspécifique induite par la plante hôte. Répondre à cette problématique implique de tester la systématique du genre dans son ensemble afin d'étudier les relations de parenté entre les différentes espèces. En prenant comme groupes externes les autres genres de la tribu des Eulachnini, cela permettra aussi de clarifier les relations entre *Essigella*, *Eulachnus* et *Pseudessigella*. Pour répondre à cette problématique, cette thèse présente cinq objectifs principaux répartis en 3 chapitres. Mon premier objectif est l'étude du problème de l'espèce chez les populations d'*Essigella* introduites en dehors du continent nord-américain. Plus particulièrement, il s'agit de savoir si une seule espèce a été introduite et si oui, s'il s'agit bien d'*E. californica* (Chapitre 1). Mon deuxième objectif est l'analyse de la phylogénie du genre *Essigella* au moyen de données moléculaires afin de connaître sa position au sein des Eulachnini et sa relation avec les genres *Eulachnus* et *Pseudessigella* (Chapitre 2). Mon troisième objectif correspond à l'étude phylogénétique du genre *Essigella* et plus particulièrement de tester sa monophylie et d'étudier sa structuration interne (Chapitre 2). Mon quatrième objectif est la délimitation des espèces du genre *Essigella* en combinant des analyses phylogénétiques avec des méthodes de délimitation d'espèces (Chapitre 2). Enfin, mon cinquième et dernier objectif correspond à la mise à jour de la taxonomie du genre *Essigella* suite aux résultats obtenus dans les chapitres 1 et 2 (Chapitre 3).

Afin de répondre à ces différents objectifs, 5 hypothèses sont avancées :

Hypothèse 1 : *Essigella* est monophylétique et *Pseudessigella* est le groupe frère d'*Essigella*.

Hypothèse 2 : La structuration du genre *Essigella* en trois sous-genres n'est pas valide.

Hypothèse 3 : Plusieurs espèces discriminées par Sorensen, notamment celles appartenant aux complexes *E. fusca* et *E. knowltoni*, qui sont très proches morphologiquement et qui vivent sur des plantes hôtes apparentées, correspondent dans chaque cas à des populations d'une seule et même espèce. Aussi, la systématique des espèces d'*Essigella* obtenue par Sorensen grâce à des données morphométriques et des analyses multivariées ne sera que partiellement retrouvée.

Hypothèse 4 : La phylogénie des espèces du genre *Essigella* ne suit pas celle des espèces de pin nord-américains et correspond plus à un schéma de changement d'hôte avec poursuite phylogénétique.

Hypothèse 5 : Une seule espèce d'*Essigella* a été introduite en dehors du continent nord-américain et il s'agit d'*Essigella californica*.

2. *EF-1α* DNA sequences indicate multiple origins of introduced populations of *Essigella californica* (Hemiptera: Aphididae)

Thomas Théry[1], Eckehard G. Brockerhoff[2], Angus J. Carnegie[3], Rui Chen[4], Stephen R. Elms[5], Maurice Hullé[6], Richard Glatz[7,8], Jaime Ortego[9], Ge-Xia Qiao[4], Évelyne Turpeau[6] and Colin Favret[1]

1. Department of Biological Sciences, Biodiversity Centre, University of Montreal, 4101 rue Sherbrooke Est, Montreal, QC, H1X 2B2, Canada

2. Scion, New Zealand Forest Research Institute, Christchurch 8440, New Zealand

3. NSW Forest Science, NSW Department of Primary Industries, Parramatta, NSW 2150, Australia

4. Chinese Academy of Sciences, Institute of Zoology, Beijing 100101, P. R. of China

5. HVP Plantations, Churchill, VIC 3842, Australia

6. Université Rennes 1, Institut de Génétique, Environnement et Protection des Plantes (IGEPP), Le Rheu 35653, France

7. School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5064, Australia

8. Terrestrial Invertebrates, South Australian Museum, Adelaide, SA 5000, Australia

9. Instituto Nacional de Tecnologia Agropecuaria (INTA), EEA Mendoza, Lujan de Cuyo 5507, Mendoza, Argentina

Publié dans Journal of Economic Entomology. 2017. 110(3) : 1269-1274

(Modifié pour les besoins de la présente thèse)

Tous les auteurs ont donné leur autorisation pour inclure ce manuscrit dans la présente thèse

Contribution respective des auteurs :

Thomas Théry : Conception du projet, collecte des données sur le terrain (USA), travaux moléculaires (extraction et amplification de l'ADN), traitement et analyse des données, interprétation et rédaction

Eckehard G. Brockerhoff : collecte des données sur le terrain (Nouvelle-Zélande), relecture du manuscrit

Angus J. Carnegie : collecte des données sur le terrain (Australie), relecture du manuscrit

Rui Chen : collecte des données sur le terrain (République Populaire de Chine), séquences partielles des gènes *ATP6*, *COI*, *EF-1α* et *Gnd* pour le matériel chinois, relecture du manuscrit

Stephen R. Elms : collecte des données sur le terrain (Australie), relecture du manuscrit

Maurice Hullé : collecte des données sur le terrain (France), relecture du manuscrit

Richard Glatz : collecte des données sur le terrain (Australie), relecture du manuscrit

Jaime Ortego : collecte des données sur le terrain (Argentine), relecture du manuscrit

Ge-Xia Qiao : collecte des données sur le terrain (République Populaire de Chine), relecture du manuscrit

Évelyne Turpeau : collecte des données sur le terrain (France), relecture du manuscrit

Colin Favret : Conception du projet, supervision et direction générales, collecte des données sur le terrain (USA), corrections

2.1 Résumé / Abstract

Résumé

Les pucerons pinicoles néarctiques du genre *Essigella* (Sternorrhyncha, Aphididae, Lachninae) ont été introduits en Europe, Afrique du Nord, Océanie, et Amérique du Sud. Les séquences ADN mitochondriales, nucléaires et endosymbiotiques de 12 populations introduites provenant de trois continents confirment que toutes appartiennent à *Essigella californica* (Essig, 1909). La variation dans les séquences des introns du gène nucléaire *EF-1α* a révélé l'existence de quatre groupes distincts. Le Groupe I rassemble une population venant de Chine, où l'espèce est nouvellement rapportée, et plusieurs venant d'Europe (France et Italie) ; le Groupe II est représenté par une population venant d'Argentine ; le Groupe III inclut deux populations provenant du Sud de l'Australie et une provenant de Nouvelle-Zélande ; et le Groupe IV correspond à cinq populations provenant de l'est et du sud-est de l'Australie. Ces résultats indiquent que les populations introduites d'*E. californica* proviennent d'au moins quatre populations distinctes. Ils montrent aussi que la variation des introns d'*EF-1α* peut être une méthode pour discriminer les populations de pucerons à reproduction asexuée.

Mots-clés : lignée asexuée, peste sylvicole, espèce envahissante, Lachninae, discrimination de populations

Abstract

Aphids in the pine-feeding Nearctic genus *Essigella* (Sternorrhyncha, Aphididae, Lachninae) have been introduced in Europe, North Africa, Oceania, and South America. Mitochondrial, nuclear, and endosymbiont DNA sequences of 12 introduced populations from three continents confirm they all belong to *Essigella californica* (Essig, 1909). Intron sequence variation of the nuclear gene *EF-1α* has revealed the existence of four distinct groups. Group I gathers one population from China, where the species is newly reported, and several from Europe (France and Italy); Group II is represented by one population from Argentina; Group III includes two populations from Southern Australia with one from New Zealand; and Group IV corresponds to five populations from Eastern and South-Eastern Australia. These results

indicate that introduced populations of *E. californica* have at least four source populations. They also show that intron variation of *EF-1α* can be a method to discriminate populations of asexually reproducing aphids.

Keywords: asexual lineage, silvicultural pest, invasive species, Lachninae, population discrimination

2.2 Introduction

Essigella Del Guercio, 1909 (Aphididae, Lachninae, Eulachnini) (Chen *et al.*, 2016) is a Nearctic genus of aphids living on the needles of Pinaceae (Sorensen, 1994). *Essigella californica* (Essig, 1909) is the only species introduced outside North America (Sorensen, 1994). It is recorded from France (Turpeau & Remaudière, 1990), Spain (Seco Fernández & Mier Durante, 1992), Australia (Carver & Kent, 2000), New Zealand (Carver & Kent, 2000; Flynn *et al.*, 2003), Brazil (Carvalho & Lazzari, 2000), Madeira (Aguiar & Ilharco, 2001), Italy (Barbagallo *et al.*, 2005), Tunisia (Boukhris-Bouhachem *et al.*, 2007), Argentina (Ortego & Mier Durante, 2012), and Great Britain (Reid *et al.*, 2015). After examination of the source material, the record from Malta (Mifsud *et al.*, 2009) was discarded due to misidentification, the specimen belonging to the genus *Eulachnus*, not *Essigella*. *Essigella californica* has been recorded on over 34 different species of *Pinus* and on some other Pinaceae (Watson & Appleton, 2007; Kimber *et al.*, 2013). Though the genus is not usually economically important in its native range (Sorensen, 1994), nor is it known to vector plant viruses (Carver & Kent, 2000), *E. californica* was recorded as causing yellowing and defoliation in France (Turpeau & Remaudière, 1990) and in New Zealand (Carver & Kent, 2000). Although it is not considered a significant pest in New Zealand (Watson *et al.*, 2008), in Australia, *E. californica* has been associated with severe chlorosis and defoliation across much of the commercial *P. radiata* D. Don plantation estate and is considered a significant silvicultural pest in that territory (May & Carlyle, 2003; May, 2004; Eyles *et al.*, 2011; Stone *et al.*, 2013a, b). Damage by *E. californica* was estimated to cause losses of up to AU$21 million per annum to the Australian forest industry (May, 2004), which led to a biological control program using *Diaeretus essigellae* Starý &

Zuparko, 2002 (Hymenoptera, Braconidae) (Kimber *et al.*, 2010) and the development of resistance breeds of *P. radiata* for commercial deployment (Sasse *et al.*, 2009).

COI is a mitochondrial gene well known for its use as a DNA barcode in animals (Hebert *et al.*, 2003a, b; Hajibabaei *et al.*, 2006). *COI* is employed in aphid species identification, notably in pest control and in phylogenetic analyses (Cœur d'acier *et al.*, 2007; Lee *et al.*, 2011). However, *COI* shows limits in some aphid groups, sometimes not being precise enough in species delimitation (Cœur d'acier *et al.*, 2014; Lee *et al.*, 2011, 2014). Thus, other genes have been investigated to improve species resolution. The gene *Gnd* of the obligate bacterial endosymbiont *Buchnera aphidicola* and the mitochondrial gene *ATP6* (Chen *et al.*, 2013; Lee *et al.*, 2014) were successfully tested. Contrary to mitochondrial genes, many nuclear genes are more stable (Simon *et al.*, 2010). They are often useful in phylogenetic analyses of higher level arthropod taxa (Caterino *et al.*, 2000; Simon *et al.*, 2010). For example, the nuclear gene *EF-1α* has been employed in the phylogeny of Hexapoda (Djernæs & Damgaard, 2006). Moreover, the variation of the *EF-1α* exon–intron structures has proven to be efficient in low level phylogenetic studies as well (Simon *et al.*, 2010). It was successfully used in phylogenetic reconstructions in several groups of insects (Cho *et al.*, 1995; Condamine *et al.*, 2013; Lin *et al.*, 2013; Cooper *et al.*, 2014) and notably in aphids (Moran *et al.*, 1999; Normark, 1999; von Dohlen *et al.*, 2006; Kim & Lee, 2008).

DNA analyses have revealed the existence of cryptic species of aphids (Depa *et al.*, 2012; Lee *et al.*, 2015). Often, the only biological distinction between morphologically identical species is their preferred host (Heie, 1986; Lee *et al.*, 2015; Mróz *et al.*, 2015). *Essigella* is a genus with a difficult taxonomy. Species are morphologically similar and several show high intraspecific variation (Sorensen, 1994). For these reasons, *Essigella californica* can be morphologically confused with *E. hoerneri* Gillette & Palmer, 1924 and *E. pini* Wilson, 1919 (Sorensen, 1994; Barbagallo *et al.*, 2005). However, several elements permit distinction between those species in North America. *Essigella californica* occurs in the same geographic vicinity as *E. hoerneri* but usually does not colonize pines of subsection Cembroides as does *E. hoerneri* (Sorensen, 1994; Blackman & Eastop, 2017). As a result, if the host is well-identified, there are few risks of misidentification between the two aphid species. In contrast, the respective native geographic ranges of *E. californica* and *E. pini* do not overlap (Sorensen, 1994), making their species identification straightforward. However, these two species are known to share

several pine species as hosts (Sorensen, 1994; Barbagallo *et al.*, 2005); thus, host identity is less useful for identification outside North America. The difficulty in confirming the identity of invasive populations of *Essigella* species has important repercussions for pest management, especially insofar as an authoritative identification is needed when searching for potential biological control agents.

Intraspecific morphological variation based on host plants was documented in *E. californica* by Sorensen (1994). This variation was considered either as being purely intraspecific (Sorensen, 1994), or suggestive that *E. californica* was actually one of a complex of species (Carvalho & Lazzari, 2000). No molecular systematic study has been carried out on the genus *Essigella*, and the existence of cryptic species within the putative *E. californica* complex has not been fully evaluated. Additionally, because of the possible confusion between *E. californica* and other *Essigella* species, and because the identity of the introduced populations was deduced only by morphology, there is no confirmation that those populations belong to a single species and that this species is indeed *E. californica*.

In this study, we used four genes, *ATP6*, *COI*, *EF-1α*, and *Gnd*, from 12 introduced populations of *Essigella* in order to confirm whether they belong to the same species and if so, that this species is *E. californica*. For this purpose, our results were compared with sequences from four North American populations of *E. californica* and from three other species-level taxa, *E. fusca ssp. voegtlini* Sorensen, 1994, *E. hoerneri*, and *E. pini*.

2.3 Materials and Methods

2.3.1 Taxon sampling

North American specimens of *Essigella* were collected during the summers of 2012 and 2013 (North Carolina and California). Overseas specimens were collected between 2012 and 2015 from Argentina, Australia, China, France, Italy, and New Zealand (Table 2.1). All specimens were preserved in 95% ethanol after collecting and subsequently kept at -20°C until DNA extraction. Species identifications were made with the species keys of genus *Essigella* published by Sorensen (1994) and Blackman & Eastop (2017). Specimens were also compared

with authoritatively identified reference material, including type specimens and material in the Sorensen Collection (Essig Museum of Entomology, Berkeley, CA). All voucher specimens are slide-mounted in Canada balsam and are deposited in the Ouellet-Robert Collection of the University of Montreal (QC, Canada); Chinese specimens are deposited in the National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing (People's Republic of China).

2.3.2 DNA Extraction, Amplification, and Sequencing

We made at least two separate extractions for each collection sample. Nondestructive DNA extractions were performed using the DNeasy Blood & Tissue kit of QIAGEN and the protocol of Favret (2005). PCR amplifications were performed at the Biodiversity Centre (University of Montreal, Montreal, QC, Canada) using Thermocycler Eppendorf Mastercycler ProS, with Phire Green Hot Start II DNA Polymerase (Thermo Fisher Scientific, Waltham, MA). Our protocols follow, with some modifications described in Table 2.2, those of Lee *et al.* (2014) for *ATP6*, Inbar *et al.* (2004) for *COI*, Favret & Voegtlin (2004b) for *EF-1α*, and Chen *et al.* (2013) for *Gnd*. Primers for each gene are the same as those published in these references. Amplicons were sequenced in both directions with the same primers at the Génome Québec Innovation Centre and the McGill University (Montreal, QC, Canada).

2.3.3 Data analyses

Chromatograms of each gene were edited using Geneious 9 software (Kearse *et al.*, 2012). Obtained sequences were compared with those in GenBank in order to confirm their general identity. Sequences were aligned and compared with Bioedit Version 7.2.5 (Hall, 1999) using the ClustalW multiple alignment program (Thompson *et al.*, 1994). Interspecific sequence divergence between species corresponds to Kimura 2 parameter (K2P) distance using MEGA 6.0 (Tamura *et al.*, 2013).

We located the *EF-1α* introns by cross-referencing our *Essigella* sequences with transcript sequences from *Acyrthosiphon pisum* (Harris, 1776) (http://www.aphid-base.com: ACYPI006711-RA).

Table 2.1. Collecting data of specimens and Genbank accession numbers of DNA sequences

Species	Country	Locality	Host plant	Genbank accession numbers			
				ATP6	*COI*	*EF-1α*	*Gnd*
E. californica	Argentina	Malargüe, Mendoza	*Pinus sp.*	KY288967	KY288911	KY288929	KY288948
E. californica	Australia	Adelaide (SA)	*Pinus sp.*	KY288966	KY288910	KY288928	KY288947
E. californica	Australia	Benalla (V)	*Pinus sp.*	KY288965	KY288909	KY288927	KY288946
E. californica	Australia	Bombala (NSW)	*Pinus patula*	KY288964	KY288908	KY288926	KY288945
E. californica	Australia	Churchill (V)	*Pinus sp.*	KY288963	KY288907	KY288925	KY288944
E. californica	Australia	Hamilton (V)	*Pinus sp.*	KY288962	KY288906	KY288924	KY288943
E. californica	Australia	Mt Mitchell (NSW)	*Pinus radiata*	KY288976	KY288920	KY288938	KY288957
E. californica	Australia	Whiporie (NSW)	*P. elliottii x P. caribea*	KY288977	KY288921	KY288939	KY288958
E. californica	China	Mt. Weibao (Yunnan)	*Pinus yunnanensis*	KY288980	KM501336	KY288942	KY288961
E. californica	France	Le Rheu (Ille et Vilaine)	*Pinus radiata*	KY288978	KY288922	KY288940	KY288959
E. californica	Italy	Turin (Piedmont)	*Pinus strobus*	KY288979	KY288923	KY288941	KY288960
E. californica	New Zealand	Christchurch (Canterbury)	*Pinus resinosa / wallachiana*	KY288970	KY288914	KY288932	KY288951
E. californica	USA	San Bernardino Co. (CA)	*Pinus coulteri*	KY288973	KY288917	KY288935	KY288954
E. californica	USA	Ventura Co. (CA)	*Pinus sp.*	KY288975	KY288919	KY288937	KY288956
E. californica	USA	Ventura Co. (CA)	*Pinus attenuata*	KY288971	KY288915	KY288933	KY288952
E. californica	USA	Placer Co. (CA)	*Pinus ponderosa*	KY288972	KY288916	KY288934	KY288953
E. hoerneri	USA	San Bernardino Co. (CA)	*Pinus monophylla*	KY288974	KY288918	KY288936	KY288955
E. fusca voegtlini	USA	Los Angeles Co. (CA)	*Pinus coulteri*	KY288968	KY288912	KY288930	KY288949
E. pini	USA	Swain Co. (NC)	*Pinus rigida*	KY288969	KY288913	KY288931	KY288950

2.4 Results

2.4.1 Mitochondrial and *Buchnera* genes

COI, *ATP6*, and *Gnd* were sequenced and analyzed for each population. The amplicon lengths were 658 base pairs (bp), 663 bp, and 749 bp for *COI*, *ATP6*, and *Gnd*, respectively (see Table 2.1 for GenBank accession numbers). All of the introduced populations of *Essigella* were genetically homogeneous for both mitochondrial genes (*COI* and *ATP6*) and the *Buchnera* gene (*Gnd*). These sequences were compared with those of North American populations of *E. californica*, *E. hoerneri*, *E. fusca voegtlini*, and *E. pini*. The *Gnd* sequences for the introduced populations matched perfectly those of *E. californica* in North America. The sequences of *COI* and *ATP6* of the introduced populations also closely matched those of North American *E. californica.* The North American *E. californica* population collected in Ventura Co. on an unidentified *Pinus* shows one nucleotide substitution in *COI.* This population and the North American one collected in Placer Co. on *Pinus ponderosa* Douglas ex Lawson each show one nucleotide substitution in *ATP6* at two different loci. Sequence divergence between introduced populations and *E. hoerneri* were 2.8%, 5.2%, and 5.7% for *COI*, *ATP6*, and *Gnd*, respectively. Rates between introduced populations and *E. fusca voegtlini* were 3.3%, 4.8%, and 8.8% for *COI*, *ATP6*, and *Gnd*, respectively. Rates between introduced populations and *E. pini* were 3.7%, 5.8%, and 9.5% for *COI*, *ATP6*, and *Gnd*, respectively. Rates between *E. hoerneri* and *E. fusca voegtlini* were 3.3%, 6.0%, and 10.3% for *COI*, *ATP6*, and *Gnd*, respectively, and those between *E. hoerneri* and *E. pini* were 3.7%, 7.2%, and 11.3% for *COI*, *ATP6*, and *Gnd*, respectively. The rates between *E. fusca voegtlini* and *E. pini* were 2.6%, 6.0%, and 8.8% for *COI*, *ATP6*, and *Gnd*, respectively. Moreover, a sequence of *COI* from British *Essigella* published on GenBank (accession number KM888108) is identical to our sequences from introduced *E. californica*.

2.4.2 Comparison of *EF-1α* Sequences

First, we analyzed *EF-1α* sequences of all *E. californica* specimens together. In that case, sequence length was 785 bp including introns and 610 bp without introns (see Table 2.1 for GenBank accession numbers). As with *COI*, *ATP6* and *Gnd*, *EF-1α* sequences were essentially

identical between all the *E. californica* populations with or without introns except for the presence of a single nucleotide substitution in three populations, two from Australia and one from New Zealand. That substitution is located at position 195 of exon 5. Second, we analyzed *EF-1α* sequences of all *Essigella* species together. Sequences lengths, including the introns, were 767 bp for *E. californica* and *E. hoerneri*, 746 bp for *E. fusca voegtlini*, and 775 bp for *E. pini*. The lengths of sequences without introns were 610 bp for all species. *EF-1α* exon sequence divergence between introduced populations of *Essigella californica* and *E. hoerneri*, *E. fusca voegtlini*, and *E. pini* was 0.3%, 1.5%, and 1.3%, respectively; respective sequence divergence including the introns was 0.3%, 2.1%, and 1.8%.

Table 2.2. Primers and PCR protocols

Genes	Primers sequences	Initial denaturation: time and T°C	Number of Cycles	Denaturation: time and T°C	Annealing: time and T°C	Elongation: time and T°C	Final Elongation: time and T°C
Lep-F1	ATTCAACCAATCATAAAGATATTGG	98 C for 30 sec	35	98 C for 10 sec	50 C for 20 sec	72 C for 20 sec	72 C for 2 min
Lep-R1	TAAACTTCTGGATGTCCAAAAAATCA						
BamHI	CGCGGATCCGGWCCWWSWATWAT GCCWGGWGG	98 C for 1 min	35	98 C for 20 sec	51 C for 40 sec	72 C for 40 sec	72 C for 3 min
ApaI	CGCGGGCCCGTATGWGCWCCAAAA TAATCWCKTTGWGCTTG						
tRNALysAf2	GACTGAAAAGCAAAGTAATGATCTCT	94 C for 3 min	35	94 C for 30 sec	55 C for 30 sec	65 C for 1 min	none
CO3WWRD	TCWCGAATWACATCWCGTCATCA						
EF1-F	GAACGTGAACGTGGTATCAC	98 C for 30 sec	35	98 C for 20 sec	51 C for 20 sec	72 C for 20 sec	72 C for 2 min
EF1-R	TGACCAGGGTGGTTTCAATAC						

EF-1α DNA sequence traces indicated the presence of heterozygosity in seven populations of *E. californica* and in the one of *E. pini*. The evidence is apparent in the form of clear and consistent double peaks in the sequencing chromatograms. In particular, there are six apparent heterozygous sites across multiple samples in the *E. californica* populations. The first two sites are located in introns 3 and 4, the next two are located in exon 5 at positions 135 and 195 and represent silent substitutions. The last two heterozygous loci are located in intron 5. The presence or absence and the nature of the heterozygous sites in populations of *E. californica* reflect four distinct groups (see Table 2.3). Group I: populations from France, Italy, and China each displayed a [1: A/T, 2: G/T, 3: C, 4: A, 5: A/G, 6: C/T] pattern; Group II: the Argentinean

population displayed a [T, T, C, A/G, A, C] pattern; Group III: two populations from southern Australia (Adelaide and Hamilton) and one from New Zealand displayed a [T, T, C/T, G, A, C] pattern; Group IV: five eastern and southeastern Australian populations (Benalla, Bombala, Churchill, Mt Mitchell, and Whiporie) displayed a [T, T, C, A, A, C] pattern. Among the North American populations of *E. californica*, one displayed the same pattern as that of Group IV and three displayed that of Group II. The Group I and Group III patterns were not recovered in our North American samples.

2.5 Discussion

2.5.1 *COI*, *ATP6*, and *Gnd*

The comparison of the K2P distances of *COI* indicates that the introduced populations of *Essigella* all belong to *E. californica*. Indeed, *COI* sequence divergence observed between the different studied species were from 2.8% to 3.7% and were superior to the rate of 2% accepted in barcoding studies to separate two species (Hebert *et al.*, 2003b). Meanwhile, the divergence between the various populations of *E. californica* never exceeded 0.2%, corresponding to a single nucleotide substitution. The separation between our four species was also confirmed with results obtained with *ATP6* and *Gnd*. However, the divergences observed for those three genes are lower than those observed between species in other genera of Eulachnini (*Eulachnus* and *Cinara*). In fact, interspecific distance values of *COI* for *Cinara* found by Chen *et al.* (2012) are 8.7% (± 2.2%) to 10.4% (± 2.4%) and those by Chen *et al.* (2013) are 8.9% (± 2.1%). For the genus *Eulachnus*, sister genus to *Essigella*, Chen *et al.* (2012, 2013) calculated values of 7.7% (± 0.9%) to 9.5% (± 1.4%) and 7.4% (± 0.9%).

K2P distances in *Gnd* seem also lower in *Essigella*. For *Gnd*, our results were between 5.7% and 11.3% whereas Chen *et al.* (2013) found values of 30.8% (± 5%) for *Cinara* and 16.9% (± 4.6%) for *Eulachnus*. We found no published values of sequence divergence of *ATP6* in the two other cinarine genera, but Lee *et al.* (2014) published an average value of 8.3% based on 20 other aphid species, whereas we found that in *Essigella* they were between 4.8% and 7.2%.

Table 2.3. Heterozygosity patterns and locations

Location in *EF-1α* sequence	Argentina	Australia							China	France	Italy	New Zealand	USA (California)				
		Adelaide (SA)	Benalla (V)	Bombala (NSW)	Churchill (V)	Hamilton (V)	Mt Mitchell (NSW)	Whiporie (NSW)					*Pinus attenuata*	*Pinus coulteri*	*Pinus ponderosa*	*Pinus* sp.	
Intron 3	T	T	T	T	T	T	T	T	W	W	W	T	T	T	T	T	
Intron 4	T	T	T	T	T	T	T	T	K	K	K	T	T	T	T	T	
Exon 51 135	C	Y	C	C	C	Y	C	C	C	C	C	Y	C	C	C	C	
Exon 51 195	R	G	A	A	A	G	A	A	A	A	A	G	R	R	A	R	
Intron 5	A	A	A	A	A	A	A	A	R	R	R	A	A	A	A	A	
Intron 5	C	C	C	C	C	C	C	C	Y	Y	Y	C	C	C	C	C	

Our lower values could be explained by the small number of species we compared with our samples of *E. californica*. Moreover, *E. hoerneri* is known to be phylogenetically close to *E. californica*. It is likely that comparing additional species of *Essigella* would yield values closer to those obtained by others authors with *Cinara* and *Eulachnus*. Having confirmed that all introduced populations belong to *E. californica*, comparison of our *COI* sequences with those available in GenBank (KM888108) confirms the presence of the species in Great Britain (Reid *et al.*, 2015).

2.5.2 Heterozygosity of *EF-1α* Sequences

Although heterozygous loci are often seen as hindrances in molecular systematics, in our case polymorphic sites provided important information. Normark (1999) also observed polymorphic sites in *EF-1α* sequences in *Trama*, another genus of Lachninae. Our results showed the same phenomenon, appearing as obvious double peaks, almost or identical in height, on our sequencing chromatograms. Like Normark (1999), we checked whether each double peak was present in at least one other specimen of the same colony. Because *E. californica* is asexual in the territories where it was introduced, there is no sexual recombination in those populations. In such conditions, each heterozygous pattern will be specific to one population or to a group of related populations (Birky, 1996; Schwander *et al.*, 2011).

Our study indicates that introduced populations of *E. californica* have at least four origins, and that at least four introductions occurred around the world: one in South America, one in Eurasia (Europe and China), and two in Oceania. However, it is hard to believe that a single introduction occurred in China, Italy, and France. It seems more likely that these countries received *E. californica* separately, although our data are unable to confirm this.

2.5.3 Retracing the Routes of Introduction

The heterozygous sites found in some introduced populations of *E. californica* were also found in North America. On the one hand, the *EF-1α* pattern found in five Australian populations (Benalla, Bombala, Churchill, Mt Mitchell and Whiporie) is also found in one populations of North America (populations collected on *Pinus ponderosa*). Sequences of *COI* and *Gnd* of this North American population matched perfectly with those of Group IV. However, this population showed a difference of one nucleotide in the ATP6 sequence in comparison with that found in Group IV. We made a similar observation with Group II, displaying the same *EF-1α* pattern as that found in the three other North American populations (on *P. attenuata* Lemmon, on *P. coulteri* D. Don and on *Pinus sp.*). Two of them (on *P. attenuata* and on *P. coulteri*) had *COI*, *ATP6*, and *Gnd* sequences identical to those found in Group II. The other (on *Pinus sp.*) had a difference of one nucleotide in the *COI* sequence and a difference of one nucleotide in the *ATP6* sequence in comparison with those of Group II. Even though our results suggest at least four origins for introduced populations of *E. californica*, the small differences we found in *COI* and in *ATP6* sequences indicate that our results may not be precise enough to prove a direct link between introduced and particular North American populations. Higher resolution population genetic methods such as microsatellites (Llewellyn *et al.*, 2003; Li *et al.*, 2015), AFLP (amplified fragment length polymorphism; Dieni *et al.*, 2016), or RFLP (restriction fragment length polymorphism; Piffaretti *et al.*, 2013) may be more useful in locating the North American points of origin of the various introduced populations.

Heterozygous base pairs in the introns of the nuclear gene *EF-1a* can discriminate populations in asexual aphid species. In our case, they showed that introduced populations of *E. californica* have at least four origins and that *E. californica* was introduced at least four times

outside Western North America, its native territory. It also confirms that the species is now present in Europe, Oceania, South America, and Asia.

2.6 Acknowledgments

We are grateful to G. L. Miller (USDA Systematic Entomology Laboratory, Beltsville, MD), P. T. Oboyski (Essig Museum of Entomology, University of California, Berkeley, CA), and R. E. Thomson (University of Minnesota, St Paul, MN) for specimen loans, S. Barbagallo for Italian specimens of *Essigella*, and M. Nagel, M. Horwood, and S. Shaw assisted with Australian collections. We also extend our thanks to J. T. Sorensen for his advice and assistance in the field. Susan Halbert (Florida Department of Agriculture and Consumer Services) and an anonymous reviewer helped improve the quality of the manuscript. E.G.B. was supported by MBIE core funding to Scion and the Better Border Biosecurity collaboration (www.b3nz.org).

3. Phylogenetic analysis and species delimitation in the pine needle-feeding aphid genus *Essigella* (Hemiptera, Sternorrhyncha, Aphididae)

Thomas Théry[1], Mariusz Kanturski[2] and Colin Favret[1]

1. Department of Biological Sciences, Biodiversity Centre, University of Montreal, 4101 rue Sherbrooke Est, Montreal, QC, H1X 2B2, Canada

2. Department of Zoology, Faculty of Biology and Environmental Protection, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland

Accepté dans Insect Systematics and Diversity

(Modifié pour les besoins de la présente thèse)

Tous les auteurs ont donné leur autorisation pour inclure ce manuscrit dans la présente thèse

Contribution respective des auteurs :

Thomas Théry : Conception du projet, collecte des données sur le terrain (USA, Canada), travaux moléculaires (extraction et amplification de l'ADN), traitement et analyse des données, interprétation et rédaction

Mariusz Kanturski : apport de données (Inde), retouche des figures et tableaux, relecture du manuscrit

Colin Favret : Conception du projet, supervision et direction générales, collecte des données sur le terrain (USA, Canada), corrections

3.1 Résumé / Abstract

Résumé

Les espèces du genre *Essigella* (Hemiptera : Aphididae) sont connues pour être spécifiques à une ou quelques espèces affiliées de Pinacées. La classification actuelle d'*Essigella* inclut 15 taxons, inférés à partir de données morphologiques et écologiques. Nous présentons une phylogénie d'*Essigella* utilisant le maximum de vraisemblance et l'inférence bayésienne en employant les séquences ADN de trois génomes : mitochondrial (*ATP6*, *COI*), nucléaire (*EF-1α*) et endosymbiotique (*Gnd*). Nous avons également testé la taxonomie d'*Essigella* en utilisant cinq méthodes de délimitation d'espèces : la méthode du barcode avec *COI* avec un seuil de 2%, celle de l'ABGD, celle du GMYC, celle du bPTP, ainsi que celle du RESL via les unités taxonomiques opérationnelles (Barcode Index Numbers ou BINs) dans la base de données BOLD (Barcode of Life Data Systems) et via son outil d'identification en ligne. Cinquante-trois populations d'*Essigella* ont été étudiées avec les genres *Cinara*, *Eulachnus* et *Pseudessigella* en groupes externes. Les analyses phylogénétiques supportent *Pseudessigella* comme le groupe frère d'*Essigella*. Elles confirment que toutes les espèces connues d'*Essigella* sont valides, majoritairement liées à une plante hôte spécifique, mais aussi qu'*Essigella pini* Wilson, 1919 renferme deux espèces, la seconde étant probablement *E. patchae* Hottes, 1957, actuellement considérée comme un synonyme. Les analyses phylogénétiques sont partiellement congruentes avec les résultats d'ABGD et ceux du barcode utilisant *COI* mais avec un seuil < 2% pour ce dernier. Elles suggèrent l'existence de plusieurs espèces cryptiques supportées également par des données écologiques. Les méthodes bPTP et GMYC ont donné des résultats incohérents, possiblement dus à un échantillonnage inadéquat. La majorité des espèces délimitées avec la méthode ABGD et celle utilisant le barcode avec un seuil < 2% sont retrouvées parmi les BINs de la base de données BOLD excepté pour les espèces cryptiques révélées au sein d'*E. califonica*. Cette comparaison de données révélent également que trois BINs correspondent à trois espèces d'*Essigella* encore non répertoriées. L'outil d'identification de BOLD ne reconnaît correctement qu'une partie des séquences soumises. Malgré leur existence dans le système, peu de BINs sont reliés aux bonnes espèces. Nos résultats soulignent que des données substantielles sont nécessaires pour utiliser convenablement les méthodes de délimitation d'espèces. Notre étude montre aussi que, pour obtenir une réponse crédible au

problème de la délimitation d'une espèce, une combinaison de différentes méthodes et données est préférable à des méthodes de délimitation d'espèces seules.

Mots-clés : Lachninae, Eulachnini, plante hôte, spéciation, barcoding

Abstract

Species of the genus *Essigella* (Hemiptera: Aphididae) are known to be specific to one or a few related species of Pinaceae hosts. The current *Essigella* classification includes 15 species-group taxa, inferred with morphological and ecological data. We present a phylogeny of *Essigella* using a maximum likelihood and Bayesian inference using DNA sequences from three genomes: mitochondrial (*ATP6*, *COI*), nuclear (*EF-1α*) and endosymbiont (*Gnd*). We also challenged the taxonomy of *Essigella* species using four species delimitation methods: the 2% *COI* barcode threshold, the ABGD, the GMYC, the bPTP methods and the one of the RESL via the operational taxonomic units (Barcode Index Numbers ou BINs) in the BOLD (Barcode of Life Data Systems) database and via its online identification tool. Fifty-three populations of *Essigella* were studied with the eulachnine genera *Cinara*, *Eulachnus* and *Pseudessigella* as outgroups. Phylogenetic analyses support *Pseudessigella* as sister-group of *Essigella*. They confirm that all the known species are valid, mostly linked to a specific host plant, but also that *E. pini* Wilson, 1919 encompasses two species, the second probably being *E. patchae* Hottes, 1957 currently considered a synonym. Phylogenetic analyses were partially congruent with the results of ABGD and those of *COI* barcoding, although the barcoding threshold was < 2% for the latter. They suggest the existence of several cryptic species also supported by ecological data. Results of bPTP and GMYC gave incoherent results, possibly due to inadequate sampling. Most of the species delimited with the ABGD method, as well as that of the barcoding with a < 2% threshold, were found amongst the BINs of the BOLD database, with the exception of the cryptic species revealed within *E. californica*. This comparison also revealed that three BINs correspond to three unknown *Essigella* species. The BOLD idntification tool correctly recognizes only a portion of the submitted sequences. Despite their existence in the system, few BINs are linked with the correct species. Our results highlight that substantial data are required to use correctly species delimitation methods. Our study also shows that, to obtain a credible

answer to the problem of species delimitation, a combination of different methods and data sources is preferable to molecular species delimitation methods alone.

Keywords: Lachninae, Eulachnini, host plant, speciation, barcoding

3.2 Introduction

Aphids (Hemiptera Aphididae) are sap-feeding insects. More than 5000 species are known (Favret, 2017) with some considered important economic pests (Eastop, 1977; Foottit *et al.*, 2006; Blackman & Eastop, 2000). Aphid taxonomy is mainly based on morphology and host plant identity, given their high host plant specificity (Blackman & Eastop, 2017). Intraspecific polymorphism and polyphenism can make species identification difficult and inaccurate (Hille Ris Lambers, 1966a; Simpson *et al.*, 2011). Aphids can have different life cycles, patterns of host alternation (von Dohlen & Moran, 2000), and different morphs depending on seasonal or climatic parameters (Hille Ris Lambers, 1966a; von Dohlen & Moran, 2000). Their morphology and general appearance can also be modified by their relationships with other organisms (Weisser *et al.*, 1999, Johnson *et al.*, 2003, Tsuchida *et al.*, 2010; Yao, 2012), most notably with their host plant (Wool & Hales, 1997; Margaritopoulos *et al.*, 2000; Favret & Voegtlin, 2004a). In some cases, the paucity or complete lack of diagnostic morphological characters remains the main issue (Sorensen, 1983, 1994; Favret, 2009). Plant host specificity is not always a reliable indicator, several aphid species being oligophagous or polyphagous.

DNA barcoding is a method used in recognition of animal species using a 658 base-pair fragment of the 5' end of the mitochondrial gene *cytochrome c oxidase I* (*COI*) (Hebert *et al.*, 2003a,b ; Hajibabaei *et al.*, 2006). The method is commonly used in integrated insect taxonomy and systematics, for example in Coleoptera (Beeren *et al.*, 2016; Magoga *et al.*, 2016), Diptera (Montagna *et al.*, 2016; Chroni *et al.*, 2017), Hymenoptera (Packer & Ruz, 2016; Schmidt *et al.*, 2017), Lepidoptera (Hajibabaei *et al.*, 2006; Buchner *et al.*, 2017) and Plecoptera (Avelino-Capistrano *et al.*, 2016). Beyond its assistance in species recognition, barcoding permits the recognition of cryptic species and several species of aphids have been discovered using this method (Miller *et al.*, 2009; Lee *et al.*, 2017). DNA barcoding is used in pathogen and pest

control by providing a rapid and accurate identification regardless of the insect's life stage (Zhang *et al.*, 2016; Cock *et al.*, 2017; Sulaiman *et al.*, 2017).

Besides its use in species identification, *COI* DNA barcoding is employed to delimit species assuming a 2% threshold of sequence divergence between species (Hebert *et al.*, 2003b). However, species delimitation using the *COI* barcode is sometimes not precise enough in Sternorrhyncha, notably for groups already known to be problematic (Zurovcová *et al.*, 2010, Cœur d'acier *et al.*, 2014, Lee *et al.*, 2014). In consequence, other barcoding genes with similar properties as *COI* have been considered in aphids: *Gnd*, a gene of the obligate bacterial endosymbiont *Buchnera aphidicola*, and *ATP6*, a mitochondrial gene, were successfully tested (Chen *et al.*, 2013; Lee *et al.*, 2014). Some nuclear genes can also be used for these same purposes; notably *EF-1α*, with its exon–intron structure, can provide relevant phylogenetic or population information (Simon *et al.*, 2010; Savory & Ramakrishnan, 2015; Théry *et al.*, 2017).

Several other methods have been developed for molecular species delimitation. For example, the Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.*, 2012) method compares the gap existing between the range of intra- and interspecific sequence distances. Other methods based on phylogenetic analyses use the theory of coalescence (Fujita *et al.*, 2012), such as the General Mixed Yule Coalescent (GMYC) (Pons *et al.*, 2006; Fujisawa & Barraclough, 2013) or the Bayesian Poisson Tree Process (bPTP) (Zhang *et al.*, 2013). The Refined Single Linkage method (RESL) is a method implemented in the Barcode of Life Data Systems (BOLD) (http://www.boldsystems.org/). It uses single linkage clustering followed by a Markov clustering (MCL) to delimit OTUs from barcode sequence records (*COI*). OTUs obtained with RESL correspond to Barcode Index Numbers (BINs) (Ratnasingham & Hebert, 2013).

Essigella Del Guercio, 1909 (Aphididae, Lachninae) (Fig. 3.1) is a small genus of narrow-bodied aphids encompassing thirteen species with two having two subspecies each (Sorensen, 1994). All species feed on the needles of *Pinus* Linnaeus, with the exception of *E. wilsoni* Hottes, 1957, which feeds only on the needles of *Pseudotsuga* Carrière, and *E. alyeska* Sorensen, 1988, which has been known to feed on *Picea* A. Dietrich as well as *Pinus*. According to Sorensen (1994), all species of *Essigella* are restricted to one or a few closely related host species, the oligophagous *E. californica* (Essig, 1909) and *E. pini* Wilson, 1919 being the only exceptions.

Figure 3.1. *Essigella hoerneri* on *Pinus monophylla* (left) and *Essigella* sp. on *Pinus ponderosa* (right)

All species are originally Nearctic, but one species, *E. californica*, was inadvertently introduced in several parts of the world (Théry *et al.*, 2017). *Essigella* exhibits high intra- and interspecific variation in external morphology making species identification difficult (Sorensen, 1994). Sorensen (1994) fully revised the genus with "discriminant function and principal component analyses, using morphometric data, and with principal coordinate analysis, multidimensional scaling and various UPGMA and single linkage clustering algorithms, using coded quantitative and qualitative data". He split *Essigella* into three subgenera: *Archeoessigella*, *Essigella* and *Lambersella* (Sorensen, 1994) (Table 3.1). He further sorted them into two species series (Series A and B) and three species complexes (*E. californica*, *E. fusca* and *E. knowltoni* complexes) (Sorensen, 1994) (Table 3.1). *Essigella* belongs to the tribe Eulachnini along with *Cinara* Curtis, 1835 (including subgenus *Schizolachnus* Mordvilko, 1909), *Eulachnus* Del Guercio, 1909 and *Pseudessigella* Hille Ris Lambers, 1966 (Chen *et al.*, 2016). Eulachnini feed on conifers either on bark (*Cinara* excluding *Schizolachnus*) or on needles (*Cinara* (*Schizolachnus*), *Eulachnus*, *Essigella* and *Pseudessigella*) (Chen *et al.*, 2016). Relationships between these genera are not yet fully resolved. *Schizolachnus* was considered a separate genus until its unification as a subgenus of *Cinara* by a recent molecular study (Chen *et al.*, 2016). Furthermore, the relationship of *Pseudessigella*, a Himalayan genus, with *Essigella* and *Eulachnus* remains to be clarified (Sorensen 1991, 1994; Kanturski *et al.*, 2017a, b).

Several *Essigella* species, notably those within each species complex, are morphologically close, some also living on closely related species of host plant. Because populations of a species

can exhibit host-induced morphological variation (Favret & Voegtlin 2004a, Jorge *et al.*, 2011; Paris *et al.*, 2016), Sorensen's taxonomy should be confirmed with molecular data. The present study tests Sorensen's (1994) taxonomy using four genes, *COI* and *ATP6* (mitochondrial), *EF-1α* (nuclear), and *Gnd* (of the bacterial primary nutritional symbiont *Buchnera aphidicola*). Phylogenetic analyses were used to study *Essigella* systematics within the genus itself and regarding its position within the Eulachnini. Notably, we sought to clarify the relationship of *Pseudessigella* with respect to *Essigella* and *Eulachnus* (Sorensen 1991, 1994; Kanturski *et al.*, 2017a, b). *Essigella* species validity was tested using both phylogenetic analyses and species delimitation methods.

3.3 Materials and Methods

3.3.1 Taxon sampling

Fifty-three populations representing thirteen species of *Essigella* were studied (Table 3.2). North American specimens were collected in Canada, Mexico and the USA, overseas ones in Argentina, Australia, France and New Zealand (Théry *et al.*, 2017). Specimens of genera used as outgroups were collected in the USA for *Cinara* and *Eulachnus* and in India for *Pseudessigella* (Table 3.2). All specimens were preserved in 95% ethanol after collecting and thereafter kept at -20°C or -80˚C until DNA extraction. Viviparous apterae specimens were slide-mounted in Canada balsam and kept as voucher specimens (Favret, 2005). They were identified with the key to the species by Sorensen (1994) and the host-based keys by Blackman & Eastop (2017). Specimens were also compared with authoritatively identified reference material, including type specimens and material in the Sorensen Collection (Essig Museum of Entomology, Berkeley, CA). Tree species were identified using field identification guides (Petrides, 1988, 1998; Farjon, 2005). We followed the pinaceous host classification of the Gymnosperm database (Earle, 2015). Voucher specimens are deposited in the Ouellet-Robert Collection of the University of Montreal (QMOR).

Table 3.1. Current classification of the genus *Essigella* (Sorensen, 1994)

		Genus *Essigella* del Guercio, 1909 : 329
		Type species : *Lachnus californicus* Essig, 1909 : 1
		Subgenus *Archeoessigella* Sorensen, 1994 : 21
		Type species *Essigella kathleenae* Sorensen, 1988 : 115
		Essigella (*Archeoessigella*) *kathleenae* Sorensen, 1988 : 115; Sorensen, 1994 : 26
		Essigella (*Archeoessigella*) *kirki* Sorensen, 1988 : 121; Sorensen, 1994 : 22
		Subgenus *Lambersella* Sorensen, 1994 : 29
		Type species : *Essigella fusca* Gillette & Palmer, 1924 : 6
	E. fusca complex	*Essigella* (*Lambersella*) *eastopi* Sorensen, 1994 : 30
	E. fusca complex	*Essigella* (*Lambersella*) *fusca fusca* Gillette & Palmer, 1924 : 6; Sorensen, 1994 : 34 = *Essigella fusca* Gillette & Palmer, 1924 : 6 = *Essigella agilis* Hottes, 1957: 71 [Synonymy by Sorensen, 1994 : 34] = *Essigella palmerae* Hottes, 1957: 96 [Synonymy by Sorensen, 1994 : 34]
	E. fusca complex	*Essigella* (*Lambersella*) *fusca voegtlini* Sorensen, 1994 : 39
	E. fusca complex	*Essigella* (*Lambersella*) *hillerislambersi* Sorensen, 1994 : 41
		Subgenus *Essigella* del Guercio, 1909 : 329
		Type Species: *Lachnus californicus* Essig, 1909: 1
Series A		*Essigella* (*Essigella*) *essigi* Hottes, 1957 : 84; Sorensen, 1994 : 45
Series A		*Essigella* (*Essigella*) *pini* Wilson, 1919 : 2; Sorensen, 1994 : 49 = *Essigella patchae* Hottes, 1957: 98 [Synonymy by Sorensen, 1994 : 49]
Series A	*E. californica* complex	*Essigella* (*Essigella*) *californica* (Essig), 1909 : 1; Sorensen, 1994 : 53 = *Lachnus californicus* Essig, 1909 : 1 = *Essigella claremontiana* Hottes, 1957 : 79 [Synonymy by Sorensen, 1994 : 53] = *Essigella cocheta* Hottes, 1957 : 82 [Synonymy by Sorensen, 1994 : 53] = *Essigella monelli* Hottes, 1957 : 95 [Synonymy by Sorensen, 1994 : 53] = *Essigella pineti* Hottes, 1957 : 101 [Synonymy by Sorensen, 1994 : 53] = *Essigella swaini* Hottes, 1957 : 105 [Synonymy by Sorensen, 1994 : 53]
Series A	*E. californica* complex	*Essigella* (*Essigella*) *hoerneri* Gillette & Palmer, 1924 : 5; Sorensen, 1994 : 62 = *Essigella gillettei* Hottes, 1957 : 88 [Synonymy by Sorensen, 1994 : 62] = *Essigella maculata* Hottes, 1957 : 93 [Synonymy by Sorensen, 1994 : 62]
Series A		*Essigella* (*Essigella*) *wilsoni* Hottes, 1957 : 106; Sorensen, 1994 : 67 = *Essigella pergandei* Hottes, 1957 : 100 [Synonymy by Sorensen, 1994 : 67] = *Essigella oregonensis* Hottes, 1958 : 155 [Synonymy by Sorensen, 1994 : 67]
Series B		*Essigella* (*Essigella*) *alyeska* Sorensen, 1988 : 118; Sorensen, 1994 : 72
Series B	*E. knowltoni* complex	*Essigella* (*Essigella*) *critchfieldi* Sorensen, 1994 : 75
Series B	*E. knowltoni* complex	*Essigella* (*Essigella*) *knowltoni knowltoni* Hottes, 1957 : 92; Sorensen, 1994 : 78 = *Essigella knowltoni* Hottes, 1957: 92 [New status by Sorensen, 1994 : 78]
Series B	*E. knowltoni* complex	*Essigella* (*Essigella*) *knowltoni braggi* Hottes, 1957 : 73; Sorensen, 1994 : 84 = *Essigella braggi* Hottes, 1957: 73 [New status by Sorensen, 1994 : 84] = *Essigella robusta* Hottes, 1957: 103 [Synonymy by Sorensen, 1994 : 84]

3.3.2 DNA Extraction, Amplification, and Sequencing

DNA extraction was non-destructive (Favret, 2005), performed using the DNeasy Blood and Tissue kit (QIAGEN, Düsseldorf, Germany). PCR amplifications were carried out using Thermocycler Eppendorf Mastercycler ProS, with Phire Green Hot Start II DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA). Our protocols and primers were those of Théry *et al.*, 2017 (Table 3.3). Amplicons were sequenced in both directions with their respective PCR primers at the McGill University and Génome Québec Innovation Centre (Montreal, Canada).

3.3.3 Phylogenetic analyses

Chromatograms of each gene were edited using Geneious 9 software (Kearse *et al.*, 2012). A GenBank BLAST search confirmed the aphid's generic identity. The sequences were aligned and compared with Bioedit Version 7.2.5 (Hall, 1999) using the ClustalW multiple alignment program (Thompson *et al.*, 1994). Alignments of *COI*, *ATP6* and *Gnd* were straightforward due to a lack of length variation. Sequences of *EF-1α* were aligned with AphidBase transcript sequences of *Acyrthosiphon pisum* (Harris, 1776) (Legeai *et al.*, 2010) providing us with the locations of introns within our *EF-1α* sequences. Phylogenetic analyses were performed with concatenated sequences, partitioned by gene. Phylogenetic trees were estimated using maximum likelihood (ML) and Bayesian inference (BI) methods using RAxML-HP BlackBox 8.2.10 (Stamakis, 2014) and MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Ronquist *et al.*, 2012), respectively. For ML analyses we used the boostrapping parameter proposed by the program (Let RAxML halt bootstrapping automatically parameter), other parameters were those by default. For BI analyses, we performed a run of 100 million generations including 4 chains (one cold chain and 3 heated chains) using Metropolis-coupled Markov Chain Monte Carlo (MCMC) with a burn-in of 25%. RAxML and MrBayes analyses were run via the CIPRES Science Gateway 3.3 (http://www.phylo.org/) (Miller *et al.*, 2010). Each gene was first analysed alone, then all four were analysed together. Because of the low number of genes and populations, the concatenations were made manually.

Table 3.2. Collecting data of specimens and Genbank accession numbers of DNA sequences

Species	Country	Locality	Host plant	Collection Number	Genbank accession numbers				Code
					ATP6	*COI*	*EF-1α*	*Gnd*	
Essigella alyeska	Canada	Lac-Édouard (QC)	*Pinus banksiana*	QMOR50670	MG579864	MG579774	MG579909	MG579819	ALY
E. californica	Argentina	Malargüe (Mendoza)	*Pinus* sp.	QMOR50043	KY288967	KY288911	KY288929	KY288948	CAL9
E. californica	Australia	Mt Mitchell (NSW)	*Pinus radiata*	QMOR50052	KY288976	KY288920	KY288938	KY288957	CAL10
E. californica	France	Le Rheu (Ille et Vilaine)	*Pinus radiata*	QMOR50054	KY288978	KY288922	KY288940	KY288959	CAL11
E. californica	New Zealand	Christchurch (Canterbury)	*Pinus resinosa / wallichiana*	QMOR50046	KY288970	KY288914	KY288932	KY288951	CAL12
E. californica	USA	San Bernardino Co. (CA)	*Pinus coulteri*	QMOR50049	KY288973	KY288917	KY288935	KY288954	CAL6
E. californica	USA	Ventura Co. (CA)	*Pinus* sp.	QMOR50051	KY288975	KY288919	KY288937	KY288956	CAL8
E. californica	USA	Ventura Co. (CA)	*Pinus attenuata*	QMOR50047	KY288971	KY288915	KY288933	KY288952	CAL5
E. californica	USA	Placer Co. (CA)	*Pinus ponderosa*	QMOR50048	KY288972	KY288916	KY288934	KY288953	CAL7
E. californica	USA	Los Angeles Co. (CA)	*Pinus coulteri*	QMOR50671	MG579865	MG579775	MG579910	MG579820	CAL1
E. californica	USA	Monterey Co. (CA)	*Pinus sabiniana*	QMOR50672	MG579866	MG579776	MG579911	MG579821	CAL2
E. californica	USA	Placer Co. (CA)	*Pinus ponderosa*	QMOR50673	MG579867	MG579777	MG579912	MG579822	CAL3
E. californica	USA	Sonoma Co. (CA)	*Pinus muricata*	QMOR50674	MG579868	MG579778	MG579913	MG579823	CAL13
E. californica	USA	Mendocino Co. (CA)	*Pinus muricata*	QMOR50675	MG579869	MG579779	MG579914	MG579824	CAL14
E. californica	USA	El Dorado Co. (CA)	*Pinus monticola*	QMOR50676	MG579870	MG579780	MG579915	MG579825	CAL15
E. californica	USA	Douglas Co. (NV)	*Pinus monticola*	QMOR50677	MG579871	MG579781	MG579916	MG579826	CAL16
E. californica	USA	Alpine Co. (CA)	*Pinus albicaulis*	QMOR50678	MG579872	MG579782	MG579917	MG579827	CAL17
E. californica	USA	Squamish (BC)	*Pinus contorta latifolia*	QMOR50679	MG579873	MG579783	MG579918	MG579828	CAL4
E. critchfieldi	USA	Curry Co. (OR)	*Pinus contorta contorta*	QMOR50680	MG579874	MG579784	MG579919	MG579829	CRI
E. eastopi	USA	Los Angeles Co. (CA)	*Pinus coulteri*	QMOR50044	KY288968	KY288912	KY288930	KY288949	EAS5
E. eastopi	USA	Los Angeles Co. (CA)	*Pinus coulteri*	QMOR50681	MG579875	MG579785	MG579920	MG579830	EAS6
E. eastopi	USA	San Bernardino Co. (CA)	*Pinus coulteri*	QMOR50682	MG579876	MG579786	MG579921	MG579831	EAS1
E. eastopi	USA	San Bernardino Co. (CA)	*Pinus coulteri*	QMOR50683	MG579877	MG579787	MG579922	MG579832	EAS2
E. eastopi	USA	San Bernardino Co. (CA)	*Pinus coulteri*	QMOR50684	MG579878	MG579788	MG579923	MG579833	EAS3
E. eastopi	USA	San Bernardino Co. (CA)	*Pinus coulteri*	QMOR50685	MG579879	MG579789	MG579924	MG579834	EAS4
E. eastopi	USA	Monterey Co. (CA)	*Pinus coulteri*	QMOR50686	MG579880	MG579790	MG579925	MG579835	EAS7
E. essigi	USA	Ventura Co. (CA)	*Pinus attenuata*	QMOR50687	MG579881	MG579791	MG579926	MG579836	ESS1
E. essigi	USA	Monterey Co. (CA)	*Pinus radiata*	QMOR50688	MG579882	MG579792	MG579927	MG579837	ESS2
E. fusca voegtlini	USA	Monterey Co. (CA)	*Pinus ponderosa*	QMOR50689	MG579883	MG579793	MG579928	MG579838	FUS1
E. fusca voegtlini	USA	Placer Co. (CA)	*Pinus ponderosa*	QMOR50690	MG579884	MG579794	MG579929	MG579839	FUS2
E. fusca voegtlini	USA	Placer Co. (CA)	*Pinus ponderosa*	QMOR50691	MG579885	MG579795	MG579930	MG579840	FUS3
E. hillerislambersi	USA	Los Angeles Co. (CA)	*Pinus jeffreyi*	QMOR50692	MG579886	MG579796	MG579931	MG579841	HIL1
E. hillerislambersi	USA	San Bernardino Co. (CA)	*Pinus jeffreyi*	QMOR50693	MG579887	MG579797	MG579932	MG579842	HIL2
E. hillerislambersi	USA	San Bernardino Co. (CA)	*Pinus jeffreyi*	QMOR50694	MG579888	MG579798	MG579933	MG579843	HIL3
E. hoerneri	USA	San Bernardino Co. (CA)	*Pinus monophylla*	QMOR50050	KY288974	KY288918	KY288936	KY288955	HOE1
E. hoerneri	USA	San Bernardino Co. (CA)	*Pinus monophylla*	QMOR50695	MG579889	MG579799	MG579934	MG579844	HOE2
E. hoerneri	USA	San Bernardino Co. (CA)	*Pinus monophylla*	QMOR50696	MG579890	MG579800	MG579935	MG579845	HOE3
E. hoerneri	USA	Ventura Co. (CA)	*Pinus monophylla*	QMOR50697	MG579891	MG579801	MG579936	MG579846	HOE4
E. hoerneri	USA	El Dorado Co. (CA)	*Pinus monophylla*	QMOR50698	MG579892	MG579802	MG579937	MG579847	HOE5
E. kathleenae	USA	Los Angeles Co. (CA)	*Pinus lambertiana*	QMOR50699	MG579893	MG579803	MG579938	MG579848	KAT1
E. kathleenae	USA	San Bernardino Co. (CA)	*Pinus lambertiana*	QMOR50700	MG579894	MG579804	MG579939	MG579849	KAT2
E. kirki	Mexico	Sierra Norte (OAX)	*Pinus* sp.	QMOR50701	MG579895	MG579805	MG579940	MG579850	KIR
E. knowltoni braggi	USA	El Dorado Co. (CA)	*Pinus contorta murrayana*	QMOR50702	MG579896	MG579806	MG579941	MG579851	KNB1
E. knowltoni braggi	USA	El Dorado Co. (CA)	*Pinus contorta murrayana*	QMOR50703	MG579897	MG579807	MG579942	MG579852	KNB2
E. knowltoni braggi	USA	Alpine Co. (CA)	*Pinus contorta murrayana*	QMOR50704	MG579898	MG579808	MG579943	MG579853	KNK1
E. knowltoni knowltoni	Canada	Whistler (BC)	*Pinus contorta latifolia*	QMOR50705	MG579899	MG579809	MG579944	MG579854	KNK2
E. knowltoni knowltoni	Canada	Squamish (BC)	*Pinus contorta latifolia*	QMOR50706	MG579900	MG579810	MG579945	MG579855	KNK3
E. pini	USA	Swain Co. (NC)	*Pinus rigida*	QMOR50045	KY288969	KY288913	KY288931	KY288950	PINNC
E. pini	Canada	St-Jérôme (QC)	*Pinus strobus*	QMOR50707	MG579901	MG579811	MG579946	MG579856	PINQC
E. wilsoni	USA	Los Angeles Co. (CA)	*Pseudotsuga macrocarpa*	QMOR50708	MG579902	MG579812	MG579947	MG579857	WIL1
E. wilsoni	USA	Ventura Co. (CA)	*Pseudotsuga macrocarpa*	QMOR50709	MG579903	MG579813	MG579948	MG579858	WIL2
E. wilsoni	USA	San Mateo Co. (CA)	*Pseudotsuga menziesii*	QMOR50710	MG579904	MG579814	MG579949	MG579859	WIL3
E. wilsoni	Canada	Vancouver (BC)	*Pseudotsuga menziesii*	QMOR50711	MG579905	MG579815	MG579950	MG579860	WIL4
Cinara sp.	USA	El Dorado Co. (CA)	*Pinus contorta murrayana*	QMOR50712	MG579906	MG579816	MG579951	MG579861	CIN
Eulachnus sp.	USA	Monterey Co. (CA)	*Pinus radiata*	QMOR50713	MG579907	MG579817	MG579952	MG579862	EUL
Pseudessigella brachychaeta	India	Yousmarg (Jammu and Kashmir)	*Pinus wallichiana*	QMOR50714	MG579908	MG579818	MG579953	MG579863	PSE

Data were partitioned into four parts: *ATP6*, *COI*, *EF-1α* and *Gnd*. For ML analyses, we considered strong bootstrap support to be > 95% and low bootstrap support to be < 70%. For BI analysis we considered strong support to be a posterior probability of > 95% and low support to be a posterior probability of < 90%.

3.3.4 Species delimitation

We compared species identified with morphological characters and morphometrical data, Operational Taxonomic Units or OTUs (Doyen & Slobodchikoff, 1974) with those discriminated with molecular data, MOTUs (Vogler & Monaghan, 2007). We used four molecular species delimitation methods: a simple 2% DNA barcode threshold using *COI* (Hebert *et al.*, 2003b; Ratnasingham & Hebert, 2013), the Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.*, 2012), the General Mixed Yule Coalescent (GMYC) (Pons *et al.*, 2006; Fujisawa & Barraclough, 2013), the Bayesian Poisson Tree Process (bPTP) (Zhang *et al.*, 2013), and the Refined Single Linkage method (RESL) (Ratnasingham & Hebert, 2013).

Assuming properties of a 658 basepair fragment of *COI* as a standard DNA barcode (Hebert *et al.*, 2003a, b), we compared Kimura 2 Parameter (K2P) distances of the *COI* sequences of all our *Essigella* populations. We chose a threshold of 2% because it was shown that *COI* divergence is usually > 2% in animal species in general (Ratnasingham & Hebert, 2013) and notably in aphids (Foottit *et al.*, 2009). Those distances were obtained and compared using MEGA 6.0 (Tamura *et al.*, 2013). Our *Essigella* sequences were analysed using the graphic web version of the ABGD method (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html). Because of their prior use in species delimitation studies and their lack of indels, we analysed *COI*, *ATP6* and *Gnd* sequences separately and compared their results. For each gene, we used MEGA 6.0 (Tamura *et al.*, 2013) to calculate distance values using a K2P model. Because a small number of populations (< 3) in some species can distort ABGD resolution (Puillandre *et al.*, 2012), we compared results obtained for all species with those for which we had ≥ 3 populations. We used a value of X = 1.25 (relative gap width), and values given by default with *pmin* = 0.001 and *pmax* = 0.1.

The GMYC method is a tree-based likelihood method using the coalescent theory. It requires ultrametric trees based on single-sequence data (Pons *et al.*, 2006; Fujisawa &

Barraclough, 2013). In consequence, we separately analysed our four genes and built our trees using BEAST 1.8 (Drummond *et al.*, 2012). We partially followed the protocol of Dumas *et al.* (2015) by using a Birth-Death model as tree prior and an uncorrelated lognormal relaxed clock as clock prior. To avoid biases of outgroups on our species delimitation results, we removed them from our analyses. We ran two independent analyses of 60 million generations for each gene, with trees sampled every 1000 generations. Substitution models were those used in our phylogenetic analyse part. LogCombiner 1.8 (Drummond *et al.*, 2012) was used to separately combine log and tree files obtained from our BEAST analyses. We used Tracer 1.6 (Rambaut *et al.*, 2014) to check the convergence of parameters. Finally, TreeAnnotator 1.8 (Drummond *et al.*, 2012) was used to summarize all obtained trees. Our output trees were converted to newick files using FigTree 1.4.2 (Rambaut, 2012) and analysed via the GMYC web server (http://species.h-its.org/gmyc/).

bPTP is also a tree-based method based on a coalescent model, but contrary to GMYC it does not require ultrametric trees as input (Zhang *et al.*, 2013). For the same reasons explained above, we ran our analyses without outgroups. We built our trees using MrBayes 3.2.6, following same protocol described above. Our output trees were converted into nexus files using FigTree 1.4.2 (Rambaut, 2012) and analysed via the bPTP web server (http://species.h-its.org/ptp/). We used 300,000 generations and a burn-in of 25% for parameters. Other parameters were those given by default.

Table 3.3. Primers and PCR protocols

Genes	Primers	Primers sequences	Initial denaturation: time and T°C	Number of Cycles	Denaturation: time and T°C	Annealing: time and T°C	Elongation: time and T°C	Final Elongation: time and T°C
ATP6	tRNALysAf2	GACTGAAAAGCAAAGTAATGATCTCT	94 C for 3 min	35	94 C for 30 sec	55 C for 30 sec	65 C for 1 min	none
	CO3WWRD	TCWCGAATWACATCWCGTCATCA						
COI	Lep-F1	ATTCAACCAATCATAAAGATATTGG	98 C for 30 sec	35	98 C for 10 sec	50 C for 20 sec	72 C for 20 sec	72 C for 2 min
	Lep-R1	TAAACTTCTGGATGTCCAAAAAATCA						
EF-1 α	EF-1-F	GAACGTGAACGTGGTATCAC	98 C for 30 sec	35	98 C for 20 sec	51 C for 20 sec	72 C for 20 sec	72 C for 2 min
	EF-1-R	TGACCAGGGTGGTTTCAATAC						
Gnd	BamHI	CGCGGATCCGGWCCWWSWATWATGCCWGGWGG	98 C for 1 min	35	98 C for 20 sec	51 C for 40 sec	72 C for 40 sec	72 C for 3 min
	ApaI	CGCGGGCCCGTATGWGCWCCAAAATAATCWCKTTGWGCTTG						

The RESL method is implemented in the BOLD Systems (http://www.boldsystems.org/) and permits the delimitation of OTUs from barcode sequence records (*COI*). This method works in two steps. First, it uses a single linkage clustering in order to allocate a provisional OTU for all *COI* sequences analyzed. Second, it uses a Markov clustering (MCL) in order to refine the results, each result corresponding to a Barcode Index Number (BIN) (Ratnasingham & Hebert, 2013). The BOLD database holds 546 *Essigella COI* sequences split into twelve BINs. Some BINs are associated with one of seven species names. Because the same aphid population can yield multiple barcode sequences, we started by sorting the sequences and chosing only one representative sequence per population. Second, we combined our own sequences with those in BOLD and analyzed them using Neighbor Joining (NJ). At the same time, we used the BOLD identification tool (http://www.boldsystems.org/ index.php/IDS_OpenIdEngine) to test if the RESL method would identify our sequences the same as we did. We chose the second BOLD identification tool option: "Species Level Barcode Records".

3.4 Results

3.4.1 DNA Extraction, Amplification, and Sequencing

All genes were sequenced and analyzed for each population. The amplicon lengths were 657 to 663 base pairs (bp) for *ATP6*, 658 bp for *COI*, 661 to 778 bp for *EF-1α* (including introns) and 749 bp for *Gnd* (see Table 3.1 for GenBank accession numbers).

3.4.2 Phylogenetic analyses

Following MrModeltest 2.3 to determine the best evolution model (Nylander, 2004), we used GTR + Γ as models for *COI*, *ATP6* and *EF-1α* and GTR + I + Γ for *Gnd*. Trees obtained for each gene separately showed few important incongruences for both ML and BI analyses. Observed incongruences coincided with low branch support. ML and BI trees of concatenated sequences were identical and are presented in a single dendrogram (Fig. 3.2). The main branches were strongly supported except for Clade A, gathering all *Essigella* species except *E. kirki* Sorensen, 1988 (posterior probabilities (*PP*) = 64%), Clade D (*pp* = 89%), and Clade E (*pp* =

53%). *Pseudessigella* appeared as the sister-group of *Essigella* (*pp* = 100%) and *Eulachnus* as the sister-group of *Pseudessigella* + *Essigella* (*pp* = 100%). Species of the subgenus *Lambersella* were clustered with strong support (*pp* = 100%) (Clade F, Fig. 3.2). They were included in the same clade (Clade C, Fig. 3.2) (*pp* = 100%) as *E. essigi* Hottes, 1957 (subgenus *Essigella*) and *E. kathleenae* Sorensen, 1988 (subgenus *Archeoessigella*). The two species of *Archeoessigella* (*E. kathleenae* and *E. kirki*) were not found together, *E. kirki* branching basally as the sister-group to all other *Essigella*, and *E. kathleenae* being found in the Clade C. Species of the subgenus *Essigella* were split into three different groups (Fig. 3.2). The first one corresponded to the Clade B (*pp* = 100%); the second was represented by *E. essigi* of the Clade C; the third corresponded to Clade D (*pp* = 89%). Sorensen's (1994) Species Series A was split into Clade B for *E. californica* and *E. hoerneri* Gillette & Palmer, 1924, Clade C for *E. essigi*, and Clade D for *E. wilsoni*. Populations of *E. pini* were divided between Canadian and US populations in Clades B and D, respectively. Sorensen's (1994) Species Series B were recovered within the Clade D (*pp* = 100%). The *Essigella californica, E. fusca* and *E. knowltoni* complexes (Sorensen, 1994) were also recovered. Among species with several populations, some showed little genetic variability, such as *E. hillerislambersi* Sorensen, 1994 and *E. hoerneri*. In contrast, others showed high variability and formed several clear infra-specific groups: notably *E. californica*, *E. eastopi* Sorensen, 1994, *E. essigi, E. fusca voegtlini* Sorensen, 1994, *E. knowltoni* Hottes, 1957 and *E. wilsoni*. *Essigella californica* was divided into two main clades, Clade G and Clade H, with a *pp* = 100% for the first and a *pp* = 81% (with a bootstrap value = 91%) for the second. Clade G was divided into two groups: the first (G1) gathered 11 populations collected on diverse pine species, the second (G2) was represented by a population collected on *P. contorta* Douglas ex Loudon. Clade H was also split into two groups: populations collected on *P. muricata* D. Don (H1) in the first instance and those collected on pines of the subsection *Strobus* (H2) in the second. *Essigella knowltoni* was divided into two clades corresponding to the subspecies *E. knowltoni braggi* Hottes, 1957 (*pp* = 87%) and *E. knowltoni knowltoni* Hottes, 1957 (*pp* = 100%). We did not observe a correlation between different populations and host plant identity with *E. eastopi, E. fusca voegtlini*, and *E. wilsoni*. However, with *E. essigi*, both populations were collected on two pine species of the subsection *Attenuatae*.

3.4.3 *EF-1α* sequences

Our *EF-1α* sequences included two introns of variable size (Introns 3 and 4), the second showing the more informative structure (Fig. 3.3). We observed a similar pattern of intron insertions and deletions (indels) for *Essigella californica*, *E. hoerneri* and the Canadian population of *E. pini*. *Essigella kirki* exhibited a similar intron indel pattern as that of the previous species, but it was completely different from that of its supposed sister species, *E. kathleenae*. Populations of the *Essigella knowltoni* sspp. and *E. critchfieldi* Sorensen, 1994 showed a similar pattern except for a deletion of 12 nucleotides in Intron 4 (5' TTAAATATACTA 3') in *E. knowltoni knowltoni*. The complete sequence was present in our populations of *E. knowltoni braggi* and *E. critchfieldi*. *Essigella alyeska* showed a similar pattern (with one nucleotide added to Intron 4) as that of *E. knowltoni braggi* and *E. critchfieldi*. All other species had unique intron indel patterns.

3.4.4 Species delimitation

3.4.4.1 *COI* barcoding

According to the 2% DNA barcode threshold, 16 MOTUs were revealed, including 13 that had been pre-identified morphologically as species-group taxa (annexes 2 et 3). Two MOTUs were found within *E. pini* with a divergence value of $p = 4.1\%$ between them.

Three MOTUs appeared within *E. californica*: sequence divergences between populations of Clade G and those of Clade H were 1.1-3.1% with more precisely, $p = 1.1\text{-}1.5\%$ between Clade G and Clade H1 and $p = 1.9\text{-}3.1\%$ between Clade G and Clade H2. Divergences between Clade H1 and Clade H2 were 1.2-2.3%, with $p = 1.2\text{-}1.5\%$ between H1 and *P. monticola* populations, and $p = 2.2\text{-}2.3\%$ between H1 and *P. albicaulis* population. Divergences within Clade H2, between *P. monticola* and *P. albicaulis* populations, were 2.0%.

3.4.4.2 Automatic Barcode Gap Discovery (ABGD)

We obtained the same number of MOTUs for taxa for which we had three or more populations (*i.e., E. californica*, *E. eastopi*, *E. fusca voegtlini*, *E. hillerislambersi*, *E. knowltoni*

Figure 3.2. Phylogenetic tree (ML and BI) of *Essigella* species using concatenated *ATP6*, *COI*, *EF-1α* and *Gnd*. *Pinus* species of subgenus *Strobus* appear in hot colors, those of subgenus *Pinus* in cold colors. Colour nuances represent infragroups within each subgenus. Non identified *Pinus* and other Pinaceae are in black. Values indicate ML bootstrap % values followed by Bayesian posterior probabilities % values

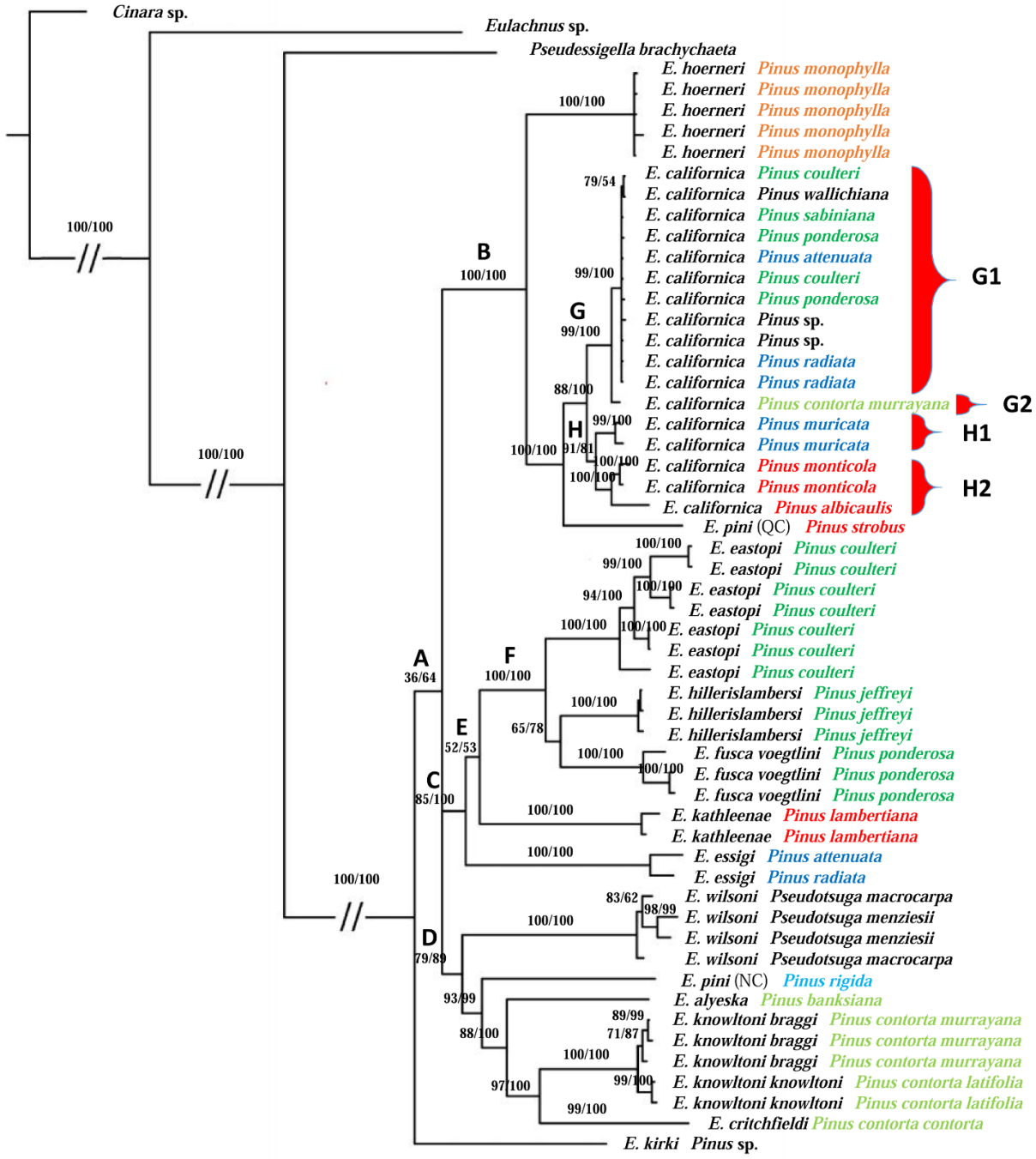
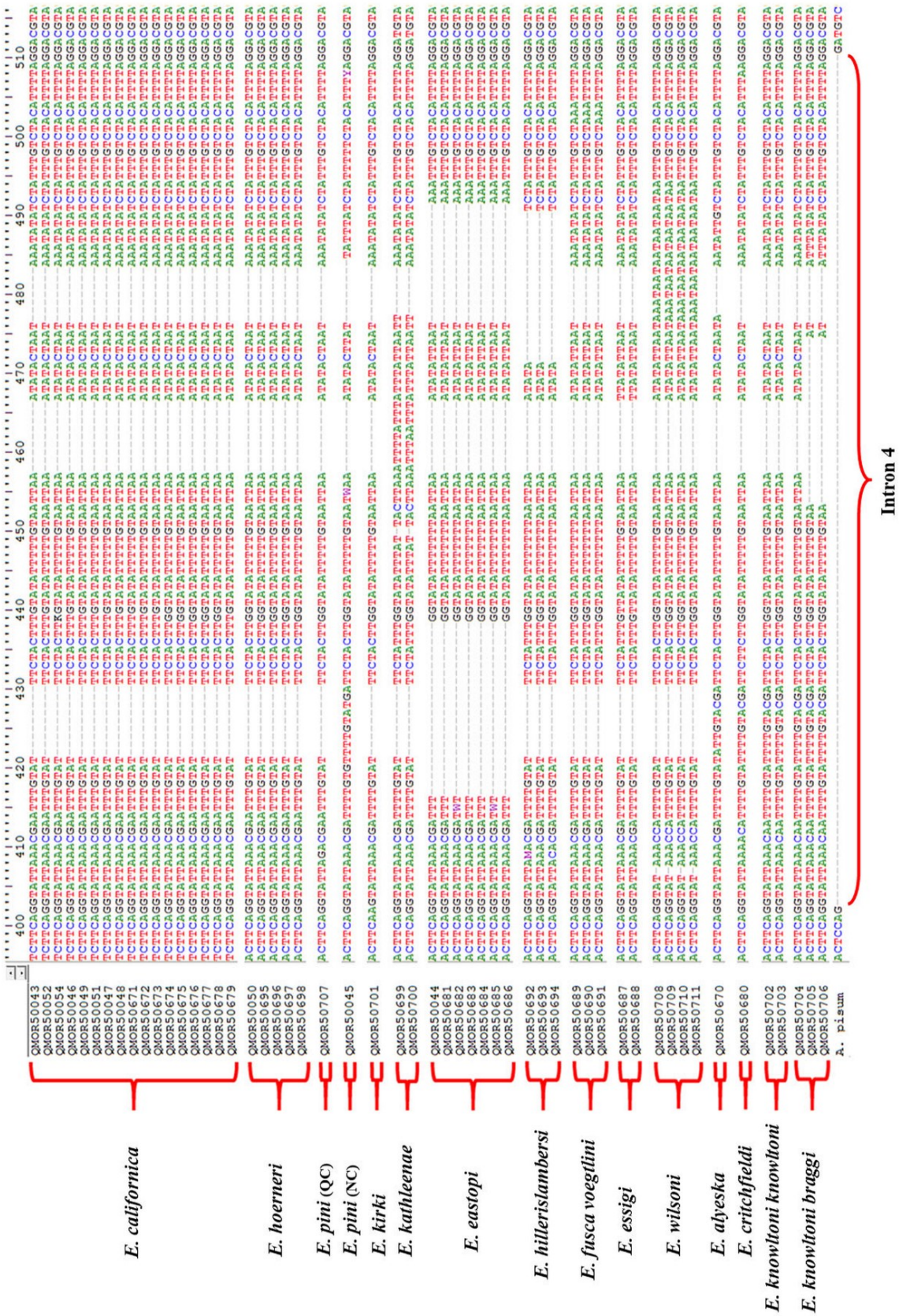


Figure 3.3. *Essigella* specific differences in intron region 4 of *EF-1α*



and *E. wilsoni*), whether or not we included the taxa with fewer than three. *Essigella pini* consisted of two MOTUs for all three genes. In all, we obtained 18, 17 and 16 MOTUs for *COI*, *ATP6* and *Gnd*, respectively (annexe 3); the variability was due to *E. californica* for which we obtained 5, 4, and 3 different MOTUs for *COI*, *ATP6* and *Gnd*, respectively.

3.4.4.3 General Mixed Yule Coalescent (GMYC) and Bayesian Poisson Tree Process (bPTP)

We obtained 15, 29, 16 and 14 MOTUs with GMYC for *ATP6*, *COI*, *EF-1α* and *Gnd*, respectively (annexe 4). In each case, *Essigella pini* was always divided into two distinct MOTUs. The bPTP web server proposes both a maximum likelihood and a Bayesian solution for each analysis. We obtained 25/23, 26/26, 14/22, and 21/21 (ML/BI) MOTUs for *ATP6*, *COI*, *EF-1α* and *Gnd*, respectively. As with GMYC, *E. pini* consistently included two MOTUs (annexe 5).

3.4.4.4 Refined Single Linkage (RESL) analysis

Of the 546 *COI Essigella* sequences in BOLD, 67 were kept and analyzed as unique populations. They represented eleven BINs, six of which were named: *E. californica*, *E. fusca*, *E. hoerneri*, *E. knowltoni*, *E. pini*, and "*Essigella* sp. A rgf-2008". Several BOLD sequences corresponded to those already published by Théry *et al.* (2017): the single sequence of *E. fusca voegtlini* and one of those of *E. hoerneri* (KY288918 and KY288912, respectively). Already being a part of our dataset, these duplicates were not downloaded for further analysis. The Neighbor Joining tree (NJ tree) matched eight proposed BINs with some of our identified sequences (annexes 6 and 7). All BOLD sequences identified to species were correctly linked to our identified sequences except for BIN BOLD:ACE3645, identified as *E. knowltoni* but representing an unlisted and possibly undescribed species. Moreover, two other BINs, BOLD:ACM1471 and BOLD:ABV1593, composed two distinct clusters with no relation to our sequences, and may thus represent two other undescribed species (annexes 6 and 7). "*Essigella* sp. A rgf-2008" corresponds to *E. californica*, the BIN named BOLD:AAI4970 corresponds to our *E. alyeska* sequence, and the one named BOLD:ACC4249 corresponds to our *E. wilsoni*

sequences (annexes 6 and 7). As with other methods, our two populations of *E. pini* were separate. In our NJ tree, the American population is linked with the BIN identified as *E. pini* (BOLD:AAL6451), whereas the Canadian one is linked with another BIN which is not identified to species (BOLD:AAI4969) (annexes 6 and 7). The BOLD identification tool gave similar identifications as ours in the case of *Essigella californica*, *E. fusca*, *E. hoerneri* and *E. knowltoni* (annexe 8). On the contrary however, our Canadian population of *E. pini* was identified as *E. californica* (annexe 8). Some of our sequences were not present in the BOLD database and either were not recognized or were recognized as a different species. For example, among our four sequences of *E. wilsoni*, two were considered *E. fusca voegtlini*, one was identified as *E. knowltoni* and one was not recognized. Strangely, even though a BIN corresponding to that species exists in BOLD, no link was made between it and our *E. wilsoni* sequences. We obtained a similar result with *E. alyeska* and for most of our specimens of *Essigella californica*. Only the populations collected on *Pinus albicaulis*, *P. contorta*, and one of the two collected on *P. muricata* were assigned in BOLD to a BIN identified as *E. californica* (BOLD:AAI4968) (annexe 8).

3.5 Discussion

Our results provide relevant information which clarify *Essigella* and Eulachnini systematics. They also bring up concerns regarding molecular species delimitation methods.

3.5.1 Species delimitation methods issues

The five species delimitation methods we used in this study presented variable and incongruent results and therefore must be considered carefully. Usually, bPTP and GMYC provide similar results (Dumas *et al.*, 2015; Jasso-Martinez *et al.*, 2016; Zhu *et al.*, 2017). GMYC may be less reliable than bPTP, overestimating the number of MOTUs (as in our case with *COI*), due to the ultrametricization of the input trees (Zhang *et al.*, 2013; Ahrens *et al.*, 2016). In contrast, for *ATP6* and *Gnd*, we observed more MOTUs with bPTP than with GMYC. The origins of these incongruities between GMYC and bPTP may be diverse, but are likely due to the small size of our population sampling. Indeed, our sampling was reduced in comparison

with those of other studies that employed both GMYC and bPTP methods (Dumas *et al.*, 2015; Ahrens *et al.*, 2016; Jasso-Martinez *et al.*, 2016; Zhu *et al.*, 2017). The problem of inadequate sampling per species was mentioned for ABGD as well: ABGD works best when there are more than to 3 to 5 populations per species (Puillandre *et al.*, 2012). However, we observed no differences in our ABGD results by calculating with species containing three or more populations alone, or with other species showing fewer populations. RESL could yield similar results as ABGD (Gibbs, 2018). However, we did not obtain the same delimitation results with the identification tool proposed by BOLD, the results appearing incongruous in comparison with BINs already delimited. Indeed, we only found links between MOTUs delimited with both ABGD and *COI* barcoding and the BINs proposed by BOLD, when we analyzed sequences with the NJ method. Because ABGD, *COI* barcoding, and RESL are distance-based methods, they may be less sensitive to sample size than bPTP and GMYC. In consequence, below we will not discuss results obtained with bPTP and GMYC. Nevertheless, because of the unexpected results obtained with the identification tool of BOLD, we will only discuss the results from *Essigella* BINs whose sequences were analyzed with the NJ method.

3.5.2 Within Eulachnini

Since its description (Hille Ris Lambers, 1966b), *Pseudessigella* has always been classified as a genus intermediate between *Eulachnus* and *Essigella*. Indeed, *Pseudessigella* shares characters with *Eulachnus*, such as simple tarsal claws (not incised), and with *Essigella*, such as 5-segmented antennae (Hille Ris Lambers, 1966b; Sorensen 1991, 1994). *Pseudessigella* also shares with *Essigella* a head fused with the pronotum (Kanturski *et al.*, 2017a) and with *Eulachnus* a membranous abdominal dorsum (Kanturski *et al.*, 2017a; Sorensen 1991, 1994). Sorensen, in his revision of the genus *Essigella* (1994), highlighted morphological proximities between *Pseudessigella* and *Essigella*, notably in the close patterns of their abdominal dorsal chaetotaxy. In contrast, a more recent morphological study pointed out that, except for its 5-segmented antennae, the general morphology of *Pseudessigella* was closer to that of *Eulachnus* (Kanturski *et al.*, 2017a). Our analyses place *Pseudessigella* as sister-group to *Essigella* (Fig. 3.2, *pp* = 100%), as predicted by Sorensen (1991, 1994). Our results also point out that the 5-segmented antennae and the head fused with the pronotum can be considered as

synapomorphies of *Essigella* and *Pseudessigella*, and the incised tarsal claws an autapomorphy of *Essigella*. In contrast, the membranous abdominal dorsum in *Eulachnus* and *Pseudessigella* is a plesiomorphic character.

Beyond the characters issue, the phylogenetic relationship between *Essigella* and *Pseudessigella* provides us with new information in understanding the historical distribution of the 3 genera. Because *Eulachnus* and *Pseudessigella* are purely Palaearctic and *Essigella* is Nearctic, their common ancestor was likely of Eurasian origin. Chen *et al.* (2016) indicated that *Essigella* and *Eulachnus* diverged during the Eocene (-56.0 to -33.9 MY). During that epoch, Asia and North America were attached (Wake, 2013). Thus, it is likely that populations of the common ancestor of *Essigella* and *Pseudessigella* diverged as the North American and Eurasian populations separated.

3.5.3 Within *Essigella*

Our phylogenetic results supported neither the division of *Essigella* into the three subgenera *Archeoessigella*, *Essigella* and *Lambersella*, nor the validity of one of the two species series created by Sorensen (1994) (*i.e.*, Series A). However, we recovered all species complexes and all species that Sorensen delimited with his multivariate analyses, the only significant exception being the two populations of *E. pini* (Clades B and D, Fig. 3.2). Several species showed clear internal cladistic structure, however, and merit discussion. A clear division of *E. californica* into several clades appeared in our results. The species appeared as two lineages represented by Clades G and H (Fig. 3.2). With a *COI* DNA barcode sequence divergence < 2% between them, Clades G1, G2 and H1 may represent one unique species. However, by excluding Clade H2, a paraphyletic species is not credible. Moreover, the *COI* barcode threshold was not congruent with our ABGD results which consistently separated the Clade H1 from the other *E. californica* populations (annexe 3). Because ABGD is a method based on the presumed gap existing between intra and interspecific divergences, results obtained with that method seemed to be more believable than those obtained with classical *COI* barcode thresholds. The classical 2% barcode threshold is a useful but arbitrary rule and several aphid species have been found showing divergence sequence less than 2% and even less than 1% (Raskauskas *et al.*, 2011). In consequence, and because of their placement in our tree (Fig. 3.2), we conclude that populations

of Clades G and H correspond to at least two distinct species. Moreover, if Clades G and H1 are to be considered two distinct species with a sequence divergence between them of 1.1-1.5%, we would have to consider that interspecific *COI* threshold is lower than 2% in *Essigella*, and possibly near 1.1%. Thus, with a *COI* sequence divergence $p = 2\%$ between them (annexe 2), we consider populations of the clade H2, *i.e.*, those found on *P. albicaulis* and those on *P. monticola*, to be distinct species as well. The populations of G1 and the population collected on *P. contorta* (G2) showed a sequence divergence of $p = 0.9\text{-}1.2\%$. These weak and transitional values and the phylogenetic position of G2 do not allow us to decide on the presence of two distinct taxa and may require additional data to confirm its species identity. It is possible that these two different groups represent subspecies or that population G2 represents an incipient species.

Morphological comparison with the type series and ecological data suggest that Clade G corresponds to the true *E. californica*. Sorensen's analyses (1983) revealed several groups within the *E. californica* species complex. He divided the complex into two groups, the one with populations developing on pinyon pines (*i.e.*, on pines of the subsection *Cembroides* like *P. monophylla*) which he discriminated as *E. hoerneri*, and populations developing on non-pinyon pines, which he discriminated as *E. californica*. Beyond this division, Sorensen (1983) mentioned several other groups within *E. californica* that were slightly distinct in comparison with the other populations and that could be linked to specific pines. More specifically, he singled out populations living on *Pinus flexilis* and *P. lambertiana*. Despite these observations and following the results of his analyses, Sorensen decided that all these populations living on non-pinyon pines belong to *E. californica* and that observed variation between them could be considered intraspecific. No populations of our discriminated MOTUs were collected on *P. flexilis* or on *P. lambertiana*. Thus, we suspect that *E. californica* may include two other cryptic species in addition to those revealed in our study.

Species that compose the *Essigella knowltoni* complex, *i.e.*, *E. knowltoni* and *E. critchfieldi* are morphologically similar. Ecological host plant and geographic data are required for species identification (Sorensen, 1994). Despite the strong proximity between these species and the *E. knowltoni* subspecies, our phylogenetic and molecular delimitation results support their validity. The subspecies appear in different clades and their *COI* barcode sequence divergence did not exceed 0.8%, whereas the divergence between *E. knowltoni* and *E.*

critchfieldi was > 4% (annexe 2). Analysis of the BOLD BINs also revealed the existence of another species belonging to the *E. knowltoni complex* (BOLD:ACE3641). This one was identified as *E. knowltoni* in BOLD but actually corresponds to a different cluster (annexe 6). This BIN may represent an undescribed species.

Two genetically distinct species were revealed within *E. pini* by phylogenetic results, regardless of the genetic locus examined. In our dendrogram, the Canadian population of *E. pini* clearly belonged to the *Essigella californica* complex, whereas the American population appeared related with species of Series B and the *Essigella knowltoni* complex (Fig. 3.2). Those two species were also revealed as different MOTUs with all species delimitation methods and exhibited a high *COI* DNA barcode sequence divergence ($p = 4.1\%$). According to these results, one of those species may correspond to *E. patchae* Hottes, 1957. Indeed, before Sorensen's revision, two species were known to occur in the Eastern part of North America. Because Sorensen's (1994) multivariate analyses did not find any difference between them, he made *E. patchae* a synonym of *E. pini*. The issue of multiple species within *E. pini* will be developed in a future publication.

Species belonging to subgenus *Lambersella* (also corresponding to the *Essigella fusca* complex (Sorensen, 1994)) are difficult to distinguish, showing high morphological variability, notably in the length of dorsal metatibial setae (Sorensen, 1994). More particularly, *E. eastopi* is itself a highly variable species, being easily confused with *E. fusca voegtlini*. Some problematic populations of both taxa occur together in southern California, suggesting to Sorensen (1994) that *E. eastopi* might be a diminutive form of *E. fusca voegtlini*. Indeed, during the initial stages of this study, we misidentified all but one population of *E. eastopi* as *E. fusca voegtlini* (both species occurring on *P. coulteri*). We also misidentified our populations of *E. fusca voegtlini* as *E. fusca fusca* Gillette & Palmer, 1924 (both subspecies occurring on *P. ponderosa*). We had initially concluded that *E. eastopi* and *E. fusca voegtlini* may represent the same species. In the light of our molecular results and following a reappraisal of our slide-mounted specimens, our populations misidentified as *E. fusca fusca* appear to be closer to *E. hillerislambersi* than to our *E. fusca voegtlini*. Moreover, the two subspecies of *E. fusca* are allopatric (Sorensen, 1994), and according to our collecting data, we collected both subspecies in relatively proximity and in a region where *E. fusca fusca* does not occur (Sorensen, 1994). Either our identifications of *Lambersella* species were inaccurate, or both subspecies occur in

sympatry. Because it is more likely we made wrong identifications, we concluded that we had only collected *E. eastopi*, *E. fusca voegtlini* and *E. hillerislambersi*. Our mistake underlines the high morphological variability of *E. eastopi*.

3.5.4 *EF-1α* sequences

Indel regions of *EF-1α* provided pertinent phylogenetic information in *Essigella* systematics. *Essigella californica*, *E. hoerneri* and the Canadian population of *E. pini* which form the *Essigella californica* complex all showed the same indel pattern (Fig. 3.3). Surprisingly, the same pattern was also found for *E. kirki*, which presented an indel pattern totally different from that of that supposed sister species *E. kathleenae*. Our first suspicion is that this might correspond to the ancestral pattern, conserved in the *Essigella californica* complex, but which was progressively modified in other species by addition or deletion of nucleotides. In contrast, the general pattern found in species of the *Essigella knowltoni* complex and in *E. alyeska* was also found in the American population of *E. pini*, with a loss of one nucleotide in the last, showing a close relationship between all those species (Fig. 3.3). The *EF-1α* intron indel patterns also indicated a divergence between *E. knowltoni* subspecies. This difference corresponded to twelve missing nucleotides in *E. knowtoni knowltoni* as compared with of *E. knowtoni braggi* but also with all species of the Series B (Fig. 3.3). Because that loss was only found in our *E. knowtoni knowltoni* sequences, it may be considered an autapomorphy of that subspecies. The indel patterns of *E. eastopi*, *E. fusca voegtlini* and *E. hillerislambersi* appeared different in Intron 4 despite their close relationship, that of *E. fusca voegtlini* being similar to that of *E. essigi.* However, the indel pattern of the three first species were similar in Intron 3, that of *E. essigi* being different by the insertion of one nucleotide.

3.5.5 The host plant issue

In general terms, our phylogenetic results did not show evidence of parallel phylogenies between *Essigella* species and their host plant (Fig. 3.2). These results corroborate the hypothesis of aphid speciation by host-shift (Tilmon, 2008; Peccoud *et al.*, 2010) with a phylogenetic tracking process (Mitter & Brook, 1983; Althoff *et al.*, 2014) as suspected by

Sorensen (tracking resource model) (1983, 1994). Although several well-known species of aphids are oligophagous or polyphagous, most aphids are associated with one or a few closely related host plants (Heie 1986; Lee *et al.*, 2015). Some related species or populations of *Essigella* do inhabit closely-related pines. For example, species of the Series B (*Essigella knowltoni* complex + *E. alyeska*) are known to develop on pine species of subsection *Contortae* (Sorensen, 1994), as shown in our phylogenetic results (Fig. 3.2). We made similar observations with the closely-related *E. eastopi*, *E. fusca voegtlini* and *E. hillerislambersi*, all feeding on pines of subsection *Ponderosae* (Sorensen, 1994) (Fig. 3.2). These observations may be explained by the fact that a host-shift speciation is more common on phylogenetically-related host plants (Percy *et al.*, 2004; Ouvrard *et al.*, 2015). Finally, except in the cases of cryptic species revealed within *E. californica* and *E. pini*, our results were consistent with the aphid-host specificity patterns proposed by Sorensen (1994).

We previously saw that a specific threshold of *COI* sequence divergence of 2% was overly conservative in the genus *Essigella* and that a threshold around 1.1% may be more credible. In our analyses, several populations showed *COI* sequence divergences nearly equal to or superior to this threshold but no MOTUs were revealed within them using ABGD. It was the case of populations of *E. eastopi* (p = 0.8-1.5%), *E. essigi* (p = 1.5%), *E. fusca voegtlini* (p = 1.4%) and *E. wilsoni* (p = 0.2-1.5%) (annexe 2). *Essigella essigi* develops on *Pinus attenuata* and *P. radiata*, both belonging to subsection *Attenuatae*. Considering the *COI* value and ecological data, it is possible that these two populations represent sub- or incipient species. Populations of the other species were collected on the same host plants. Because it is less likely to have several cryptic species on the same host plant than on different ones, we cannot conclude that *E. eastopi* and *E. fusca voegtlini* include cryptic species. The same can be said for *E. wilsoni*. We found a p = 1.1-1.5% between populations collected on *Pseudotsuga macrocarpa* and *Ps. menziesii*. But we also had a p = 1.1% between populations on *Ps. menziesii* alone.

We revealed that several cryptic species occurred within *E. californica* and that a *COI* barcode threshold of 2% was overly conservative in *Essigella*. However, the re-assessment of that threshold challenged our ABGD results and our ecological observations as the presence or absence of cryptic species within *E. eastopi*, *E. essigi* and *E. wilsoni*, notably. Despite the fact that we found no differences in our ABGD results by testing our analyses with or without species with fewer than three populations, our weak sampling may have had a negative effect on the

ABGD resolution. We recognized several MOTUs in *E. californica* because it was the species for which we had the most populations. Perhaps our conclusions would have been different for other species with more populations. In addition to the population size issue, ABGD can also be affected by recent speciation events (Puillandre *et al.*, 2012). If the speciation event is not old enough, not all species will be delimited (Puillandre *et al.*, 2012). In consequence, ABGD may not have detected speciation events in *E. eastopi*, *E. essigi* and *E. wilsoni*.

In addition to the three cryptic species, analysis of the BOLD BINs permitted us to reveal three more potential new species. However, morphological study of the voucher specimens and comparison with type material of the several synonyms would be required to confirm their validity.

A comparison of several species delimitation methods combined with other approaches appears necessary to better understand the complexity of the reality of species. In short, the use of as much data as possible is important. An additional study using substantial material and taxa would be required to resolve the issue of cryptic species in *E. californica*, but also to resolve those which appeared in other species of the genus. Because we did not have populations of *E. fusca fusca* in our study, we were unable to confirm the validity of both subspecies of *E. fusca*, nor to conclude on the relationship between the two subspecies of *E. fusca*, *E. eastopi*, and *E. hillerislambersi*. Likewise, populations of *E. californica* collected on *Pinus flexilis* and *P. lambertiana* would have provided more complete understanding of the cryptic species belonging to that complex.

3.6 Acknowledgments

We are grateful to G. L. Miller (USDA Systematic Entomology Laboratory, Beltsville, MD), P. T. Oboyski (Essig Museum of Entomology, University of California, Berkeley, CA), and R. E. Thomson (University of Minnesota, St Paul, MN) for specimen loans, and S. A. Akbar (Central Institute of Temperate Horticulture, Jammu and Kashmir, India) for specimens of *Pseudessigella*. We also extend our thanks to J. T. Sorensen for his advice and assistance in the field, E. Jousselin (CBGP-INRA, Montferrier sur Lez, France) for her advice and her hospitality of TT at the CBGP-INRA lab, E. Gagnon (University of Montréal, Montréal Canada) and B.

Domenech (University of Montréal, Montréal Canada) for their support and their comments in molecular analyses.

4. Molecular species diagnoses in *Essigella* Del Guercio, 1909 (Sternorrhyncha : Aphididae : Lachninae), with the description of three new species

Thomas Théry[1], Mariusz Kanturski[2] and Colin Favret[1]

1. Department of Biological Sciences, Biodiversity Centre, University of Montreal, 4101 rue Sherbrooke Est, Montreal, QC, H1X 2B2, Canada

2. Department of Zoology, Faculty of Biology and Environmental Protection, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland

Accepté dans ZooKeys

(Modifié pour les besoins de la présente thèse)

Tous les auteurs ont donné leur autorisation pour inclure ce manuscrit dans la présente thèse

Contribution respective des auteurs :

Thomas Théry : Conception du projet, collecte des données sur le terrain (USA, Canada), préparation des spécimens, traitement et analyse des données, prise de photos (habitus), interprétation et rédaction

Mariusz Kanturski : prise de photos (détails au SEM), réalisation des planches, relecture du manuscrit

Colin Favret : Conception du projet, supervision et direction générales, collecte des données sur le terrain (USA, Canada), corrections.

4.1 Résumé / Abstract

Résumé

Des données morphologiques et moléculaires sont utilisées pour décrire trois nouvelles espèces d'*Essigella* : *Essigella domenechi* n. sp., *Essigella gagnonae* n. sp. et *Essigella sorenseni* n. sp.; et pour rétablir comme valide *Essigella patchae* Hottes, 1957 stat. nov., jusqu'à présent considérée comme synonyme d'*E. pini* Wilson, 1919. Le catalogue des espèces d'*Essigella* est mis à jour. Cette étude souligne la nécessité et l'utilité d'employer des caractères ADN discrets dans les diagnoses d'espèces de pucerons.

Mots-clés : Espèce cryptique, séquences ADN, Hemiptera, taxonomie

Abstract

Morphological and molecular data are used to describe three new species of *Essigella*: *Essigella domenechi* n. sp., *Essigella gagnonae* n. sp. and *Essigella sorenseni* n. sp.; and to re-establish as valid *Essigella patchae* Hottes, 1957 stat. nov., until now considered a synonym of *E. pini* Wilson, 1919. The catalogue of *Essigella* species is updated. This study highlights the need and utility to use discreet DNA characters in aphid species diagnoses.

Keywords: Cryptic species, DNA sequences, Hemiptera, taxonomy

4.2 Introduction

Morphological characters remain the commonest way to separate animal species and they are conspicuously used in diagnoses and descriptions of new taxa. However, in the case of cryptic species, no or few morphological differences are available, and other kinds of taxon-related attributes must be employed as valuable diagnostic characters. DNA sequences permit the discovery of cryptic species and are used to separate them from their relatives (Hebert *et al.*, 2003a, b; Cœur d'acier *et al.*, 2014; Lukhtanov *et al.*, 2017; Morinière *et al.*, 2017). However, despite their reliability, they are seldom used specifically in diagnoses of new species, notably

because they are not specifically recommended in the International Code of Zoological Nomenclature (Renner, 2016).

Essigella (Sternorrhyncha, Aphididae, Lachninae) is an aphid genus found on the needles of various pinaceous hosts. Most species feed on true pines, *Pinus* Linnaeus, but *E. wilsoni* Hottes, 1957, is found only on Douglas firs, *Pseudotsuga* Carrière. *Essigella alyeska* Sorensen, 1988 is recorded on spruce, *Picea* A. Dietrich, although its typical host is *Pinus banksiana* Lamb. (Sorensen, 1994). Most species of *Essigella* are considered monophagous except *E. californica* (Essig, 1909) and *E. pini* Wilson, 1919, which are oligophagous on *Pinus*. Although all species are Nearctic in origin, *E. californica* was accidently introduced in several countries around the world (Théry *et al.*, 2017). *Essigella* currently encompasses 15 valid taxa, with an additional thirteen synonyms (Wilson, 1919; Gillette & Palmer, 1924; Hottes 1957, 1958; Sorensen, 1988; Sorensen, 1994). Species are variable and show few diagnostic characters (Sorensen, 1994). The genus was revised by Sorensen (1994) using morphometric data and multivariate analyses. Besides the 15 taxa he recognized, Sorensen (1994) notably divided *Essigella* into three subgenera: *Archeoessigella*, *Essigella* and *Lambersella*, two species series, and three species complexes. A recent molecular phylogenetic study did not support the validity of the three subgenera and of one of the species series (Théry *et al.*, submitted). Moreover, the phylogenetic results, combined with molecular species delimitation, revealed that two species, *Essigella californica* and *E. pini*, actually encompass four and two species, respectively. In the case of *E. pini*, one of the two species is suspected to be *E. patchae* Hottes, 1957, considered a synonym of *E. pini* by Sorensen (1994). Examination of type material of *E. califonica* and *E. pini*, as well as that of their respective synonyms and reference specimens, indicates that the three cryptic species found within *E. californica* are new to science and confirm the validity of *E. patchae*.

In the present work, we describe as new the three cryptic species revealed by Théry *et al.* (submitted): *Essigella domenechi* n. sp., *E. gagnonae* n. sp. and *E. sorenseni* n. sp. In addition, we re-establish *E. patchae* stat. nov. and provide diagnostic characters to separate it and *E. pini*. Because these four species are difficult to distinguish morphologically, discreet DNA sequence data supplement classical morphological characters in the diagnoses.

4.3 Materials and Methods

4.3.1 Abbreviations used

CTT: Private Collection of T. Théry: Fleury les Aubrais, France

EMEC: Essig Museum of Entomology, University of California, Berkeley, CA, USA

QMOR: Ouellet-Robert Entomological Collection, University of Montreal, QC, Canada

UMSP: University of Minnesota Insect Collection, St Paul, MN, USA

USNM: National Aphid Collection, National Museum of Natural History, Beltsville, MD, USA.

4.3.2 Taxon sampling

All *Essigella* specimens published here were collected recently in the USA and Canada (TT and CF), or are found in the Sorensen Collection at EMEC. Specimens studied were mainly viviparous apterae. Some viviparous alatae were also studied in the case of *E. patchae* for which the holotype is an alate. Recently collected specimens were preserved in 95% ethanol after collecting and subsequently kept at -20°C or -80°C. DNA extraction of at least one specimen per population was realized. It was non-destructive (Favret, 2005), permitting us to keep the specimen as voucher. Those specimens were identified using the keys of Sorensen (1994) and Blackman & Eastop (2017). We compared our material with the type specimens of the valid species, *E. californica* (EMEC) and *E. pini* (UMSP), as well as those of their synonyms *E. claremontiana* Hottes, 1957, *E. cocheta* Hottes, 1957, *E. monelli* Hottes, 1957, *E. pineti* Hottes, 1957, *E. swaini* Hottes, 1957 (EMEC, USNM). We also compared specimens of new taxa and of *E. patchae* with other *E. californica* specimens from the Sorensen Collection (EMEC), and of *E. pini* from UMSP and USNM.

4.3.3 Preparation, measurements and pictures

All new material was slide-mounted in Canada balsam and deposited in QMOR, CTT, and USNM, in the case of holotypes. Preparations were thick to reduce deformation due to compression. As far as possible, appendices were placed so that they be strictly horizontal permitting correct length and width measurements as well as to ascertain the correct location of

dorsal and ventral setae of the hind femora and tibiae. Body length was measured from the frontal margin of the head to the posterior margin of the 7th abdominal segment. Because of the likely deformation of the body due to a variable number of embryos, width measurements were taken only of the head, between the frontal interior margins of the compound eyes. Lengths of appendages were measured at their longest, including condyles, widths were measured at the widest part of the appendage. The length of the processus terminalis was taken from the terminal margin of primary rhinarium to the apex of the antenna. The following abbreviations are applied (see annexe 9): BL - body length; LAIII - length of third antennal segment; LAIV - length of fourth antennal segment; LAV - length of fifth antennal segment; LPRIV - length of primary rhinarium on fourth antennal segment; LPRV - length of primary rhinarium on fifth antennal segment; LPT - length of processus terminalis; HWE - head width at eyes; LURS – length of ultimate rostral segment; LMF – length of metafemur; WMF – width of metafemur; LMT – length of metatibia; WMT – width of metatibia; WS – width of siphunculus at external edges; LMB - length of metabasitarsus; LMD - length of metadistitarsus; LFS – length of longest frontal seta; LDMFS – length of longest dorsal metafemoral seta; LVMFS – length of longest ventral metafemoral seta; LDMTS – length of longest dorsal metatibial seta; LVMTS – length of longest ventral metatibial seta.

Entire non–prepared specimens were photographed with a Carl Zeiss Discovery.V20 stereoscope using an AxioCam HRc camera and a Zen 2012 Carl Zeiss Software, version 1.1.1.0. Pictures of slide-mounted specimens were realised using light microscope Nikon Eclipse E600 with differential interference contrast (DIC) and photographed by Nikon DS-Fi camera. Scanning electron microscope (SEM) photos were taken using a Hitachi SU8010 field emission scanning electron microscope FESEM (Hitachi High-Technologies Corporation, Tokyo, Japan) at 5, 10 and 15 kV accelerating voltage with a secondary electron detector (ESD). For specimen preparation for SEM pictures, we followed the protocol of Kanturski *et al.*, 2015. Measurements in diagnoses and descriptions are given in microns (μm) with standard deviation (annexe 10).

4.3.4 Molecular data

The three new species were primarily revealed in the study of Théry *et al.* (submitted) using DNA sequences of the mitochondrial genome (*ATP6*, *COI*) and the obligate bacterial endosymbiont *Buchnera aphidicola* (*Gnd*) within populations of *E. californica* sensu lato. Indeed, *ATP6* and *Gnd* show similar properties as *COI* in species discrimination in barcoding (Hebert *et al.*, 2003a, b; Chen *et al.*, 2013; Lee *et al.*, 2014). Sequence lengths were 663 base pairs (bp), 658 bp and 749 bp for *ATP6*, *COI* and *Gnd*, respectively (see Théry *et al.*, submitted for GenBank accession numbers and other details).

4.4 Taxonomy

The following species, including *E. patchae*, belong to the *E. californica* species complex, which also includes *E. hoerneri* Gillette & Palmer, 1924 (Sorensen, 1994) (see discussion). All of these species, as well as *E. pini*, exhibit 6 dorsal setae on their 3rd and 4th abdominal segments (Sorensen, 1994). However, this character is homoplastic within *Essigella* as *E. pini* and the *E. californica* complex are not closely related (Théry *et al.*, submitted); it is used here to distinguish the species of the *E. californica* complex and *E. pini* from the other species of the genus. Morphological and ecological (host plant identity) comparisons of specimens of the new species with type material of synonym species of *E californica* and *E. hoerneri* allowed us to reject the possibility that our new species correspond to one of those synonyms.

Essigella domenechi **n. sp.**

(Fig. 1d)

Holotype: viviparous aptera, USA, California, Alpine Co., N38.328 - W119.637, 10.vii.2013, on *Pinus albicaulis*, T. Théry & C. Favret *leg.* (USNM). Paratypes: 8 viviparous apterae, same data as holotype (QMOR, CTT).

Diagnosis

Like species of the *E. californica* complex and *E. pini*, *E. domenechi* n. sp. has its 3rd and 4th dorsal terga usually bearing 6 setae. It can be distinguished from *E. patchae* by the presence of rows of minute teeth on URS (absent or faint in *E. patchae*; Figs. 2b, d); from *E. pini* by a relatively elongate URS with subparallel lateral margins (URS with lateral side rounded and convergent at base in *E. pini*; Figs. 2a, c); from *E. gagnonae* n. sp. and *E. sorenseni* n. sp. with the following characters: tibiae concolorous showing almost or same color than that of body (pro- and metatibiae conspicuously darkened in *E. sorenseni* n. sp., sometimes slightly darkened in *E. gagnonae* n. sp.), dorsal tegument thick; width of head between eyes = 300.7 ± 14.2 (289.0 ± 13.3 for *E. gagnonae* n. sp., and 353.6 ± 15.3 for *E. sorenseni* n. sp.); ratio of 3rd / 5th antennal segment < 1.6 (< 1.6 for *E. gagnonae* n. sp. but > 1.6 in *E. sorenseni* n. sp.); overall pubescence short or medium-sized with average length of the longest dorsal seta of metafemur = 29.7 ± 4.2 (59.8 ± 9.8 for *E. gagnonae* n. sp., and 51.2 ± 10.7 for *E. sorenseni* n. sp.; average length of the longest ventral seta of metafemur = 32.6 ± 4.5 (43.1 ± 5.4 for *E. gagnonae* n. sp., and 54.4 ± 5.6 for *E. sorenseni* n. sp.); average length of the longest dorsal seta of metatibia = 44.0 ± 8.1 (85.7 ± 10.8 for *E. gagnonae* n. sp., and 76.4 ± 15.8 for *E. sorenseni* n. sp.); average length of the longest ventral seta of metatibia = 37.5 ± 7.0 (49.4 ± 9.5 for *E. gagnonae* n. sp., and 67.7 ± 12.0 for *E. sorenseni* n. sp.); and average length of the longest frontal seta = 32.6 ± 7.5 (58.7 ± 8.3 for *E. gagnonae* n. sp., and 53.4 µm ± 11.9 for *E. sorenseni* n. sp.). *E. domenechi* n. sp. is not morphologically distinguishable from *E. californica*, that latter being highly variable, nor from *E. hoerneri*. *E. domenechi* n. sp. can be separated from *E. californica*, *E. gagnonae* n. sp., *E. hoerneri* and *E. sorenseni* n. sp. with the following DNA characters:

Gene	*ATP6* (663 bp)				*COI* (658 bp)								*Gnd* (749 bp)	
Site	4	71	227	324	190	229	334	386	418	565	619	625	219	621
E. domenechi n. sp.	**C**	**C**	**C**	**G**	**G**	**G**	**A**	**G**	**C**	**G**	**G**	**G**	**C**	**C**
E. gagnonae n. sp.	T	T	T	A	A	A	T	A	T	A	A	A	A	A
E. sorenseni n. sp.	T	T	T	A	A	A	T	A	T	A	A	A	A	A
E. californica	T	T	T	A	A	A	T	A	T	A	A	A	A	A
E. hoerneri	T	T	T	A	A	A	T	A	T	A	A	A	A	A

Table 4.1. Diagnostic nucleotides differences between *E. domenechi* n. sp. and *E. californica*, *E. gagnonae* n. sp., *E. hoerneri* and *E. sorenseni* n. sp. for *ATP6*, *COI* and *Gnd*

Description

Viviparous apterae (prepared specimens): body with pale tegument, with visible pigmented scleroites; dorsal tegument visibly thicker, sclerotized. Legs quite pale, concolorous, tibiae slightly darker than body. Antennae pale, the 5th, the 4th and the apical third part of the 3rd article of antennae darkened. URS elongated, with lateral margins subparallel, bearing rows of small teeth. Overall pubescence short to medium-sized, dorsal setae of appendices incrassate, ventral ones acute. Terga of abdominal segment 3 and 4 with 6 dorsal setae. Cauda obvious but not too protruding, apically rounded, slightly erected upwards. BL: 1600-2100 (1800 ± 170) (n = 7). HWE: 283.2-326.0 (300.7 ± 14.2) (n = 7), LAIII: 162.2-184.6 (171.8 ± 6.5) (n = 13), LAIV: 96.4-106.7 (101.0 ± 4.1) (n = 9), LAV: 113.7-124.4 (120.2 ± 4.1) (n = 5), LPRIV: 20.8-25.8 (23.0 ± 1.6) (n = 9), LPRV: 17.6-21.9 (19.6 ± 1.4) (n = 9), LPT: 8.5-14.5 (11.9 ± 1.9) (n = 9), LURS: 71.4-79.2 (75.2 ± 2.8) (n = 6), LMF: 675.7-728.8 (708.3 ± 24.2) (n = 6), WMF: 68.2-77.3 (74.9 ± 2.9) (n = 11), LMT: 975.1-1074.4 (1027.8 ± 38.2) (n = 9), WMT: 36.8-43.9 (41.4 ± 2.2) (n = 12), WS: 36.7-43.5 (40.4 ± 2.2) (n = 9), LMB: 107.2-114.4 (110.9 ± 2.3) (n = 11), LMD: 189.4-206.4 (194.9 ± 6.9) (n = 11), LFS: 18.7-39.5 (32.6 ± 7.5) (n = 7), LDMFS: 25.2-36.8 (29.7 ± 4.2) (n = 12), LVMFS: 26.1-44.0 (32.6 ± 4.5) (n = 12), LDMTS: 33.7-61.8 (44.0 ± 8.1) (n = 12), LVMTS: 24.7-48.1 (37.5 ± 7.0) (n = 12).

Host plant and distribution

USA, California, on *Pinus albicaulis* Engelm., known from Stanislaus National Forest at high elevation (type series). The species probably occurs in other high mountains where *P. albicaulis* is present.

Etymology

This species is dedicated to Boris Domenech, PhD student at the University of Montreal (QC, Canada) for his comments on the genetic analyses with which the species was discovered.

Essigella gagnonae **n. sp.**

(Fig. 1e)

Holotype: viviparous aptera, USA, Nevada, Douglas Co., N38.999 - W119.896, 10.vii.2013, on *Pinus monticola*, T. Théry & C. Favret *leg.* (USNM). Paratypes: 1 viviparous aptera, same data as holotype (QMOR); 12 viviparous apterae, California, El Dorado Co., N38.834 - W120.042, 09.vii.2013, on *Pinus monticola*, T. Théry & C. Favret *leg.*, specimens on 10 slides (QMOR, CTT); 5 viviparous apterae, California, Lassen Co., HWY 89, 6 km N Jct HWY 36 & 89, 6600', S of Lassen Nat'l Park (77G20), 10.vii.1977, on *Pinus monticola*, J. T. Sorensen *leg.*, specimens on 1 slide (EMEC); 5 viviparous apterae, Californica, Alpine Co., E side Ebbett's Pass, HWY 4, 3 km E summit (77G41), 17.vii.1977, on *Pinus monticola*, J. T. Sorensen *leg.*, specimens on 1 slide (EMEC); 13 viviparous apterae, Washington, Kitsap Co., 8 km S Hood Canal Bridge, HWY 3 (78G49), 09.vii.1978, on *Pinus monticola*, J. T. Sorensen *leg.*, specimens on 3 slides (4 + 4 + 5) (EMEC); 8 viviparous apterae, Washington, Grays Harbor Co., 16 km W Amanda Park, HWY 101 (78G54), 10.vii.1978, on *Pinus monticola*, J. T. Sorensen leg., specimens on 2 slides (4 + 4) (EMEC); 5 viviparous apterae, Nevada, Washoe Co., Mt Rose, Summit, Cmpgd, Toiyabe Nat'l Forest (78H9), 02.viii.1978, on *Pinus monticola*, J. T. Sorensen *leg.*, specimens on 2 slides (2 + 3) (EMEC).

Diagnosis

Like species of the *E. californica* complex and *E. pini*, *E. gagnonae* n. sp. has its 3rd and 4th dorsal terga usually bearing 6 setae. It can be distinguished from *E. patchae* by the presence of minute teeth on URS (absent or faint in *E. patchae*; Figs. 2b, d); from *E. pini* by a relatively elongate URS with subparallel lateral margins (URS with lateral side rounded and convergent at base in *E. pini*; Figs. 2a, c); from *E. domenechi* n. sp. and *E. sorenseni* n. sp. with the following characters: legs from concolorous slightly darker than body to with pro- and metatibiae slightly darkened with mesotibiae lighter (tibiae concolorous in *E. domenechi* n. sp., pro- and metatibiae conspicuously darkened in *E. sorenseni* n. sp.); width of head between eyes = 289.0 ± 13.3 (300.7 ± 14.2 for *E. domenechi* n. sp., and 353.6 ± 15.3 for *E. sorenseni* n. sp.); ratio of 3rd / 5th antennal segments < 1.6 (< 1.6 for *E. domenechi* n. sp. but > 1.6 in *E. sorenseni* n. sp.); overall pubescence medium-sized to long with average length of the longest dorsal setae of metafemora

= 59.8 ± 9.8 (29.7 ± 4.2 for *E. domenechi* n. sp., and 51.2 ± 10.7 for *E. sorenseni* n. sp.); average length of the longest ventral seta of metafemur = 43.1 ± 5.4 (32.6 ± 4.5 for *E. domenechi* n. sp., and 54.4 ± 5.6 for *E. sorenseni* n. sp.); average length of the longest dorsal seta of metatibia = 85.7 ± 10.8 (44.0 ± 8.1 for *E. domenechi* n. sp., and 76.4 ± 15.8 for *E. sorenseni* n. sp.); average length of the longest ventral seta of metatibia = 49.4 ± 9.5 (37.5 ± 7.0 for *E. domenechi* n. sp., and 67.7 ± 12.0 for *E. sorenseni* n. sp.); and average length of the longest frontal seta = 58.7 ± 8.3 (32.6 ± 7.5 for *E. domenechi* n. sp., and 53.4 ± 11.9 for *E. sorenseni* n. sp.). *Essigella gagnonae* n. sp. is for now morphologically indistinguishable from *E. californica*, that latter being very variable, nor from *E. hoerneri*. *Essigella gagnonae* n. sp. can be separated from *E. californica*, *E. domenechi* n. sp., *E. hoerneri* and *E. sorenseni* n. sp. with the following DNA characters:

Gene	*ATP6* (663 bp)	*COI* (658 bp)			*Gnd* (749 bp)
Site	260	28	235	271	665
E. gagnonae n. sp.	**G**	**G**	**C**	**C**	**C**
E. domenechi n. sp.	A	A	T	A	T
E. sorenseni n. sp.	A	A	T	A	A
E. californica	A	A	T	A	A
E. hoerneri	A	A	T	A	T

Table 4.2. Diagnostic nucleotides differences between *E. gagnonae* n. sp. and *E. californica*, *E. domenechi* n. sp., *E. hoerneri* and *E. sorenseni* n. sp. for *ATP6*, *COI* and *Gnd*

Description

Viviparous apterae (prepared specimens): body with pale tegument sometimes slightly yellowish, with visible pigmented scleroites. Legs from concolorous, slightly darker than body to with pro- and metatibiae slightly darkened, darker than body and mesotibiae. Antennae pale, the 5th, the 4th and the apical third part of the 3rd article of antennae darkened. URS elongated, with lateral margins subparallel, bearing rows of small teeth. Overall pubescence medium-sized to long, dorsal setae of appendices incrassate, ventral ones acute, in specimens with very long dorsal setae in metafemora and metatibiae (> 100 µm), these setae almost acute to seemly acute, straight to sinuated. Terga of abdominal segment 3 and 4 with 6 dorsal setae. Cauda obvious but not too protruding, apically rounded, slightly erected upwards. BL: 1600-2000 (1800 ± 130) (n

= 19). HWE: 271.0-311.9 (289.0 ± 13.3) (n = 13), LAIII: 157.6-197.4 (178.1 ± 11.1) (n = 29), LAIV: 90.2-111.6 (99.7 ± 6.3) (n = 33), LAV: 116.0-141.6 (125.4 ± 5.8) (n = 20), LPRIV: 21.5-29.1 (24.3 ± 1.8) (n = 21), LPRV: 18.5-22.6 (20.6 ± 1.2) (n = 18), LPT: 7.6-16.8 (12.1 ± 2.5) (n = 23), LURS: 64.5-79.8 (72.0 ± 3.8) (n = 18), LMF: 650.3-798.5 (707.3 ± 38.6) (n = 22), WMF: 69.5-104.6 (87.0 ± 10.8) (n = 29), LMT: 876.1-1104.2 (999,9 ± 67.4) (n = 25), WMT: 33.8-52.5 (42.3 ± 4.1) (n = 40), WS: 34.4-42.6 (38,9 ± 2.5) (n = 18), LMB: 101.8-131.0 (116.1 ± 8.0) (n = 36), LMD: 180.3-209.9 (195.0 ± 8.6) (n = 34), LFS: 44.4-80.2 (58.7 ± 8.3) (n = 26), LDMFS: 42.0-82.9 (59.8 ± 9.8) (n = 43), LVMFS: 31.5-52.6 (43.1 ± 5.4) (n = 42), LDMTS: 60.9- 107.7 (85.7 ± 10.8) (n = 46), LVMTS: 30.5-74.5 (49.4 ± 9.5) (n = 46).

Host plant and distribution

USA, California, Nevada and Washington, on *Pinus monticola* Douglas ex D. Don. The species occurs in elevated areas where *P. monticola* is present.

Etymology

This species is dedicated to Édeline Gagnon, PhD student at the University of Montreal (QC, Canada) for her help on the genetic analyses with which the species was discovered.

Essigella sorenseni **n. sp.**

(Fig. 1f)

Holotype: viviparous aptera, USA, California, Sonoma Co., N38.534 - W123.276, 02.vii.2013, on *Pinus muricata*, T. Théry & C. Favret *leg.* (QMOR). Paratypes: 14 viviparous apterae, same data than holotype, specimens on 14 slides (QMOR, CTT); 3 viviparous apterae, California, Mendocino Co., N38.984 - W123.696, 03.vii.2013, on *Pinus muricata*, T. Théry & C. Favret *leg.*, specimens on 3 slides (QMOR, CTT); 6 viviparous apterae, California, Mendocino Co., HWY 1, 5 km of Albion, Little River Road, 23.vii.1977, on *Pinus muricata*, 77G52, J. T. Sorensen *leg.*, specimens on 3 slides (2 + 2 + 2) EMEC); 13 viviparous apterae, California, Humbodlt Co., nr Little River State Beach, 17 km N Arcata, HWY 101, 04.vii.1978, on *Pinus muricata*, 78G3, J. T. Sorensen *leg.*, specimens on 4 slides (4 + 4 + 4 + 1) (EMEC).

Diagnosis

Like species of the *E. californica* complex and *E. pini*, *E. sorenseni* n. sp. has its 3rd and 4th dorsal terga usually bearing 6 setae. It can be distinguished from *E. patchae* by the presence of minute teeth on URS (absent or faint in *E. patchae*; Figs. 2b, d); from *E. pini* by a relatively elongate URS with subparallel lateral margins (URS with lateral side rounded and convergent at base in *E. pini*; Figs. 2a, c); from *E. domenechi* n. sp. and *E. gagnonae* n. sp. with the following characters: usually pro- and metatibiae conspicuously darkened with mesotibiae always lighter (tibiae concolorous in *E. domenechi* n. sp., concolorous or with pro- and metatibiae slightly darkened with mesotibiae lighter in *E. gagnonae* n. sp.); width of head between eyes = 353.6 ± 15.3 (300.7 ± 14.2 for *E. domenechi* n. sp., and 289.0 ± 13.3 for *E. gagnonae* n. sp.); ratio of 3rd / 5th antennal segments > 1.6 (< 1.6 for *E. domenechi* n. sp. and *E. gagnonae* n. sp.); overall pubescence medium-sized to long with average length of the longest dorsal setae of metafemora = 51.2 ± 10.7 (29.7 ± 4.2 for *E. domenechi* n. sp., and 59.8 ± 9.8 for *E. gagnonae* n. sp.); average length of the longest ventral seta of metafemur = 54.4 ± 5.6 (32.6 ± 4.5 for *E. domenechi* n. sp., and for 43.1 ± 5.4 for *E. gagnonae* n. sp.); average length of the longest dorsal seta of metatibia = 76.4 ± 15.8 (44.0 ± 8.1 for *E. domenechi* n. sp., and 85.7 ± 10.8 for *E. gagnonae* n. sp.); average length of the longest ventral seta of metatibia = 67.7 ± 12.0 (37.5 ± 7.0 for *E. domenechi* n. sp., and 49.4 ± 9.5 for *E. gagnonae* n. sp.); and average length of the longest frontal setae = 53.4 ± 11.9 (32.6 ± 7.5 for *E. domenechi* n. sp., and for 58.7 ± 8.3 *E. gagnonae* n. sp.). *E. sorenseni* n. sp. is for now morphologically indistinguishable from *E. californica*, that latter being very variable, nor from *E. hoerneri*. *E. sorenseni* n. sp. can be separated from *E. californica*, *E. domenechi* n. sp., *E. gagnonae* n. sp. and *E. hoerneri* with the following DNA characters:

Gene	*ATP6* (663 bp)		*COI* (658 bp)	*Gnd* (749 bp)		
Site	110	399	247	198	407	431
E. sorenseni n. sp.	**C**	**C**	**T**	**T**	**C**	**G**
E. domenechi n. sp.	T	T	C	C	T	T
E. gagnonae n. sp.	T	T	C	C	T	T
E. californica	T	T	C	C	T	T
E. hoerneri	T	T	C	C	T	T

Table 4.3. Diagnostic nucleotides differences between *E. sorenseni* n. sp. and *E. californica*, *E. domenechi* n. sp., *E. gagnonae* n. sp. and *E. hoerneri* for *ATP6*, *COI* and *Gnd*

Description

Viviparous apterae (prepared specimens): body with a yellowish tegument more or less darkened at joints according the specimens, with conspicuous and pigmented scleroites. Legs usually with pro- and metatibiae conspicuously darkened, much darker than body and mesotibiae. Antennae pale, the 5th, the 4th and the apical third part of the 3rd article of antennae darkened. URS elongated, with lateral margins subparallel, bearing rows of small teeth. Overall pubescence medium-sized to long, dorsal setae of appendices incrassate, ventral ones acute, in specimens with very long dorsal setae in metafemora and metatibiae (> 100 µm), these setae not acute or seemly acute but still incrassate, the setae sometimes well curved at base. Terga of abdominal segment 3 and 4 with 6 dorsal setae. Cauda obvious but not too protruding, apically rounded, slightly erected upwards. BL: 1900-2300 (2200 ± 110) (n = 21). HWE: 322.3-376.1 (353,6 ± 15.3) (n = 17), LAIII: 207.5-256.3 (233.6 ± 12.8) (n = 25), LAIV: 98.3-130.0 (112.2 ± 7.1) (n = 34), LAV: 120.1-139.8 (127.9 ± 4.7) (n = 23), LPRIV: 19.9-27.8 (24.1 ± 1.8) (n = 28), LPRV: 17.4-23.4 (19.5 ± 1.5) (n = 21), LPT: 11.6-15.7 (13.8 ± 1.4) (n = 21), LURS: 74.1-86.4 (80.5 ± 3.2) (n = 21), LMF: 702.3-927.8 (810.8 ± 58.9) (n = 26), WMF: 87.5-128.9 (103.1 ± 11.3) (n = 36), LMT: 1064.2-1450.4 (1233.4 ± 95.1) (n = 26), WMT: 49.5-76.0 (55.1 ± 5.1) (n = 37), WS: 39.0-44.6 (41.4 ± 1.7) (n = 22), LMB: 118.5-140.3 (130.3 ± 6.4) (n = 38), LMD: 183.4-212.5 (198.1 ± 7.9) (n = 34), LFS: 31.9-82.7 (53.4 ± 11.9) (n = 25), LDMFS: 34.2-79.4 (51.2 ± 10.7) (n = 45), LVMFS: 43.4-66.0 (54.4 ± 5.6) (n = 44), LDMTS: 47.7-113.8 (76.4 ± 15.8) (n = 46), LVMTS: 45.9-92.2 (67.7 ± 12.0) (n = 45).

Host plant and distribution

USA, California, on *Pinus muricata* D. Don, known from Humboldt, Mendocino and Sonoma counties (type series), but probably present everywhere on coastal range in California where *P. muricata* occurs.

Etymology

This species is dedicated to John T. Sorensen, aphid specialist who eminently revised the genus *Essigella* in 1994, for his advice and the hospitality accorded to the authors (TT and CF) in California.

Essigella patchae Hottes, 1957 **stat. nov.**

(Figs. 1b, 2b, d, f, h).

Essigella patchae Hottes, 1957: 98 (Type locality: "Stillwater, Maine"). Holotype viviparous alate in USNM. Sorensen, 1994: 49 [synonymy with *E. pini* Wilson]. **Status re-established**.

Other examined material: 1 viviparous alate and 1 viviparous aptera, Canada, Québec, Saint-Hippolyte, N45.991 - W74.009, ix.2015, on *Pinus strobus*, C. Favret *leg.* (QMOR); 1 viviparous aptera, Saint-Hippolyte, N45.989 - W74.005, ix.2016, on *Pinus strobus*, T. Théry *leg.* (QMOR); 1 viviparous aptera, Saint-Hippolyte, N45.989 - W74.005, ix.2017, on *Pinus strobus*, T. Théry *leg.* (QMOR).

Diagnosis

Like species of the *E. californica* complex and *E. pini*, *E. patchae* has its 3rd and 4th dorsal terga usually with 6 setae. *Essigella patchae* can be distinguished from the other species of the *E. californica* complex species and from *E. pini* by its ultimate rostral segment (URS) exhibiting no or barely visible rows of small teeth (Figs. 2b, d), which are well visible in other species of the *E. californica* complex and also in *E. pini* (Figs. 2a, c); it can also be differentiated from *E. pini* by having the general shape of the URS more elongated with lateral margins almost parallel (Figs. 2b, d) (lateral sides more rounded and convergent at base in *E. pini*; Figs. 2a, c); shorter cauda than that of *E. pini* which can be elongated and acute; a genital plate with fewer setae which are longer in *E. patchae* in comparison with *E. pini* (Figs. 2e, f) and with teeth of the genital plate tegument more developed in *E. patchae* (Figs. 2g, h).

Host plant and distribution

The species is currently known from its type locality in Maine (USA) and from one locality in Quebec (Canada) on *Pinus strobus* Linnaeus (see discussion).

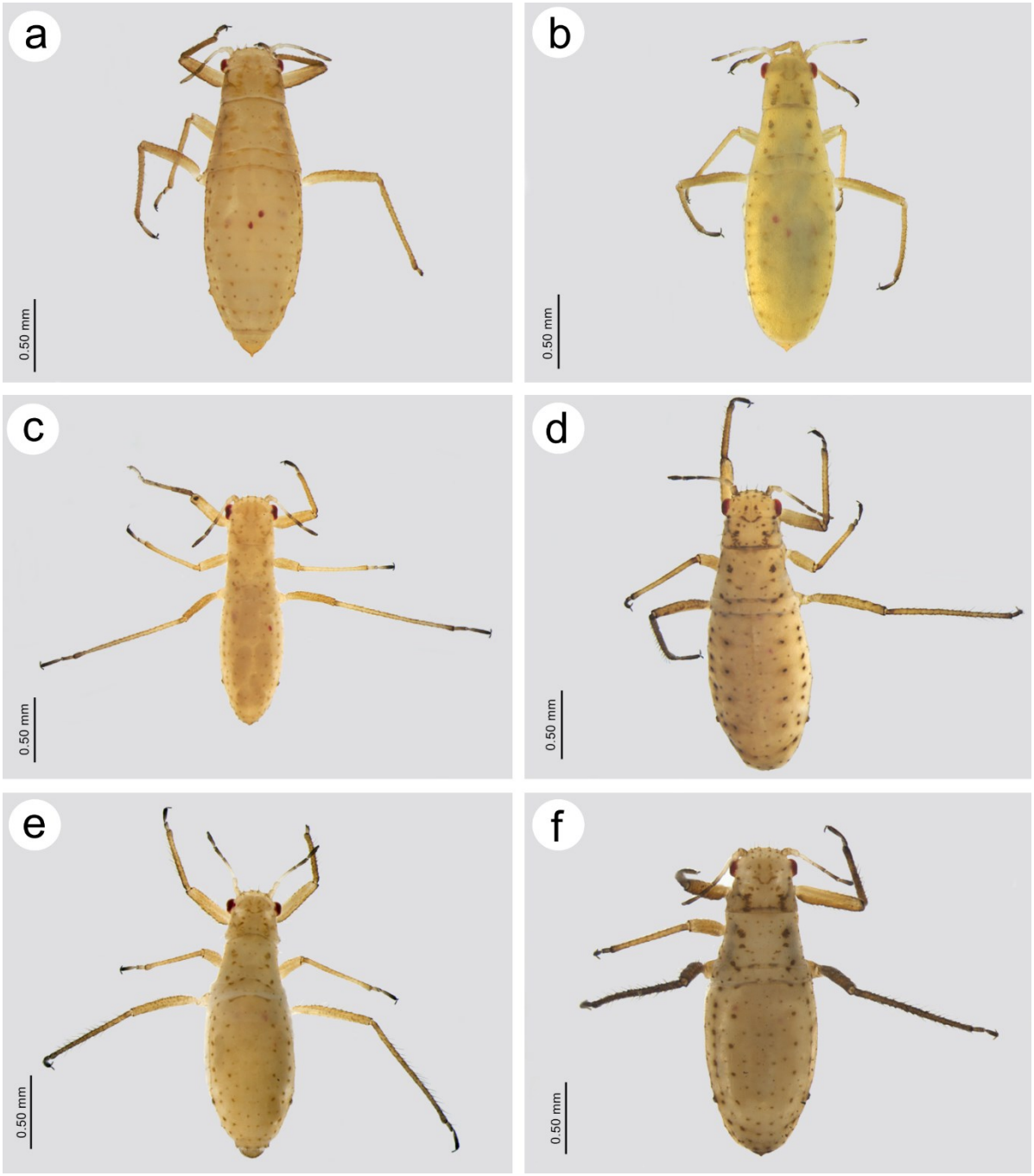


Figure 4.1. Habitus of viviparous apterae: a. *Essigella pini*; b. *E. patchae*; c. *E. californica*; d. *E. domenechi* n. sp.; e. *E. gagnonae* n. sp.; f. *E. sorenseni* n. sp.

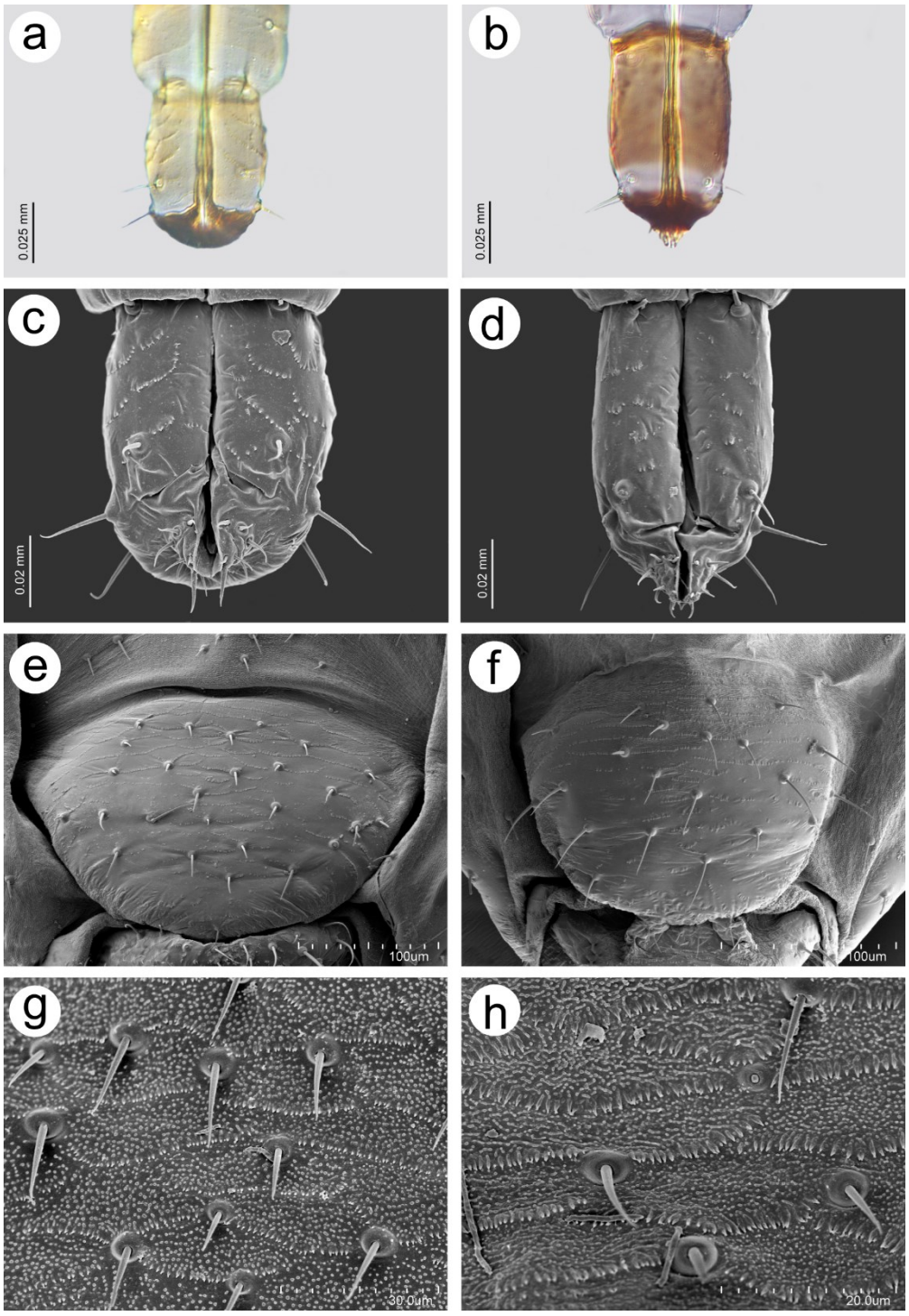


Figure 4.2. Morphological structures in *Essigella pini* and in *E. patchae*: a. URS in *E. pini* (slide-mounted specimen); b. URS in *E. patchae* (slide-mounted specimen); c. URS in *E. pini* (SEM); d. URS in *E. patchae* (SEM); e. genital plate in *E. pini* (SEM); f. genital plate in *E. patchae* (SEM); g. details in genital plate in *E. pini* (SEM); h. details in genital plate in *E. patchae* (SEM)

Simplified key to species of the *Essigella californica* complex, for viviparous apterae.

Due to the variability of preparation, notably cover slip-induced deformations, teneral specimens and general morphological variability, several specimens and the identity of the host plant are required to best use this key.

1a. Dorsal terga 3 and 4 usually with 6 setae……...*E. californica* complex, *E. pini*……2

1b. Dorsal terga 3 and 4 usually with more than 6 setae……..……

…….....other *Essigella* species (cf. Sorensen, 1994)

2a. Western North America species…..……...……3

2b. Eastern North America species..……...……..……...7

3a. On pinyon pines (*Pinus cembroides*, *P. edulis*, *P. monophylla*, *P. quadrifolia*) ……..*E. hoerneri*

3b. Not on pinyon pines…….……...4

4a. On *Pinus albicaulis*, *P. monticola* or *P. muricata*…….……..5

4b. On other pine species…….……*E. californica*

5a. Ratio of LAIII / LAV > 1.6; on *P. muricata*…….……..*E. sorenseni* n. sp.

5b. Ratio of LAIII / LAV < 1.6……..……...6

6a. Dorsal setae of metatibiae short (44.0 ± 8.1), on *P. albicaulis*…….…….....*E. domenechi* n. sp.

6b. Dorsal setae of metatibiae long (85.7 ± 10.8), on *P. monticola*……...…*E. gagnonae* n. sp.

7a. Ultimate Rostral Segment (URS) with conspicuous rows of small teeth; lateral sides of URS convex, convergent at base (Figs. 2a, c)……..……...……*E. pini*

7b. Ultimate Rostral Segment (URS) without or with barely visible rows of small teeth (Figs. 2b, d); lateral sides of URS subparallel, not convergent at base……*E. patchae*

Catalogue of *Essigella* species

Genus *Essigella* Del Guercio, 1909 : 329

Type species : *Lachnus californicus* Essig, 1909 : 1

= *Archeoessigella* Sorensen, 1994 [**New Synonymy**]

Type species : *Essigella kathleenae* Sorensen, 1988 : 115; Sorensen, 1994 : 21

= *Lambersella* Sorensen, 1994 [**New Synonymy**]

Type species : *Essigella fusca* Gillette & Palmer, 1924 : 6; Sorensen, 1994 : 29

Essigella alyeska Sorensen, 1988 : 118; Sorensen, 1994 : 72

Essigella californica (Essig), 1909 : 1; Sorensen, 1994 : 53

= *Lachnus californicus* Essig, 1909 : 1

= *Essigella claremontiana* Hottes, 1957 : 79 [Synonymy by Sorensen, 1994 : 53]

= *Essigella cocheta* Hottes, 1957 : 82 [Synonymy by Sorensen, 1994 : 53]

= *Essigella monelli* Hottes, 1957 : 95 [Synonymy by Sorensen, 1994 : 53]

= *Essigella pineti* Hottes, 1957 : 101 [Synonymy by Sorensen, 1994 : 53]

= *Essigella swaini* Hottes, 1957 : 105 [Synonymy by Sorensen, 1994 : 53]

Essigella critchfieldi Sorensen, 1994 : 75

Essigella domenechi Théry *et al.*, **n. sp.**

Essigella eastopi Sorensen, 1994 : 30

Essigella essigi Hottes, 1957 : 84; Sorensen, 1994 : 45

Essigella fusca fusca Gillette & Palmer, 1924 : 6 ; Sorensen, 1994 : 34

= *Essigella fusca* Gillette & Palmer, 1924 : 6

= *Essigella agilis* Hottes, 1957: 71 [Synonymy by Sorensen, 1994 : 34]

= *Essigella palmerae* Hottes, 1957: 96 [Synonymy by Sorensen, 1994 : 34]

Essigella fusca voegtlini Sorensen, 1994 : 39

Essigella gagnonae Théry *et al.*, **n. sp.**

Essigella hillerislambersi Sorensen, 1994 : 41

Essigella hoerneri Gillette & Palmer, 1924 : 5; Sorensen, 1994 : 62

= *Essigella gillettei* Hottes, 1957 : 88 [Synonymy by Sorensen, 1994 : 62]

= *Essigella maculata* Hottes, 1957 : 93 [Synonymy by Sorensen, 1994 : 62]

Essigella kathleenae Sorensen, 1988 : 115; Sorensen, 1994 : 26

Essigella kirki Sorensen, 1988 : 121; Sorensen, 1994 : 22

Essigella knowltoni braggi Hottes, 1957 : 73; Sorensen, 1994 : 84

= *Essigella braggi* Hottes, 1957: 73 [New status by Sorensen, 1994 : 84]

= *Essigella robusta* Hottes, 1957: 103 [Synonymy by Sorensen, 1994 : 84]

Essigella knowltoni knowltoni Hottes, 1957: 92 [New status by Sorensen, 1994 : 78]

= Essigella knowltoni Hottes, 1957: 92

Essigella patchae Hottes, 1957 : 98; Sorensen, 1994 : 49; [**Stat. Nov.]**

Essigella pini Wilson, 1919 : 2; Sorensen, 1994 : 49

Essigella sorenseni Théry *et al.*, **n. sp.**

Essigella wilsoni Hottes, 1957 : 106; Sorensen, 1994 : 67

= *Essigella pergandei* Hottes, 1957 : 100 [Synonymy by Sorensen, 1994 : 67]

= *Essigella oregonensis* Hottes, 1958: 155 [Synonymy by Sorensen, 1994 : 67]

4.5 Discussion

4.5.1 *Essigella californica*

Sorensen, in his revision of the genus *Essigella* (1994), had already documented the existence of different host-associated groups within *E. californica*. He notably mentioned populations living on *Pinus flexilis* E. James and *P. lambertiana* Douglas, populations that he nevertheless considered as exhibiting intraspecific variation (Sorensen, 1983, 1994). Populations from those two pine species were not considered in the study of Théry *et al.* (submitted) and it is possible that they correspond to yet two more cryptic species. *Essigella californica* is known to live on at least 34 *Pinus* species (Kimber *et al.*, 2013) and it is likely other cryptic species await discovery. We are unable to fully evaluate the species complex due to a lack of material. *Essigella californica* continues to be a complex issue meriting further study. Such a study would require substantial material of representative populations from as many known host plants as possible. A redescription of this species and the members of its complex would require morphometric data and multivariate analyses as per Sorensen (1994), combined with molecular phylogenetic and species delimitation methods as per Théry *et al.* (submitted).

4.5.2 *Essigella patchae* and *E. pini*

Essigella pini is known to be oligophagous on *Pinus* and according to Sorensen (1994), this species can be found on pine species of the sugbenus *Pinus*, section *Trifoliae*, subsection *Contortae* (notably on *P. virginiana* Miller), subection *Australes* (notably on *P. taeda* Linnaeus), and on pine species of the sugbenus *Strobus*, section *Quinquefoliae*, subsection *Strobus* (notably on *P. strobus*). It could also be found on species of subsection *Sylvestres* (Sorensen 1994). The type specimen of *E. pini* was collected in Maryland on *P. virginiana* (Wilson, 1919; Sorensen, 1994) whereas that of *E. patchae* was collected in Maine on *P. strobus* (Hottes, 1957; Sorensen, 1994). Genetic material analysed by Théry *et al.* (submitted) came from a Canadian specimen of *E. patchae* collected on *P. strobus* and a US specimen of *E. pini* collected on *P. rigida* (subsection *Australes*). Our first suspicions are that *E. patchae* could be a more northern species that would feed on pines of subsection *Strobus* whereas *E. pini* would be more southern developing both on pines of subsections *Australes* and *Contortae*. It could appear curious that Sorensen did not discriminate both species, even though they are morphologically very close. Actually, Sorensen himself collected only species occurring in the western part of USA. Because *E. pini* and *E. patchae* are the only species occurring in the East, all *E. pini* and *E. patchae* specimens that Sorensen studied came from other collections and represented a smaller specimen sample in comparison with other species. Considering the list of specimens Sorensen (1994) studied and those we verified from both USNM and UMSP collections, it is likely Sorensen studied no more than two specimens identified as *E. patchae*, notably the type specimen in poor condition. Those conditions made revelation of significant differences between the two species difficult.

Molecular data in aphid diagnoses

Aphids represent a relatively well-studied insect group mostly because of their economic importance. Molecular data are most often used in population genetics (Wongsa *et al.*, 2017; Medina *et al.*, 2017). They are used also in works linked with species recognition using barcodes because of their small size and their difficult systematics (Cœur d'acier *et al.*, 2014; Lee *et al.*, 2011). As in other animal groups, new aphid species can be discovered or confirmed using DNA analyses (Depa *et al.*, 2012; Chen *et al.*, 2015; Jiang *et al.*, 2015). The present paper represents

the first time that DNA sequence characters have been used in an aphid species diagnosis. Indeed, use of this kind of data and especially substitutions of nucleotides as characters is rare in animal diagnoses (Renner, 2016), and rarer in insects. The precedent was established 8 years ago (Brower, 2010). The International Code of Zoological Nomenclature does not explicitly recommend DNA sequence data to establish animal taxa, nor does it forbid it (ICZN, 1999). Other kinds of non-morphological characters are commonly used in other groups. For example, songs or acoustic signals are used to differentiate species in several animal groups and can be considered good diagnostic characters in frogs (Brown & Richards, 2008) or in Orthopteran insects (Hertach *et al.*, 2015; Iorgu *et al.*, 2017). In consequence, we judge that the absence, the presence, or the identity of a nucleotide or of a DNA sequence fragment are the molecular equivalent to the absence, the presence, or the shape of a seta, a puncture, or of any other morphological character. We thus support that this kind of DNA character can be used unambiguously in a diagnosis.

4.6 Acknowledgments

We are grateful to G. L. Miller (USDA Systematic Entomology Laboratory, Beltsville, MD), P. T. Oboyski (Essig Museum of Entomology, University of California, Berkeley, CA), and R. E. Thomson (University of Minnesota, St Paul, MN) for specimen loans. We also extend our thanks to J. T. Sorensen for his advice and assistance in the field.

5. Conclusion

En prenant comme sujet d'étude un groupe d'insectes spécialisés dans l'exploitation d'un nombre limité de plantes hôtes, cette thèse avait pour but d'étudier la problématique de l'espèce dans un cadre évolutif de spéciation écologique et ce, en utilisant des données moléculaires. Le choix du genre *Essigella* permettait de focaliser cette problématique sur la nature de la plante hôte et sa potentielle influence sur la variation morphologique chez ces insectes. Le choix d'*Essigella* permettait d'élargir cette recherche à la révision du genre, mais également de préciser la place de celui-ci au sein de la tribu des Eulachnini. Il ébauchait aussi une étude au niveau populationnel.

5.1 *Essigella* au sein des Eulachnini

Mes analyses phylogénétiques confirment l'hypothèse de Sorensen qui considère *Pseudessigella* comme le groupe frère d'*Essigella* (1991, 1994). Par conséquent, la présence de 5 articles antennaires ainsi que la fusion de la tête avec le pronotum sont des synapomorphies du groupe *Pseudessigella* + *Essigella*, et la sclérotinisation des tergites abdominaux et l'incision des tarses sont des autapomorphies d'*Essigella*. Cette proximité phylogénétique entre *Essigella* et *Pseudessigella* permet également d'apporter de nouvelles informations pour expliquer la répartition géographique de ces trois genres et plus particulièrement la présence du genre *Essigella* en Amérique du Nord. En effet, bien que *Pseudessigella* soit le groupe frère d'*Essigella*, *Pseudessigella* et *Eulachnus* sont paléarctiques alors qu'*Essigella* est uniquement néarctique. Ceci laisserait alors à penser que l'ancêtre commun de ces trois genres serait asiatique. D'après Chen *et al.* (2016), les lignées respectives d'*Eulachnus* et *Essigella* auraient divergé au tout début de l'Éocène (-56.0 à -33.9 MA), période durant laquelle l'Asie et l'Amérique du Nord était en contact (Wake, 2013). Il est alors possible d'imaginer qu'après une première séparation entre un premier groupe, ancêtre d'*Eulachnus*, et un second, ancêtre commun à *Essigella* et *Pseudessigella*, des populations du second aurait colonisé l'Amérique du Nord alors que ce continent était en contact avec l'Eurasie. Après une nouvelle séparation des deux continents, ces deux groupes auraient alors évolué indépendamment pour donner

Pseudessigella en Asie et *Essigella* en Amérique du Nord. Toutefois, Asie et Amérique du Nord ont de nouveau été en contact à différentes périodes après l'Éocène (Wake, 2013). De plus, les travaux de Chen *et al.* (2016) ne prennent pas en compte *Pseudessigella*. Aussi, de nouvelles études similaires prenant en compte ce genre seraient nécessaires afin de dater précisément la séparation des lignées respectives d'*Essigella* et *Pseudessigella*.

5.2 Monophylie et structuration interne du genre *Essigella*

Selon Sorensen, le genre *Essigella* peut être structuré en 3 sous-genres (*Archeoessigella*, *Essigella* et *Lambersella*), 2 séries d'espèces (Série A et Série B) et 3 complexes d'espèces (complexes *E. californica*, *E. fusca* et *E. knowltoni*). Mes analyses phylogénétiques confirment l'hypothèse d'un genre *Essigella* monophylétique, ainsi que celle voulant qu'une structuration d'*Essigella* en trois sous-genres ne soit pas valide. Toutefois, elle indique l'existence d'un regroupement d'espèces qui correspond à la Série B et corrobore la validité des trois complexes d'espèces. L'infirmation de l'existence des trois sous-genres était prévisible puisqu'*Archeoessigella* était considéré comme plésiomorphe et paraphylétique par Sorensen (1994). Mes résultats montrent qu'*E. kirki*, initialement placée au sein du sous-genre *Archeoessigella*, correspond au groupe frère de l'ensemble des autres espèces d'*Essigella*. En outre, les espèces du sous-genre *Essigella* se répartissent en différents groupes, alors qu'au contraire, les espèces du sous-genre *Lambersella* forment un groupe monophylétique. Toutefois, ces dernières sont regroupées dans le même clade qu'*E. kathleenae* (sous-genre *Archeoessigella*) et qu'*E. essigi* (sous-genre *Essigella*).

5.3 La délimitation d'espèces au sein d'*Essigella*

Mes résultats infirment mon hypothèse de départ quant à l'existence de différentes populations présentant des variations liées à la nature de leur plante hôte. Ils indiquent au contraire l'existence de différentes espèces, corroborant ainsi la systématique de Sorensen. Toutefois mes études ont clairement montré que le genre *Essigella* comporte plus d'espèces que ce que ne laissait supposer le travail de Sorensen et que le genre ne renferme pas 13 mais au

moins 20 espèces. En effet, plusieurs éléments laissent à penser que d'autres espèces cryptiques pourraient exister. En démontrant qu'un seuil de 2% était surévalué dans le barcode utilisant *COI* chez *Essigella*, j'ai mis en avant la possibilité que d'autres populations de cette étude pouvaient correspondre à des espèces cryptiques, notamment chez *E. californica*, *E. eastopi*, *E. essigi* et *E. wilsoni*. Toutefois, ces résultats n'ayant pas été retrouvés avec toutes les méthodes de délimitation d'espèces utilisées, celles-ci mériteraient d'autres investigations pour pouvoir conclure sur leurs statuts.

En plus des trois espèces cryptiques révélées au sein d'*E. californica*, mon étude a permis de discriminer et de réhabiliter l'espèce *E. patchae*, qui avait été mise en synonymie avec *E. pini* par Sorensen (1994). Le matériel dont disposait Sorensen pour ces deux espèces était très limité en comparaison avec les autres espèces étudiées dans sa révision. Plus particulièrement, il semble que Sorensen n'ait étudié que deux spécimens pour *E. patchae*, dont le type qui est en mauvais état. Aussi, au vu des caractères ténus qui distinguent les deux espèces et du peu de matériel mis à sa disposition, il était presque impossible pour Sorensen de discriminer les deux espèces. Il est donc possible qu'avec un échantillonnage plus important, Sorensen aurait conclu en la validité des deux taxons.

Enfin, l'analyse des séquences de *COI* hébergées sur le site de BOLD a également permis de révéler l'existence d'autres espèces d'*Essigella* non répertoriées par la science. En effet, ces séquences, rassemblées en différents BINs ne correspondent à aucune des espèces que nous avons collectées. L'une d'elles (BOLD:ACE3641) semble être cryptique au sein de l'espèce *E. knowltoni* et, comme elle et les autres espèces du complexe *E. knowltoni*, vit sur *Pinus contorta*. Pour les deux autres (BOLD:ACM1471 et BOLD:ABV2593), faute de n'avoir pour le moment que leur séquence *COI* d'accessible, il nous est impossible de discuter de leur place dans la phylogénie du genre *Essigella*. Toutefois, l'étude morphologique des ces trois espèces et leur comparaison avec les types d'espèces d'*Essigella* considérées actuellement comme des synonymes seraient nécessaires afin de confirmer leurs statuts.

La comparaison des séquences des quatre gènes étudiés a mis en évidence la présence de différences génétiques caractéristiques sur trois d'entre eux entre les espèces nouvellement décrites et *E. californica*. De par la grande proximité morphologique entre ces différents taxons, ces caractères ont été inclus dans la diagnose de chacune de ces trois espèces. Ce type de caractères est très rarement utilisé dans des diagnoses spécifiques et, à ma connaissance, je suis

le premier à utiliser ce genre de caractères pour des pucerons. Mes recherches montrent que le gène *EF-1α* peut également apporter des informations utiles dans la discrimination de certaines espèces et sous-espèces, la structuration de certains introns apparaissant caractéristique de certains taxons. La comparaison des patrons d'hétérozygotie de ce gène a permis, quant à elle, la discrimination de populations.

5.4 *Essigella* et ses plantes hôtes

Comme attendu, mes analyses n'ont pas révélé de parallélisme étroit entre la phylogénie des espèces d'*Essigella* et celle de leur plante hôte. Toutefois, la spécificité de chaque espèce d'*Essigella* décrite par Sorensen a été retrouvée, excepté dans le cas des espèces cryptiques révélées au sein d'*E. californica* et *E. pini*. En effet, celles-ci n'étaient pas connues de l'auteur. On note toutefois pour les complexes *E. fusca* et *E. knowltoni* que les plantes hôtes de chaque espèce sont phylogénétiquement proches, ce qui suggère un modèle de poursuite phylogénétique avec changement d'hôte sur des plantes apparentées.

5.5 *Essigella californica* en dehors du continent nord-américain

Malgré la découverte des trois espèces cryptiques que j'ai décrites sous les noms *E. domenechi*, *E gagnonae* et *E. sorenseni*, il a été montré que les populations exotiques d'*Essigella* appartenaient toutes à *E. californica*, conformément à mon hypothèse. Toutefois, malgré des similitudes évidentes dans la séquence d'*EF-1α* entre certaines populations exotiques et nord-américaines, je n'ai pas pu définir leurs origines respectives, les méthodes employées n'étant pas adaptées.

5.6 Perspectives

Cette thèse a apporté de précieuses informations quant à la systématique d'*Essigella* et celle des Eulachnini. Toutefois, vu l'échantillonnage limité, il a été difficile de statuer sur tous

les taxons d'*Essigella*, ou de répondre à certaines interrogations apparues en cours d'étude. Elle laisse ainsi plusieurs questions en suspens.

Premièrement, je n'ai pas pu statuer sur la nature des deux sous-espèces d'*E. fusca*. En effet, aucun spécimen de la sous-espèce *E. fusca fusca* n'a pu être étudié. Deuxièmement, au-delà du problème du statut de certaines populations, la découverte des trois espèces cryptiques au sein d'*E. californica*, la forte oligophagie de cette espèce, ainsi que les remarques de Sorensen concernant certaines de ses populations (sur *P. flexilis* et *P. lambertiana*) amènent à penser que l'espèce pourrait encore renfermer d'autres taxons cryptiques. Aussi, cette étude mériterait d'être poursuivie avec un plus grand effectif de spécimens, afin de confirmer mes résultats et de conclure à la fois sur les populations qui s'avèrent problématiques et sur celles appartenant potentiellement à des espèces nouvelles. Toutefois, *E. californica* mériterait une étude à part entière, en employant une méthodologie semblable à celles de Sorensen, combinée avec des analyses moléculaires comme celles que j'ai effectuées. L'espèce étant connue pour vivre sur plus de 30 espèces de pins, un échantillonnage conséquent des populations vivant sur ces différentes essences est indispensable. Une telle étude permettrait de conclure sur l'étendue de l'oligophagie de cette espèce et sur le nombre réel de taxons au sein de celle-ci. Elle permettrait aussi de redécrire plus précisément l'espèce. Enfin, bien que j'aie montré que seule *E. californica* avait été introduite en dehors du continent nord-américain, une analyse populationnelle utilisant d'autres méthodes plus adaptées (AFLP, RFLP et microsatellites) permettrait de retracer les routes d'introduction et l'origine de ces populations introduites.

Au-delà de répondre à ma problématique, cette thèse aura également permis d'aborder certains défis actuels de la taxonomie liés à l'utilisation de techniques modernes, comme celles utilisant des données moléculaires. En effet, près de 260 ans après la publication par Linné de la dixième édition de son *Systema Naturae*, la taxonomie demeure une science vivante qui se doit d'évoluer avec son temps. Elle doit savoir adopter dans son fonctionnement de nouvelles règles afin de mieux répondre aux besoins de ceux qui l'utilise dans l'étude et la description du monde vivant. Le cas des espèces cryptiques en est bon exemple.

Bibliographie

Aguiar, A. M. F., & F. A. Ilharco. 2001. Aphids (Homoptera: Aphidoidea) from Madeira Island [Portugal]: New records and corrections. Boletín de Sanidad Vegetal. Plagas. 27 : 323-336.

Ahrens, D., T. Fujisawa, H. J. Krammer, J. Eberle, S. Fabrizi, & A. P. Vogler. 2016. Rarity and incomplete sampling in DNA-based species delimitation. Systematic Biology. 65(3) : 478–494.

Althoff, D. M., K. A. Segraves, & M. T. J. Johnson. 2014. Testing for coevolutionary diversification: linking pattern with process. Trends in Ecology and Evolution. 22 : 82-89.

Althoff, D. M. 2016. Specialization in the yucca–yucca moth obligate pollination mutualism: A role for antagonism? American journal of botany. 103(10) : 1803-1809.

Avelino-Capistrano, F., L. S. Barbosa, & D. M. Takiya. 2016. Description of a new *Kempnyia* Klapálek from Brazil (Plecoptera : Perlidae) with life stages associated using DNA barcodes. Zootaxa. 4079(3) : 372–380.

Barbagallo, S., A. Binazzi, & S. Ortu. 2005. On the presence in Italy of the Nearctic aphid *Essigella californica* (Essig) living on american pines. Redia. 88 : 79-84.

Beeren, C., M. Maruyama, & D. J. Kronauer. 2016. Cryptic diversity, high host specificity and reproductive synchronization in army ant-associated *Vatesus* beetles. Molecular Ecology. 25(4) : 990–1005.

Berlocher, S. H., & J. L. Feder. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annual review of entomology. 47(1) : 773-815.

Birky, C. W. 1996. Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. Genetics. 144 : 427-437.

Blackman, R. L., & V. F. Eastop. 2000. Aphids on the World's Crops : an Identification and Information Guide, 2nd edn. John Wiley & Sons Ltd., England.

Blackman, R. L., & V. F. Eastop. 2017. Aphids on the world's plants : An online identification and information guide. http://www.aphidinwordsplants.info/ (accessed 1st November 2017).

Boukhris-Bouhachem, S., R. Souissi, E. Turpeau, J. Rouzé-Jouan, M. Fahem, N. Ben Brahim, & M. Hullé. 2007. Aphid (Hemiptera : Aphidoidea) diversity in Tunisia in relation to seed potato production. Annales de la Société Entomologique de France (N.S.). 43(3) : 311–318.

Brooks, D. R. 1981. Hennig's parasitological method : a proposed solution. Systematic Zoology. 30 : 229-249.

Brower, A. V. 2010. Alleviating the taxonomic impediment of DNA barcoding and setting a bad precedent : names for ten species of '*Astraptes fulgerator*' (Lepidoptera: Hesperiidae: Eudaminae) with DNA-based diagnoses. Systematics and Biodiversity. 8(4) : 485-491.

Brown, R. M., & Richards, S. J. 2008. Two new frogs of the genus *Platymantis* (Anura : Ceratobatrachidae) from the Isabel Island group, Solomon Islands. Zootaxa. 1888(1) : 47-68.

Buchner, P., M. Corley, & J. Junnilainen. 2017. Three new species and one new subspecies of Depressariinae (Lepidoptera) from Europe. ZooKeys. 684: 119–154.

Burke, H. E. 1937. Important insect enemies of the Monterey pine. Proceedings of the Western Shade Tree Conference. 4 : 21–31.

Carletto, J., E. Lombaert, P. Chavigny, T. Brévault, L. Lapchin, & F. Vanlerberghe-Masutti. 2009. Ecological specialization of the aphid *Aphis gossypii* Glover on cultivated host plants. Molecular ecology. 18(10) : 2198-2212.

Carstens, B. C., T. A. Pelletier, N. M. Reid, & J. D. Satler. 2013. How to fail at species delimitation. Molecular ecology. 22(17) : 4369-4383.

Carvalho R. C. Z., & S. M. N. Lazzari. 2000. First Record of the California Pine Needle Aphid, *Essigella* (*Essigella*) *californica* (Essig) (Homoptera : Aphididae : Lachninae), in Southern Brazil. Proceedings of the Entomological Society of Washington. 102(3) : 757-758.

Carver, M., & D. S. Kent. 2000. *Essigella californica* (Essig) and *Eulachnus thunbergii* Wilson (Hemiptera : Aphididae : Lachninae) on *Pinus* in south-eastern Australia. Australian Journal of Entomology. 39 : 62–69.

Caterino, M. S., S. Cho, & F. A. H. Sperling. 2000. The current state of insect molecular systematics : a thriving tower of Babel. Annual Review of Entomology. 45 : 1–54.

Chen, R., L. Y. Jiang, & G. X. Qiao. 2012. The effectiveness of three regions in mitochondrial genome for aphid DNA barcoding : A case in Lachninae. PLoS ONE. 7(10) : e46190.

Chen, R., L. Y. Jiang, L. Liu, Q. H. Liu, J. Wen, R. L. Zhang, X. Y. Li, Y. Wang, F. M. Lei, & G. X. Qiao. 2013. The *gnd* gene of *Buchnera* as a new, effective DNA barcode for aphid identification. Systematic Entomology. 38 : 615–625.

Chen, J., B. Zhang, X. Zhu, L. Jiang, & G. X. Qiao. 2015. Review of the aphid genus *Aspidophorodon* Verma, 1967 with descriptions of three new species from China (Hemiptera: Aphididae: Aphidinae). Zootaxa. 4028(4) : 551-576.

Chen, R., C. Favret, L. Jiang, Z. Wang, & G. X. Qiao. 2016. An aphid lineage maintains a bark-feeding niche while switching to and diversifying on conifers. Cladistics. 32(5) : 555-572.

Chen, R., Z. Wang, J. Chen, L. Y. Jiang, & G. X. Qiao. 2017. Insect-bacteria parallel evolution in multiple-co-obligate-aphid association : a case in Lachninae (Hemiptera: Aphididae). Scientific Reports. 7(1) : 10204.

Cho, S., A. Mitchell, J. C. Regier, C. Mitter, R. W. Poole, T. P. Friedlander, & S. Zhao. 1995. A highly conserved nuclear gene for low-level phylogenetics : *Elongation Factor-1α* recovers morphology-based tree for heliothine moths. Molecular Biology and Evolution. 12(4) : 650-656.

Chroni, A., M. Djan, D. O. Vidaković, T. Petanidou, & A. Vujić. 2017. Molecular species delimitation in the genus *Eumerus* (Diptera : Syrphidae). Bulletin of Entomological Research. 107(1) : 126–138.

Cock, M. J., P. K. Beseh, A. G. Buddie, G. Cafá, & J. Crozier. 2017. Molecular methods to detect *Spodoptera frugiperda* in Ghana, and implications for monitoring the spread of invasive species in developing countries. Scientific Reports. 7(1) : 4103.

Cocuzza, G., V. Cavalieri, E. Jousselin, A. Cœur d'acier, & S. Barbagallo. 2007. Morphological and molecular analysis of *Brachycaudus*, subgenus *Appelia*-complex (Rhynchota Aphididae). Redia. 90 : 33-49.

Cœur d'acier, A., E. Jousselin, J. F. Martin, & J. Y. Rasplus. 2007. Phylogeny of the genus *Aphis* Linnaeus, 1758 (Homoptera : Aphididae) inferred from mitochondrial DNA sequences. Molecular Phylogenetics and Evolution. 42(3) : 598–611.

Cœur d'acier, A., A. Cruaud, E. Artige, G. Genson, A. L. Clamens, É. Pierre, S. Hudaverdian, J. C. Simon, E. Jousselin, & J. Y. Rasplus. 2014. DNA barcoding and the associated

PhylAphidB@se website for the identification of european aphids (Insecta : Hemiptera: Aphididae). PLoS ONE. 9(6) : e97620.

Condamine, F. L., E. F. A. Toussaint, A. M. Cotton, G. S. Genson, F. A. H. Sperling, & G. J. Kergoat. 2013. Fine-scale biogeographical and temporal diversification processes of peacock swallowtails (*Papilio* subgenus *Achillides*) in the Indo-Australian Archipelago. Cladistics. 29 : 88–111.

Cooper, S. J., C. H. Watts, K. M. Saint, & R. Leijs. 2014. Phylogenetic relationships of Australian Scirtidae (Coleoptera) based on mitochondrial and nuclear sequences. Invertebrate Systematics. 28(6) : 628-642.

Cruaud, A., N. Rønsted, B. Chantarasuwan, L. S. Chou, W. L. Clement, A. Couloud, A. Couloud, B. Cousins, G. Genson, R. D. Harrison, P. E. Hanson, M. Hossaert-Mckey, R. Jabbour-Zahab, E. Jousselin, C. Kerdelhué, F. Kjellberg, C. Lopez-Vaamonde, J. Peebles, Y. Q. Peng, R. A. Santinelo Pereira, T. Schramm, R. Ubaidillah, S. van Noort, G. D. Weiblen, D. R. Yang, A.Yodpinyanee, R. Libeskind-Hadas, J. M. Cook, J. Y. Rasplus, & V. Savolainen. 2012. An extreme case of plant-insect codiversification : figs and figs-pollinating wasps. Systematic Biology. 61(6) : 1029–1047.

Davison, A., R. L. Blackie, & G. P. Scothern. 2009. DNA barcoding of stylommatophoran land snails : a test of existing sequences. Molecular Ecology Resources. 9(4) : 1092-1101.

Del Guercio, G. 1909. Intorno a due nuovi generi e a tre specie nuove di afidi di California. Rivista di Patologia Vegetale. 3 : 328-332.

Depa, Ł., E. Mróz, & K. Szawaryn. 2012. Molecular identity of *Stomaphis quercus* (Hemiptera : Aphidoidea : Lachnidae) and description of a new species. European Journal of Entomology. 109 : 435-444.

De Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation : A conceptual unification and terminological recommendations. Pages 57-75 in Endless forms : Species and speciation (D. J. Howard, and S. H. Berlocher, eds.). Oxford University Press, New York.

De Queiroz K. 2005a. Ernst Mayr and the modern concept of species. Proceedings of the National Academy of Sciences of the United States of America. 102 : 6600-6607.

De Queiroz K. 2005b. A unified concept of species and its consequences for the future of taxonomy. Proceedings of the California Academy of Sciences. 56 : 196-215.

De Queiroz K. 2005c. Different species problems and their resolution. BioEssays. 27 : 1263-1269.

De Queiroz K. 2007. Species concept and species delimitation. Systematic Biology. 56(6) : 879-886.

De Vienne, D. M., G. Refrégier, M. López-Villavicencio, A. Tellier, M. E. Hood, & T. Giraud. 2013. Cospeciation vs host-shift speciation : methods for testing, evidence from natural associations and relation to coevolution. New Phytologist. 198(2) : 347-385.

Dixon, A. F. G. 1998. Aphid ecology : an optimization approach. London : Chapman & Hall.

Dieni, A., J. Brodeur, & J. Turgeon. 2016. Reconstructing the invasion history of the lily leaf beetle, *Lilioceris lilii*, in North America. Biological Invasions. 18 : 31-44.

Djernæs, M., & J. Damgaard. 2006. Exon-intron structure, paralogy and sequenced regions of elongation factor-1 alpha in Hexapoda. Arthropods Systematics & Phylogeny. 64(1) : 45-52.

Dobzhansky T. 1976. Organismic and molecular aspects of species formation. In : Ayala FJ, editor. Molecular Evolution. Sunderland, MA : Sinauer Associates. 95–105.

Downey, M. H., & C. C. Nice. 2011. Experimental evidence of host race formation in *Mitoura* butterflies (Lepidoptera : Lycaenidae). Oikos. 120(8) : 1165-1174.

Doyen, J. T., & C. N. Slobodchikoff. 1974. An operational approach to species classification. Systematic Biology. 23(2) : 239-247.

Drummond, A. J., M. A. Suchard, D. Xie, & A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution. 29(8) : 1969–1973.

Dumas, P., J. Barbut, B. Le Ru, J. F. Silvain, A. L. Clamens, E. d'Alençon, & G. J. Kergoat. 2015. Phylogenetic molecular species delimitations unravel potential new species in the pest genus *Spodoptera* Guenée, 1852 (Lepidoptera, Noctuidae). PLoS ONE. 10(4) : e0122407.

Earle, J. C. 2015. The Gymnosperm database. http://conifers.org/pi/Pinus.php (accessed 1st November 2017).

Eastop, V. F. 1977. Worldwide importance of aphids as virus vectors, pp. 3-62. In K.F. Harris and K. Maramorosch (eds.), Aphids as virus vectors. Academic Press, London.

Ebert, T. A., & B. Cartwright. 1997. Biology and ecology of *Aphis gossypii* Glover (Homoptera : aphididae). Southwestern Entomologist. 22(1) : 116-153.

Ehrlich, P. R., & P. H. Raven. 1964. Butterflies and plants: A study in coevolution. Evolution. 18(4) : 586-608.

Ereshefsky, M. 2011. Mystery of mysteries: Darwin and the species problem. Cladistics. 27(1) : 67-79.

Essig, E. O. 1909. Aphididae of southern California I. Pomona College Journal of Entomology. 11-10.

Eyles, A., D. Smith, E. A. Pinkard, I. Smith, R. Corkrey, S. Elms, C. Beadle, & C. Mohammed. 2011. Photosynthetic responses of field-grown *Pinus radiata* trees to artificial and aphid-induced defoliation. Tree physiology. 31(6) : 592-603.

Farjon, A. 2005. Pines: drawings and descriptions of the genus *Pinus* (No. Ed. 2). Brill Academic Publishers.

Favret, C., & D. J. Voegtlin. 2004a. Host-based morphometric differentiation in three *Cinara* species (Insecta : Hemiptera : Aphididae) feeding on *Pinus edulis* and *P. monophylla*. Western North American Naturalist. 64(3) : 364–375.

Favret, C., & D. J. Voegtlin. 2004b. Speciation by host-switching in pinyon *Cinara* (Insecta : Hemiptera : Aphididae). Molecular Phylogenetics and Evolution. 32 : 139-151.

Favret, C. 2005. A new non-destructive DNA extraction and specimen clearing technique for aphids (Hemiptera). Proceedings of the Entomological Society of Washington. 107 : 469–470.

Favret, C. 2009. Wing morphometry helps diagnose cryptic species and resurrect *Mindarus pinicolus* (Hemiptera : Aphididae). Annals of the Entomological Society of America. 102(6) : 970–981.

Favret, C. 2017. Aphid species file. Version, 5 (5.0). http://Aphid.SpeciesFile.org. (accessed 1st November 2017).

Febvay G., Y. Rahbé, M. Rynkiewicz, J. Guillaud, & G. Bonnot. 1999. Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, *Acyrthosyphon pisum,* reared on different diets. The Journal of Experimental Biology. 202 : 2639–2652.

Flynn, A. R., D. A. J. Teulon, & M. A. W. Stufkens. 2003. Distribution and flight activity of the Monterey pine aphid in New Zealand. New Zealand Plant Protection. 56 : 33-38.

Floyd, R., E. Abebe, A. Papert, & M. Blaxter. 2002. Molecular barcodes for soil nematode identification. Molecular ecology. 11(4) : 839-850.

Foottit, R. G., S. E. Halbert, G. L. Miller, E. Maw, & L. M. Russell. 2006. Adventive aphids (Hemiptera : Aphididae) of America North of Mexico. Proceedings-Entomological Society of Washington. 108(3) : 583–610.

Foottit, R. G., H. E. L Maw, & K. S. Pike. 2009. DNA barcodes to explore diversity in aphids (Hemiptera : Aphididae and Adelgidae). Redia. 92 : 87–91.

Freudenstein, J. V., M. B. Broe, R. A. Folk, & B. T. Sinn. 2017. Biodiversity and the Species Concept—Lineages are not Enough. Systematic biology. 66(4) : 644-656.

Fujisawa, T., & T. G. Barraclough. 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach : a revised method and evaluation on simulated data sets. Systematic Biology. 62(5) : 707–724.

Fujita, M. K., A. D. Leaché, F. T. Burbrink, J. A. McGuire, & C. Moritz. 2012. Coalescent-based species delimitation in an integrative taxonomy. Trends in Ecology & Evolution. 27(9) : 480–488.

Gibbs, J. 2018. DNA barcoding a nightmare taxon: assessing barcode index numbers and barcode gaps for sweat bees. Genome. 61 : 21-31.

Gillette, C. P., & M. A. Palmer. 1924. New Colorado Lachnini. Annals of the Entomological Society of America. 17(1) : 1-57.

Hajibabaei, M., D. H. Janzen, J. M. Burns, W. Hallwachs, & P. D. N. Hebert. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America. 103 : 968–971.

Hall, T. A. 1999. BioEdit : a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symposium Series. 41 : 95–98.

Hausdorf, B. 2011. Progress toward a general species concept. Evolution. 65(4) : 923-931.

Hebert, P. D. N., A. Cywinska, S. L. Ball, & J. R. de Waard. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B. 270(1512) : 313–321.

Hebert, P. D. N., S. Ratnasingham, & J. R. de Waard. 2003b. Barcoding animal life : cytochrome *c* oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B : Biological Sciences. 270(Suppl 1) : S96–S99.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen, & W. Hallwachs. 2004. Ten species in one : DNA barcoding reveals cryptic species in the neotropical skipper butterfly

Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America. 101(41) : 14812-14817.

Heie, O. E., 1986. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. III. Family Aphididae : subfamily Pterocommatinae & tribe Aphidini of subfamily Aphidinae. E.J. Brill/Scandinavian Science Press Ltd., Leiden.

Hembry, D. H., J. B. Yoder, & K. R. Goodman. 2014. Coevolution and the Diversification of Life. The American Naturalist. 184(4) : 425-438.

Herre, E. A., C. A. Machado, E. Bermingham, J. D. Nason, D. M. Windsor, S. S. McCafferty, & K. Bachmann. 1996. Molecular phylogenies of figs and their pollinator wasps. Journal of Biogeography. 23(4) : 521-530.

Hertach, T., Trilar, T., Wade, E. J., Simon, C., & Nagel, P. 2015. Songs, genetics, and morphology : revealing the taxonomic units in the European *Cicadetta cerdaniensis* cicada group, with a description of new taxa (Hemiptera : Cicadidae). Zoological Journal of the Linnean Society. 173(2) : 320-351.

Hille Ris Lambers, D. 1966a. Polymorphism in Aphididae. Annual Review of Entomology. 11(1) : 47–78.

Hille Ris Lambers, D. 1966b. New and little known Aphids from Pakistan (Homoptera, Aphididae). Tijdschrift voor Entomologie. 109 : 193–220.

Hohenegger, J. 2014. Species as the basic units in evolution and biodiversity : Recognition of species in the Recent and geological past as exemplified by larger foraminifera. Gondwana Research. 5(2) : 707-728.

Hook, E. J., & G. W. Griffiths. 1980. Intracellular symbiotes of the Homoptera. Annual Review of Entomology. 25 : 161-87.

Hottes, F. C. 1957. A synopsis of the genus *Essigella* (Aphidae). Proceedings of the Biological Society of Washington. 70 : 69-109.

Hottes, F. C. 1958. A new species of *Essigella* from Oregon (Aphidae). Proceedings of the Biological Society of Washington. 71 : 155-156.

Hu, L., Y. Yang, Y. Zhao, D. Niu, R. Yang, R. Wang., *et al.* 2017. DNA barcoding for molecular identification of *Demodex* based on mitochondrial genes. Parasitology research, 116(12) : 3285-3290.

Huelsenbeck, J. P. & F. Ronquist. 2001. MRBAYES : Bayesian inference of phylogeny. Bioinformatics.17 : 754–755.

ICZN. 1999. International Code of Zoological Nomenclature. 4th ed. London, UK: The International Trust for Zoological Nomenclature. 306 pp. Available from: http://iczn.org/iczn/index.jsp (accessed 1st November 2017).

Inbar, M., M. Wink, & D. Wool. 2004. The evolution of host plant manipulation by insects : molecular and ecological evidence from gall-forming aphids on *Pistacia*. Molecular Phylogenetics and Evolution. 32 : 504–511.

Iorgu, I. Ş., E. I. Iorgu, G. Szövényi, & K. M. Orci. 2017. A new, morphologically cryptic bush-cricket discovered on the basis of its song in the Carpathian Mountains (Insecta, Orthoptera, Tettigoniidae). ZooKeys. 680 : 57-72.

Jasso-Martínez, J. M., A. N. Castañeda-Sortibrán, C. Pozo, R. García-Sandoval, B. R. Prado, A. Luis-Martínez, J. Lorente-Bousquets, & R. Rodríguez-Arnaiz. 2016. The *Enantia jethys* Complex : Insights from *COI* Confirm the Species Complex and Reveal a New Potential Cryptic Species. Southwestern Entomologist. 41(4) : 1005-1020.

Jiang, L., Chen, J., Guo, K., & G. X. Qiao. 2015. Review of the genus *Ceratovacuna* (Hemiptera : Aphididae) with descriptions of five new species from China. Zootaxa. 3986(1) : 35-60.

Johnson, S. N., A. E. Douglas, S. Woodward, & S. E. Hartley. 2003. Microbial impacts on plant-herbivore interactions : the indirect effects of a birch pathogen on a birch aphid. Oecologia. 134(3) : 388–396.

Jorge, L. R., P. Cordeiro-Estrela, L. B. Klaczko, G. R. Moreira, & A. V. Freitas. 2011. Host-plant dependent wing phenotypic variation in the neotropical butterfly *Heliconius erato*. Biological Journal of the Linnean Society. 102(4) : 765-774.

Jousselin, E., Y. Desdevises, & A. Cœur d'acier. 2009. Fine-scale cospeciation between *Brachycaudus* and *Buchnera aphidicola* : bacterial genome helps define species and evolutionary relationships in aphids. Proceedings of the Royal Society of London B : Biological Sciences. 276 : 187–196.

Jousselin, E., A. Cruaud A., G. Genson, F. Chevenet, R. G. Foottit, & A. Cœur d'acier. 2013. Is ecological speciation a major trend in aphids? Insights from a molecular phylogeny of the conifer-feeding genus *Cinara*. Frontiers in Zoology. 10(1) : 56.

Joy, J. B. 2013. Symbiosis catalyses niche expansion and diversification. Proceedings of the Royal Society B- Biological Sciences. 280(1756) : 20122820.

Kanturski, M., J. Karcz, & K. Wieczorek, 2015. Morphology of the European species of the aphid genus *Eulachnus* (Hemiptera : Aphididae : Lachninae) – a SEM comparative and integrative study. Micron. 76 : 23–36.

Kanturski, M., S. A. Akbar, & C. Favret. 2017a. Morphology and sensilla of the enigmatic Bhutan pine aphid *Pseudessigella brachychaeta* Hille Ris Lambers (Hemiptera : Aphididae) – A SEM study. Zoologischer Anzeiger - A Journal of Comparative Zoology. 266 : 1–13.

Kanturski, M., S. A. Akbar, & C. Favret. 2017b. The Bhutan pine aphid *Pseudessigella brachychaeta* Hille Ris Lambers (Hemiptera : Aphididae : Lachninae) from India reveals the hitherto unknown oviparous female and dwarfish male. Zoological Studies. 56(12).

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies, & A. Drummond. 2012. Geneious Basic : an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28*(*12) : 1647-1649.

Kergoat, G. J., A. S. Meseguer, & E. Jousselin. 2017. Evolution of Plant–Insect Interactions: Insights From Macroevolutionary Approaches in Plants and Herbivorous Insects. In N. Sauvion, D. Thiéry, & P.-A. Calatayud (Eds.), Insect-Plant interactions in a Crop Protection Perspective (pp. 25–53).

Kim, H., & S. Lee. 2008. Molecular systematics of the genus *Megoura* (Hemiptera : Aphididae) using mitochondrial and nuclear DNA sequences. Molecules and Cells. 25(4) : 510-522.

Kimber, W., R. Glatz, G. Caon, & D. Roocke. 2010. *Diaeretus essigellae* Starý and Zuparko (Hymenoptera : Braconidae : Aphidiini), a biological control for Monterey pine aphid, *Essigella californica* (Essig) (Hemiptera: Aphididae : Cinarini) : host-specificity testing and historical context. Australian Journal of Entomology. 49 : 377–387.

Kimber, W., R. Glatz, & S. Shaw. 2013. Introduction of the wasp *Diaeretus essigellae*, for biological control of Monterey Pine Aphid *Essigella californica*, in Australia. Final Report. Forest & Wood Products Australia, Resources. Project Number PNC063-0607. Melbourne. VIC. 72 p.

Kull, K. 2016. The biosemiotic concept of the species. Biosemiotics. 9(1) : 61-71.

Lee, W., H. Kim, J. Lim, H.-R. Choi, Y. Kim, Y.-S. Kim, J.-Y. Ji, R. G. Foottit, & S. Lee. 2011. Barcoding aphids (Hemiptera : Aphididae) of the Korean Peninsula : updating the global data set. Molecular Ecology Resources. 11 : 32–37.

Lee, W., Y. Lee, H. Kim, S. I. Akimoto, & S. Lee. 2014. Developing a new molecular marker for aphid species identification : Evaluation of eleven candidate genes with species-level sampling**.** Journal of Asia-Pacific Entomology. 17 : 617–627.

Lee, Y., W. Lee, S. Lee, & H. Kim. 2015. A cryptic species of *Aphis gossypii* (Hemiptera : Aphididae) complex revealed by genetic divergence and different host plant association. Bulletin of Entomological Research. 105 : 40-51.

Lee, Y., W. Lee, M. Kanturski, R. G. Foottit, S. I. Akimoto, & S. Lee. 2017. Cryptic diversity of the subfamily Calaphidinae (Hemiptera : Aphididae) revealed by comprehensive DNA barcoding. PloS ONE. 12(4) : e0176582.

Legeai, F., S. Shigenobu, J. P. Gauthier, J. Colbourne, C. Rispe, O. Collin, S. Richards, A. C. C. Wilson, & D. Tagu. 2010. Aphidbase : http://www.aphidbase.com: ACYPI006711-RA (accessed 1st November 2017).

Lesage, L. 1995. Revision of the costate species of *Altica* Müller of North America north of Mexico (Coleoptera : Chrysomelidae). The Canadian Entomologist. 127(3) : 295–411.

Lherminier, P. & M. Solignac. 2005. De l'espèce, Paris, Éditions Syllepse. 695 pp.

Li, J., J. Cao, J. Niu, X. Liu, & Q. Zhang. 2015. Identification of the Population Structure of *Myzus persicae* (Hemiptera : Aphididae) on Peach Trees in China Using Microsatellites. Journal of Insect Science. 15(1) : 73.

Lin, Y. P., T. Kondo, P. Gullan, & L. G. Cook. 2013. Delimiting genera of scale insects : molecular and morphological evidence for synonymising *Taiwansaissetia* Tao, Wong and Chang with *Coccus* Linnaeus (Hemiptera : Coccoidea : Coccidae). Systematic Entomology. 38 : 249-264.

Llewellyn, K. S., H. D. Loxdale, R. Harrington, C. P. Brookes, S. J. Clark, & P. Sunnucks. 2003. Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. Molecular Ecology. 12 : 21-34.

Lozier, J. D., G. K. Roderick, & N. J. Mills. 2007. Genetic evidence from mitochondrial, nuclear, and endosymbiont markers for the evolution of host plant associated species in the aphid genus *Hyalopterus* (Hemiptera : Aphididae). Evolution. 6 : 1353–1367.

Lukhtanov, V. A., & A. V. Dantchenko. 2017. A new butterfly species from south Russia revealed through chromosomal and molecular analysis of the *Polyommatus* (*Agrodiaetus*) *damonides* complex (Lepidoptera, Lycaenidae). Comparative Cytogenetics. 11(4) : 769-795.

Machado, C. A., E. Jousselin, F. Kjellberg, S. G. Compton, & E. A. Herre. 2001. Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. Proceedings of the Royal Society of London B: Biological Sciences. 268(1468) : 685-694.

Madeira, P. M., R. M. Chefaoui, R. L. Cunha, F. Moreira, S. Dias, G. Calado, & R. Castilho. 2017. High unexpected genetic diversity of a narrow endemic terrestrial mollusc. PeerJ. 5 : e3069.

Magoga, G., D. Sassi, M. Daccordi, C. Leonardi, M. Mirzaei, R. Regalin, G. Lozzia, & M. Montagna. 2016. Barcoding Chrysomelidae : a resource for taxonomy and biodiversity conservation in the Mediterranean Region. ZooKeys. 597 : 27–38.

Margaritopoulos, J. T., J. A. Tsitsipis, E. Zintzaras, & R. L. Blackman. 2000. Host-correlated morphological variation of *Myzus persicae* (Hemiptera : Aphididae) populations in Greece. Bulletin of Entomological Research. 90(3) : 233–244.

May, B. M. 2004. Assessment of the causality of *Essigella*-ascribed defoliation of mid-rotation radiata pine and its national impact in terms of cost of lost wood production. Forest and Wood Products Research and Development Corporation. p. 43.

May, B. M., & J. C. Carlyle. 2003. Effect of defoliation associated with Essigella californica on growth of mid-rotation *Pinus radiata*. Forest Ecology and Management. 183(1) : 297-312.

Mayden, R. L. 1997. A hierarchy of species concepts: The denouement in the saga of the species problem. Pp. 381–424 in M. F. Claridge, H. A. Dawah, and M. R. Wilson, eds. Species: The units of biodiversity. London: Chapman and Hall.

Mayr, E., 1942. Systematics and the Origin of Species. Columbia University Press, New York.

Medina, R. F., Armstrong, S. J., & K. Harrison. 2017. Genetic population structure of sugarcane aphid, *Melanaphis sacchari*, in sorghum, sugarcane, and Johnsongrass in the continental USA. Entomologia Experimentalis et Applicata. 162(3) : 358-365.

Mifsud, D., N. Pérez Hidalgo, & S. Barbagallo. 2009. Aphids (Hemiptera : Aphidoidea) associated with native trees in Malta (Central Mediterranean). Bulletin of the Entomological Society of Malta. 2 : 81-93.

Miller, G. L., C. Favret, A. Carmichael, & D. J. Voegtlin. 2009. Is there a cryptic species within *Aulacorthum solani* (Hemiptera : Aphididae) ? Journal of Economic Entomology. 102(1) : 398–400.

Miller, M.A., W. Pfeiffer, & T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, pp 1-8. In Proceedings, Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA.

Mitter, C., & D. R. Brooks. 1983. Phylogenetic aspects of coevolution. DJ Futuyma, M. Slatkin (Eds.), Coevolution, Sinauer Publications, Sunderland, MA. 65–98.

Montagna, M., V. Mereghetti, V. Lencioni, & B. Rossaro. 2016. Integrated taxonomy and DNA barcoding of Alpine midges (Diptera : Chironomidae). PloS ONE. 11(3) : e0149673.

Moran, N. A., M. A. Munson, P. Baumann, & H. Ishikawa. 1993. A molecular clock in endosymbiotic bacteria is calibrated using the insect hosts. Proceedings of the Royal Society of London B : Biological Sciences. 253 : 167–171.

Moran, N. A., M. E. Kaplan, M. J. Gelsey, T. G. Murphy, & E. A. Scholes. 1999. Phylogenetics and evolution of the aphid genus *Uroleucon* based on mitochondrial and nuclear DNA sequences. Systematic Entomology. 24 : 85–93.

Morinière, J., L. Hendrich, M. Balke, A. J. Beermann, T. König, M. Hess, S. Koch, R. Müller, F. Leese, P. D. N. Hebert, A. Hausmann, C. D. Schubart, & G. Haszprunar. 2017. A DNA barcode library for Germany′ s mayflies, stoneflies and caddisflies (Ephemeroptera, Plecoptera & Trichoptera). Molecular Ecology Resources. 17(6) : 1293-1307.

Mróz, E., J. Trela, & Ł. Depa. 2015. Taxonomic analysis of *Lachnus pallipes/longirostris–roboris* complex (Hemiptera, Aphididae, Lachninae), with the redescription of sexual morphs and new synonymy. Zoologischer Anzeiger. 254 : 51-61.

Munson M. A., P. Baumann, & M. G. Kinsey. 1991 *Buchnera* gen. nov. and *Buchnera aphidicola* sp. nov., a Taxon Consisting of the Mycetocyte-Associated, Primary Endosymbionts of Aphids. International Journal of Systematic Bacteriology. 41(4) : 566-568.

Normark, B. B. 1999. Evolution in a putatively ancient asexual aphid lineage : Recombination and rapid karyotype change. Evolution. 53 : 1458–1469.

Nosil, P. 2012. Ecological speciation. Oxford University Press, Oxford.

Nováková, E., V. Hypša, J. Klein, R. G. Foottit, C. D. von Dohlen, & N. A. Moran. 2013. Reconstructing the phylogeny of aphids (Hemiptera : Aphididae) using DNA of the obligate symbiont *Buchnera aphidicola*. Molecular Phylogenetics and Evolution. 68 : 42–54.

Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Ohmart, C. P. 1981. An annotated list of insects associated with *Pinus radiata* D. Don in California. CSIRO Division of Forest Research, Divisional Report. 8 : 1–50.

Ortego, J., & M. Pilar Mier Durante. 2012. Primera cita de *Essigella californica* (Essig, 1909) (Hemiptera : Aphididae) en Argentina. Revista de la Facultad de Ciencias Agrarias Universidad Nacional de Cuyo. 44 (1) : 199-204.

Ouvrard, D., P. Chalise, & D. M. Percy. 2015. Host-plant leaps versus host-plant shuffle : a global survey reveals contrasting patterns in an oligophagous insect group (Hemiptera, Psylloidea). Systematics and Biodiversity. 13 : 434–454.

Packer, L., & L. Ruz. 2016. DNA barcoding the bees (Hymenoptera : Apoidea) of Chile : species discovery in a reasonably well known bee fauna with the description of a new species of *Lonchopria* (Colletidae). Genome. 60(5) : 414–430.

Paris, T. M., S. A. Allan, D. G. Hall, M. G. Hentz, G. Hetesy, & P. A. Stansly. 2016. Host plant affects morphometric variation of *Diaphorina citri* (Hemiptera : Liviidae). PeerJ. 4 : e2663.

Peccoud, J., J. C. Simon, C. Von Dohlen, A. Cœur d'acier, M. Plantegenest, F. Vanlerberghe-Masutti, & E. Jousselin. 2010. Evolutionary history of aphid-plant associations and their role in aphid diversification. Comptes Rendus Biologies. 333(6) : 474–487.

Pellmyr, O. 2003. Yuccas, yucca moths and coevolution : a review. Annals of the Missouri Botanical Garden. 90 : 35-55.

Percy, D. M. 2003. Radiation, diversity and host-plant interactions among island and continental legume feeding Psyllids. Evolution. 57(11) : 2540–2556.

Percy, D. M., R. D. Page, & Q. C. B. Cronk. 2004. Plant-insect interactions : double-dating associated insect and plant lineages reveals asynchronous radiations. Systematic Biology. 53(1) : 120–127.

Petrides, G. A. 1988. A field guide to eastern trees: eastern United States and Canada, including the Midwest. The Peterson field guide series.

Petrides, G. A. 1998. A field guide to western trees: western United States and Canada (Vol. 44). Houghton Mifflin Harcourt.

Piffaretti, J., J. P. Rossi, F. Vanlerberghe-Masutti, G. Genson, A. Cœur d'acier, & E. Jousselin. 2013. Molecular identification and ecological characteristics of two cryptic lineages within a cosmopolitan aphid pest, *Brachycaudus helichrysi* (Hemiptera : Aphididae). Applied Entomology and Zoology. 48 : 155-164.

Pigliucci, M. 2003. Species as family resemblance concepts: the (dis-)solution of the species problem? BioEssays. 25 : 596–602.

Pons, J., T. G. Barraclough, J. Gomez-Zurita, A. Cardoso, D. P. Duran, S. Hazell, S. Kamoun, W. D. Sumlin, & A. P. Vogler. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology. 55(4) : 595–609.

Puillandre N, A. Lambert, S. Brouillet, & G. Achaz. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology. 21(8) : 1864–1877.

Pušić, B. 2017. Species as Individuals: Just another Class View of Species. Organon f. 24(1) : 37-53.

Rabatel, A., G. Febvay, K. Gaget, G. Duport, P. Baa-Puyoulet, P. Sapountzis, N. Bendridi, M. Rey, Y. Rahbé, H. Charles, F. Calevro, & S. Colella. 2013. Tyrosine pathway regulation is host-mediated in the pea aphid symbiosis during late embryonic and early larval development. BMC Genomics. 14(1) : 235.

Rakauskas, R., J. Turčinavičienė, & J. Bašilova. 2011. How many species are there in the subgenus *Bursaphis* (Hemiptera : Sternorrhyncha : Aphididae)? CO-I evidence. European Journal of Entomology. 108(3) : 469–479.

Rambaut, A. 2012. FigTree v1. 4. Molecular evolution, phylogenetics and epidemiology. Edinburgh, UK : University of Edinburgh, Institute of Evolutionary Biology.

Rambaut, A, M. A. Suchard, D. Xie, & A. J. Drummond. 2014. Tracer v1.6. Available from http://tree.bio.ed.ac.uk/software/tracer (accessed 1st November 2017).

Ramírez, W. 1974. Coevolution of *Ficus* and Agaonidae. Annals of the Missouri Botanical Garden, 770-780.

Ratnasingham, S., & P. D. N. Hebert. 2013. A DNA-based registry for all animal species : the Barcode Index Number (BIN) system. PloS ONE. 8(7) : e66213.

Reid, S., R. D. Dransfield, & R. Brightwell. 2015. *Essigella californica* (Aphidoidea, Aphididae), a pine-feeding aphid new to Britain. British Journal of Entomology and Natural History. 28 : 121-124.

Reid, C. A. M., & M. Beatson. 2015. Disentangling a taxonomic nightmare: a revision of the Australian, Indomalayan and Pacific species of *Altica* Geoffroy, 1762 (Coleoptera : Chrysomelidae : Galerucinae). Zootaxa. 3918(4) :503-551.

Renner, S. S. 2016. A return to Linnaeus's focus on diagnosis, not description : the use of DNA characters in the formal naming of species. Systematic biology. 65(6) : 1085-1095.

Richards, R. A. 2010. The Species Problem. Cambridge University Press, Cambridge, UK.

Ronquist, F., & J. P. Huelsenbeck. 2003. MRBAYES 3 : Bayesian phylogenetic inference under mixed models. Bioinformatics. 19 : 1572–1574.

Ronquist, F, M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Hohna, B. Larget, L. Liu, M. A. Suchard, & J. P. Huelsenbeck. 2012. MrBayes 3.2 : efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology. 61 : 539–542.

Rønsted, N., G. D. Weiblen, J. M. Cook, N. Salamin, C. A. Machado, & V. Savolainen. 2005. 60 million years of co-divergence in the fig–wasp symbiosis. Proceedings of the Royal Society of London B : Biological Sciences. 272(1581) : 2593-2599.

Rundle, H. D., & P. Nosil. 2005. Ecological speciation. Ecology Letters. 8 : 336-352.

Sabri, A., P. Leroy, E. Haubruge, T. Hance, I. Frère, J. Destain, & P. Thonart, P. 2011. Isolation, pure culture and characterization of *Serratia symbiotica* sp. nov., the R-type of secondary endosymbiont of the black bean aphid *Aphis fabae*. International journal of systematic and evolutionary microbiology. 61(9) : 2081-2088.

Sasse, J., S. Elms, & P. Kube. 2009. Genetic resistance in *Pinus radiata* to defoliation by the pine aphid *Essigella californica*, Australian Forestry. 72(1) : 25-31.

Savory, F. R., & U. Ramakrishnan, 2015. Cryptic diversity and habitat partitioning in an economically important aphid species complex. Infection, Genetics and Evolution. 30 : 230-237.

Schmidt, S., A. Taeger, J. Morinière, A. Liston, S. M. Blank, K. Kramp, M. Kraus, O. Schmidt, E. Heibo, M. Prous, T. Nyman, T. Malm, & J. Stahlhut. 2017. Identification of sawflies and horntails (Hymenoptera, 'Symphyta') through DNA barcodes : successes and caveats. Molecular Ecology Resources. 17(4) : 670–685.

Schwander, T., L. Henry, & B. J. Crespi. 2011. Molecular evidence for ancient asexuality in *Timema* stick insects. Current Biology. 21 : 1129-1134.

Seco Fernandez, M. V., & M. P. Mier Durante. 1992. Presencia en España del pulgón verde de los pinos americanos : *Essigella* (Hom., Aphididae : Cinarinae). Boletín de la Asociación Española de Entomología. 16 : 255-256.

Simon, S., B. Schierwater, & H. Hadrys. 2010. On the value of Elongation factor-1α for reconstructing pterygote insect phylogeny. Molecular Phylogenetics and Evolution. 54 : 651–656.

Simpson, S. J., G. A. Sword, & N. Lo. 2011. Polyphenism in insects. Current Biology. 21(18) : R738-R749.

Sokal, R. R., & T. J. Crovello. 1970. The biological species concept: a critical evaluation. The American Naturalist. 104(936) : 127-153.

Sorensen, J. T. 1983. Cladistic and phenetic analysis of *Essigella* aphids : systematics and phylogeny in relation to their Pinaceae host plants (Homoptera : Aphididae, Lachninae). Ph.D. dissertation, University of California, Berkeley.

Sorensen, J. T. 1988. Three new species of *Essigella* (Homoptera : Aphididae). Pan-Pacific Entomologist. 64(2) : 115-125.

Sorensen, J. T. 1991. Phylogenetic character responses for shape component variance during the multivariate evolution of Eulachnine aphids : redescription of *Pseudessigella* Hille Ris Lambers (Homoptera : Aphididae : Lachninae). Pan-Pacific Entomologist. 67(1) : 28–54.

Sorensen. J. T. 1994. A revision of the aphid genus *Essigella* (Homoptera : Aphididae : Lachninae) : its ecological associations with, and evolution on, Pinaceae hosts. Pan-Pacific Entomologist. 70(1) : 1-102.

Stamatakis, A. 2014. RAxML version 8 : a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9) : 1312–1313.

Stone, C., G. Melville, A. Carnegie, D. Smith, A. Eyles, & M. Nagel. 2013a. Crown damage by the aphid *Essigella californica* in a *Pinus radiata* plantation in southern New South Wales : causality and related management issues. Australian Forestry. 76(1) : 16-24.

Stone, C., A. Carnegie, G. Melville, D. Smith, & M. Nagel. 2013b. Aerial mapping canopy damage by the aphid *Essigella californica* in a *Pinus radiata* plantation in southern New South Wales : what are the challenges ? Australian Forestry. 76 (2) : 101-109.

Sulaiman, I. M., E. Jacobs, S. Simpson, & K. Kerdahi. 2017. Identification of 18 vector species belonging to Group I, Group II, and Group III 'Dirty 22' species known to contaminate food and spread foodborne pathogens : DNA barcoding study of public health importance. International Journal of Tropical Insect Science. 37(1) : 1–10.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, & S. Kumar. 2013. MEGA6 : Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution. 30 : 2725–2729.

Teulon, D. A. J., T. J. B. Herman, & M. M. Davidson. 2003. Monitoring Monterey pine aphid in Hawke's Bay Forests. New Zealand Plant Protection. 56 : 39-44.

Thompson, J. N. 1989. Concepts of coevolution. Trends in Ecology & Evolution. 4(6) : 179-183.

Thompson, J. D., D. G. Higgins, & T. J. Gibson. 1994. CLUSTAL W : improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 22(22) : 4673-4680.

Thompson, J. N., C. Schwind, P. R. Guimarães, & M. Friberg. 2013. Diversification through multitrait evolution in a coevolving interaction. Proceedings of the National Academy of Sciences. 110(28) : 11487-11492.

Tilmon, K. J. (Ed.). 2008. Specialization, speciation, and radiation : the evolutionary biology of herbivorous insects. Univ of California Press.

Triapitsyn, S. V., V. V. Berezovskiy, & P. S. Tretiakov. 2015. Complex of natural enemies of *Essigella californica* (Essig, 1909) (Hemiptera : Aphididae, Lachninae) in Riverside, California, USA. Boletín de la Asociación Española de Entomología. 39(1/2) : 175-183.

Tsuchida, T, R. Koga, M. Horikawa, T. Tsunoda, T. Maoka, S. Matsumoto, J. C. Simon, & T. Fukatsu. 2010. Symbiotic bacterium modifies aphid body color. Science. 330(6007) : 1102–1104.

Turcotte, M. M., T. J. Davies, C. J. Thomsen, & M. T. Johnson. 2014. Macroecological and macroevolutionary patterns of leaf herbivory across vascular plants. Proceedings of the Royal Society of London B : Biological Sciences. 281(1787) : 20140555.

Turpeau, E., & G. Remaudière. 1990. Découverte en France d'un puceron des pins américains du genre *Essigella* (Hom. *Aphididae*). Comptes rendus de l'Academie d'Agriculture de France. 76(8) : 131-132.

Vogler, A. P., & M. T. Monaghan. 2007. Recent advances in DNA taxonomy. Journal of Zoological Systematics and Evolutionary Research. 45 : 1–10.

Von Dohlen, C. D., & N. A. Moran. 2000. Molecular data support a rapid radiation of aphids in the Cretaceous and multiple origins of host alternation. Biological Journal of the Linnean Society. 71(4) : 689–717.

Von Dohlen, C. D., C. A. Rowe, & O. E. Heie. 2006. A test of morphological hypotheses for tribal and subtribal relationships of Aphidinae (Insecta : Hemiptera : Aphididae) using DNA sequences. Molecular Phylogenetics and Evolution. 38 : 316–329.

Wake, D. B. 2013. The enigmatic history of the European, Asian and American plethodontid salamanders. Amphibia-Reptilia. 34(3) : 323-336.

Watson, M., & C. Appleton. 2007. Monterey pine aphid (*Essigella californica*). Forest Pathology in New Zealand. 65. (http://www.nzffa.org.nz/farm-forestry-model/the-essentials/forest-health-pests-and-diseases/Pests/Essigella-californica/monterey-pine-aphid-essigella-californica Accessed on 15 June 2016).

Watson, M. C., D. J. Kriticos, G. M. Drayton, D. A. J. Teulon, & E. G. Brockerhoff. 2008. Assessing the effect of *Essigella californica* on *Pinus radiata* at two sites in New Zealand. New Zealand Plant Protection. 61 : 179-184.

Weisser, W. W., C. Braendle, & N. Minoretti. 1999. Predator-induced morphological shift in the pea aphid. Proceedings of the Royal Society of London B : Biological Sciences. 266(1424) : 1175–1181.

Wharton, T., P. Cooper, & R. Floyd. 2004. Life stage development of *Essigella californica* (Aphidoidea : Lachnidae : Cinarinae). Annals of the Entomological Society of America. 97(4) : 697-700.

Wharton, T. N., & D. J. Kriticos. 2004. The fundamental and realized niche of the Monterey Pine aphid, *Essigella californica* (Essig) (Hemiptera : Aphididae) : implications for managing softwood plantations in Australia. Diversity and Distributions. 10(4) : 253-262.

Wiens, J. J. 2007. Species Delimitation : New Approaches for Discovering Diversity. Systematic Biology. 56 (6) : 875–87.

Wilson, H. F. 1919. Three new lachnids with comparative notes on three others (Homop.). Entomological News. 30(1) : 1-7.

Wilson J. S., M. L. Forister, L. A. Dyer, J. M. O'Connor, K. Burls, C. R. Feldman, M. A. Jaramillo, J. S. Miller, G. Rodríguez-Castañeda, E. J. Tepe, J. B. Whitfield, & B. Young. 2012. Host conservatism, host shifts and diversification across three trophic levels in two Neotropical forests. Journal of Evolutionary Biology. 25(3) : 532-546.

Wittgenstein, L. 1953. Philosophical Investigations. New York, NY: Macmillan.

Wongsa, K., Duangphakdee, O., & A. Rattanawannee. 2017. Genetic Structure of the *Aphis craccivora* (Hemiptera : Aphididae) From Thailand Inferred From Mitochondrial *COI* Gene Sequence. Journal of Insect Science. 17(4) : 84.

Wool, D., & D. F. Hales. 1997. Phenotypic plasticity in Australian cotton aphid (Homoptera : Aphididae) : host-plant effects on morphological variation. Annals of the Entomological Society of America. 90(3) : 316–328.

Yao, I. 2012. Ant attendance reduces flight muscle and wing size in the aphid *Tuberculatus quercicola*. Biology Letters. 8 : 624–627.

Yoder, J. B., & S. L. Nuismer. 2010. When does coevolution promote diversification ? The American Naturalist. 176(6) : 802-817.

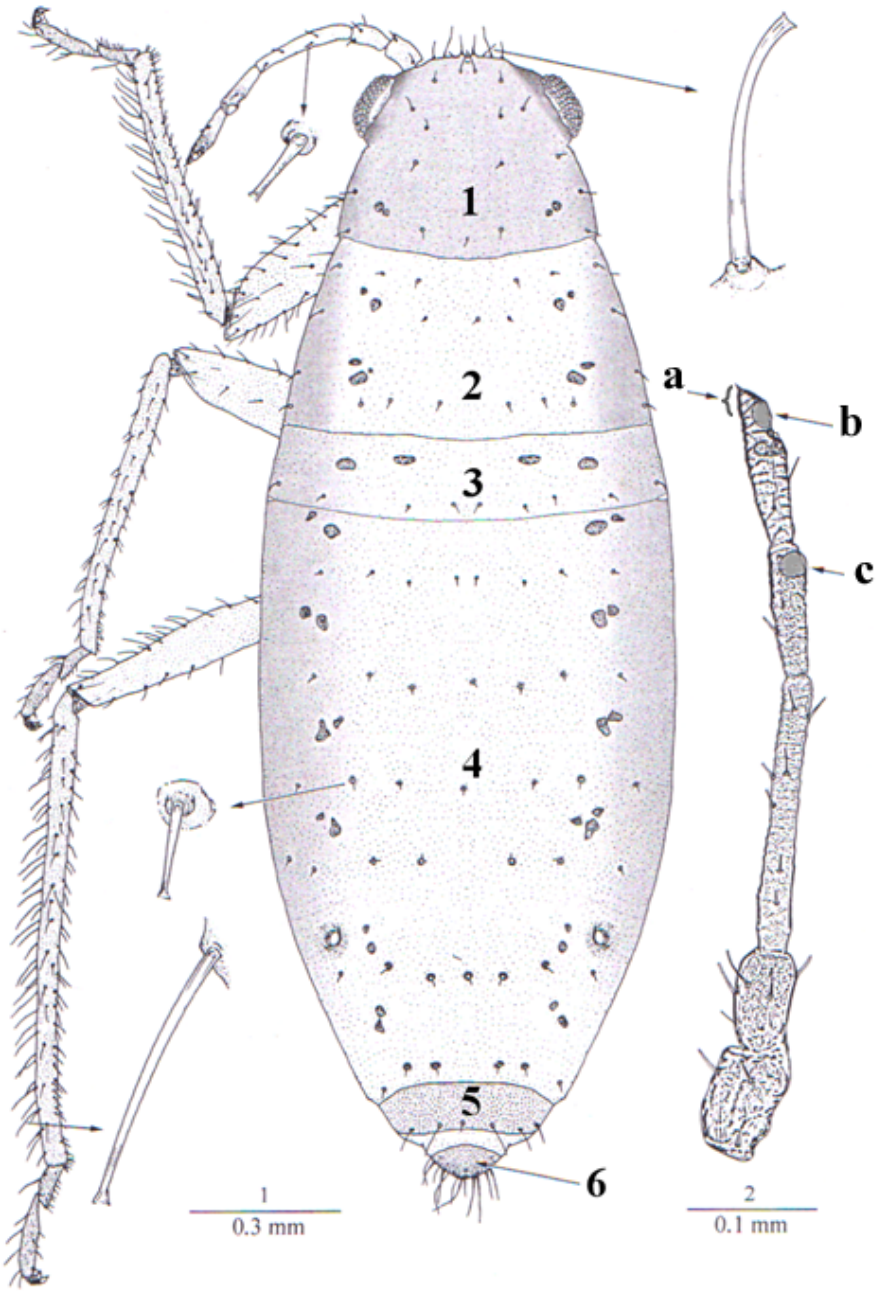
Zhang, J., P. Kapli, P. Pavlidis, & A. Stamatakis 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics. 29(22) : 2869–2876.

Zhang, T., Y. J. Wang, W. Guo, D. Luo, Y. Wu, Z. Kučerová, V. Stejskal, G. Opit, Y. Cao, F. J. Li, & Z. H. Li. 2016. DNA barcoding, species-specific PCR and real-time PCR techniques for the identification of six *Tribolium* pests of stored products. Scientific Reports. 6 : 28494.

Zhu, X. C., J. Chen, R. Chen, L. Y. Jiang, & G. X. Qiao. 2017. DNA barcoding and species delimitation of Chaitophorinae (Hemiptera, Aphididae). ZooKeys. 656 : 25–50.

Zurovcová, M., J. A. N. Havelka, P. Starý, P. Vechtová, D. Chundelová, A. Jarosová, & L. Kucerová. 2010. "DNA barcoding" is of limited value for identifying adelgids (Hemiptera : Adelgidae) but supports traditional morphological taxonomy. European Journal of. Entomology. 107(2) : 147–156.

Annexe 1. Morphologie d'*Essigella californica*



Essigella californica (Essig), habitus avec détails de l'antenne et des soies. 1. fusion tête/pronotum; 2. fusion mesonotum/metanotum; 3. tergite abdominal I; 4. tergites abdominaux II-VII; 5. tergite abdominal VIII; 6. Cauda; a. processus terminalis; b. rhinarium primaire de l'antennomère V; c. rhinarium primaire de l'antennomère IV (d'après Barbagallo *et al.*, modifié).

Annexe 2. *COI* distance results

E. essigi		***E. fusca voegtlini***	***E. hillerisi-lambersi***	***E. hoerneri***	***E. kathleenae***	***E. kirki***	***E. k. braggi***	***E. k. knowltoni***	***E. pini***		***E. wilsoni***	
P. attenuata	*P. radiata*								*P. rigida*	*P. strobus*	*Ps. macrocarpa*	*Ps. menziesii*
0,000												
0,015	0,000											
0,026 - 0,034	0,030 - 0,031	0 - 0,014										
0,028 - 0,030	0,028 - 0,030	0,023 - 0,026	0 - 0,002									
0,039	0,033	0,042 - 0,044	0,039 - 0,041	0,000								
0,041 - 0,044	0,038 - 0,041	0,046 - 0,051	0,041 - 0,042	0,049	0 - 0,009							
0,047	0,047	0,057 - 0,069	0,052 - 0,054	0,056	0,059 - 0,061	0,000						
0,035 - 0,036	0,033 - 0,035	0,039 -0,043	0,036 - 0,039	0,041 - 0,046	0,049 - 0,052	0,049 - 0,051	0 - 0,006					
0,039	0,030	0,039 -0,044	0,039 - 0,041	0,044	0,047 - 0,051	0,051	0,005 - 0,008	0,000				
0,033	0,026	0,029 - 0,034	0,033 - 0,034	0,037	0,042 - 0,046	0,049	0,029 - 0,033	0,028	0,000			
0,044	0,038	0,047 - 0,049	0,044 - 0,046	0,036	0,054 - 0,057	0,055	0,051 - 0,054	0,046	0,041	0,000		
0,039 - 0,041	0,033 - 0,035	0,039 - 0,046	0,039 - 0,043	0,044 - 0,046	0,047 - 0,049	0,056 - 0,057	0,038 - 0,041	0,034 - 0,036	0,036 - 0,038	0,041 - 0,042	0 - 0,002	
0,038 - 0,043	0,031 - 0,039	0,034 - 0,046	0,034 - 0,044	0,042 - 0,049	0,047 - 0,052	0,056	0,036 - 0,039	0,033 - 0,038	0,036 - 0,039	0,044 - 0,049	0,009 - 0,015	0 - 0,011

		Cinara sp.	*Eulachnus* sp.	*Pseudessigella brachychaeta*	*E. alyeska*	*E. californica*					*E. critchf.*	*E. eastopi*
						Pinus spp.	*P. contorta*	*P. muricata*	*P. monticola*	*P. albicaulis*		
***Cinara* sp.**		0,000										
***Eulachnus* sp.**		0,133	0,000									
Pseudessigella brachychaeta		0,087	0,084	0,000								
E. alyeska		0,102	0,089	0,055	0,000							
E. californica	*Pinus* spp.	0,099 - 0,102	0,087 - 0,090	0,054 - 0,057	0,046 - 0,049	0 - 0,003						
	P. contorta	0,101	0,087	0,052	0,046	0,09 - 0,012	0,000					
	P. muricata	0,096 - 0,097	0,087 - 0,089	0,049 - 0,050	0,044 - 0,046	0,011 - 0,015	0,011 - 0,012	0 - 0,002				
	P. monticola	0,101 - 0,102	0,094 - 0,097	0,060 - 0,062	0,052 - 0,054	0,019 - 0,023	0,019 - 0,020	0,012 - 0,015	0 - 0,003			
	P. albicaulis	0,104	0,103	0,063	0,052	0,028 - 0,031	0,028	0,022 - 0,023	0,020	0,000		
E. critchfieldi		0,104	0,101	0,065	0,051	0,051 - 0,054	0,051	0,046 - 0,047	0,052 - 0,054	0,064	0,000	
E. eastopi		0,090 - 0,097	0,073 - 0,078	0,042 - 0,047	0,031 - 0,036	0,031 - 0,039	0,031 - 0,034	0,028 - 0,033	0,037 - 0,044	0,044 - 0,049	0,038 - 0,046	0 - 0,015
E. essigi	*P. attenuata*	0,092	0,084	0,049	0,041	0,034 - 0,038	0,034	0,033 - 0,034	0,041 - 0,042	0,041	0,041	0,025 - 0,030
	P. radiata	0,085	0,082	0,044	0,041	0,034 - 0,038	0,031	0,030 - 0,031	0,038 - 0,039	0,047	0,038	0,019 - 0,026
E. fusca voegtlini		0,092 - 0,096	0,080 - 0,085	0,050 - 0,054	0,047 - 0,049	0,039 - 0,044	0,0391 - 0,041	0,036 - 0,039	0,046 - 0,049	0,054 - 0,056	0,047 - 0,049	0,025 - 0,031
E. hillerislambersi		0,089 - 0,090	0,085	0,047	0,044	0,031 - 0,036	0,034 - 0,036	0,030- 0,033	0,038 - 0,041	0,047 - 0,049	0,044 - 0,046	0,022 - 0,028
E. hoerneri		0,099	0,090	0,049	0,046	0,026 - 0,030	0,026	0,019 - 0,020	0,030 - 0,031	0,036	0,049	0,033 - 0,038
E. kathleenae		0,099 - 0,104	0,099	0,065 - 0,067	0,056 - 0,059	0,047 - 0,051	0,047	0,042 - 0,044	0,049 - 0,051	0,060	0,051	0,038 - 0,046
E. kirki		0,109	0,103	0,070	0,059	0,051 - 0,054	0,054	0,049 - 0,051	0,047 - 0,049	0,059	0,061	0,049 - 0,054
E. knowltoni braggi		0,096 - 0,101	0,090 - 0,092	0,057 - 0,059	0,044 - 0,047	0,042 - 0,047	0,042 - 0,044	0,034 - 0,04114	0,042 - 0,049	0,047 - 0,051	0,044 - 0,048	0,030 - 0,036
E. k.nowltoni knowltoni		0,096	0,090	0,057	0,043	0,042 - 0,046	0,042	0,038 - 0,039	0,049- 0,051	0,052	0,046	0,026 - 0,034
E. pini	*P. rigida*	0,089	0,085	0,044	0,042	0,036 - 0,039	0,036	0,031 - 0,033	0,039 - 0,042	0,047	0,044	0,026 - 0,031
	P. strobus	0,095	0,097	0,058	0,054	0,033 - 0,034	0,033	0,030 -0,031	0,039 - 0,041	0,046	0,054	0,034 - 0,041
E. wilsoni	*Ps. macrocarapa*	0,103 - 0,104	0,087 - 0,089	0,059 - 0,060	0,046 - 0,047	0,042 - 0,047	0,039 - 0,041	0,041 - 0,044	0,047 - 0,051	0,054 - 0,056	0,049 - 0,051	0,025 - 0,036
	Ps. menziesii	0,101 - 0,104	0,087 - 0,090	0,059 - 0,065	0,044 - 0,052	0,041 - 0,047	0,041	0,039 - 0,044	0,047 - 0,049	0,054 - 0,057	0,051 - 0,053	0,025 - 0,038

Annexe 3. OTUs and MOTUs according to morphology, 2% threshold *COI* and ABGD

Morphology (Sorensen, 1994)	Barcoding (Hebert *et al.*, 2003a, b)	ABGD (Puillandre *et al.*, 2012)		
		ATP6	*COI*	*Gnd*
OTU 1 *E. alyeska* (QMOR50670) **OTU 2** *E. californica* (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673, QMOR50674, QMOR50675, QMOR50676, QMOR50677, QMOR50678, QMOR50679) **OTU 3** *E. critchfieldi* (QMOR50680) **OTU 4** *E. eastopi* (QMOR50044, QMOR50681, QMOR50682, QMOR50683, QMOR50684, QMOR50685, QMOR50686) **OTU 5** *E. essigi* (QMOR50687, QMOR50688) **OTU 6** *E. fusca voegtlini* (QMOR50689, QMOR50690, QMOR50691) **OTU 7** *E. hillerislambersi* (QMOR50692, QMOR50693, QMOR50694) **OTU 8** *E. hoerneri* (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698) **OTU 9** *E. kathleenae* (QMOR50699, QMOR50700) **OTU 10** E. kirki (QMOR50701) **OTU 11** *E. knowltoni braggi* (QMOR50702, QMOR50703, QMOR50704) *E. knowltoni knowltoni* (QMOR50705, QMOR50706) **OTU 12** *E. pini* (QMOR50045, QMOR50707) **OTU 13** *E. wilsoni* (QMOR50708, QMOR50709, QMOR50710, QMOR50711)	**MOTU 1** *E. alyeska* (QMOR50670) **MOTU 2** *E. californica* (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673, QMOR50674, QMOR50675, QMOR50679) **MOTU 3** *E. californica* (QMOR50676, QMOR50677) **MOTU 4** *E. californica* (QMOR50678) **MOTU 5** *E. critchfieldi* (QMOR50680) **MOTU 6** *E. eastopi* (QMOR50044, QMOR50681, (QMOR50682, QMOR50683, QMOR50684, QMOR50685, QMOR50686) **MOTU 7** *E. essigi* (QMOR50687, QMOR50688) **MOTU 8** *E. fusca voegtlini* (QMOR50689, QMOR50690, QMOR50691) **MOTU 9** *E. hillerislambersi* (QMOR50692, QMOR50693, QMOR50694) **MOTU 10** *E. hoerneri* (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698) **MOTU 11** *E. kathleenae* (QMOR50699, QMOR50700) **MOTU 12** *E. kirki* (QMOR50701) **MOTU 13** *E. knowltoni braggi* (QMOR50702, QMOR50703, QMOR50704) *E. knowltoni knowltoni* (QMOR50705, QMOR50706) **MOTU 14** *E. pini* (QMOR50045) **MOTU 15** *E. pini* (QMOR50707) **MOTU 16** *E. wilsoni* (QMOR50708, QMOR50709, QMOR50710, QMOR50711)	**MOTU 1** *E. alyeska* (QMOR50670) **MOTU 2** *E. californica* (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673,QMOR50679) **MOTU 3** *E. californica* (QMOR50674, QMOR50675) **MOTU 4** *E. californica* (QMOR50676, QMOR50677) **MOTU 5** *E. californica* (QMOR50678) **MOTU 6** *E. critchfieldi* (QMOR50680) **MOTU 7** *E. eastopi* (QMOR50044, QMOR50681, (QMOR50682, QMOR50683, QMOR50684, QMOR50685, QMOR50686) **MOTU 8** *E. essigi* (QMOR50687, QMOR50688) **MOTU 9** *E. fusca voegtlini* (QMOR50689, QMOR50690, QMOR50691) **MOTU 10** *E. hillerislambersi* (QMOR50692, QMOR50693, QMOR50694) **MOTU 11** *E. hoerneri* (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698) **MOTU 12** *E. kathleenae* (QMOR50699, QMOR50700) **MOTU 13** *E. kirki* (QMOR50701) **MOTU 14** *E. knowltoni braggi* (QMOR50702, QMOR50703, QMOR50704) *E. knowltoni knowltoni* (QMOR50705, QMOR50706) **MOTU 15** *E. pini* (QMOR50045) **MOTU 16** *E. pini* (QMOR50707) **MOTU 17** *E. wilsoni* (QMOR50708, QMOR50709, QMOR50710, QMOR50711)	**MOTU 1** *E. alyeska* (QMOR50670) **MOTU 2** *E. californica* (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673) **MOTU 3** *E. californica* (QMOR50674, QMOR50675) **MOTU 4** *E. californica* (QMOR50676, QMOR50677) **MOTU 5** *E. californica* (QMOR50678) **MOTU 6** *E. californica* (QMOR50679) **MOTU 7** *E. critchfieldi* (QMOR50680) **MOTU 8** *E. eastopi* (QMOR50044, QMOR50681, (QMOR50682, QMOR50683, QMOR50684, QMOR50685, QMOR50686) **MOTU 9** *E. essigi* (QMOR50687, QMOR50688) **MOTU 10** *E. fusca voegtlini* (QMOR50689, QMOR50690, QMOR50691) **MOTU 11** *E. hillerislambersi* (QMOR50692, QMOR50693, QMOR50694) **MOTU 12** *E. hoerneri* (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698) **MOTU 13** *E. kathleenae* (QMOR50699, QMOR50700) **MOTU 14** *E. kirki* (QMOR50701) **MOTU 15** *E. knowltoni braggi* (QMOR50702, QMOR50703, QMOR50704) *E. knowltoni knowltoni* (QMOR50705, QMOR50706) **MOTU 16** *E. pini* (QMOR50045) **MOTU 17** *E. pini* (QMOR50707) **MOTU 18** *E. wilsoni* (QMOR50708, QMOR50709, QMOR50710, QMOR50711)	**MOTU 1** *E. alyeska* (QMOR50670) **MOTU 2** *E. californica* (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673,QMOR50679) **MOTU 3** *E. californica* (QMOR50674, QMOR50675) **MOTU 4** *E. californica* (QMOR50676, QMOR50677, QMOR50678) **MOTU 5** *E. critchfieldi* (QMOR50680) **MOTU 6** *E. eastopi* (QMOR50044, QMOR50681, (QMOR50682, QMOR50683, QMOR50684, QMOR50685, QMOR50686) **MOTU 7** *E. essigi* (QMOR50687, QMOR50688) **MOTU 8** *E. fusca voegtlini* (QMOR50689, QMOR50690, QMOR50691) **MOTU 9** *E. hillerislambersi* (QMOR50692, QMOR50693, QMOR50694) **MOTU 10** *E. hoerneri* (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698) **MOTU 11** *E. kathleenae* (QMOR50699, QMOR50700) **MOTU 12** *E. kirki* (QMOR50701) **MOTU 13** *E. knowltoni braggi* (QMOR50702, QMOR50703, QMOR50704) *E. knowltoni knowltoni* (QMOR50705, QMOR50706) **MOTU 14** *E. pini* (QMOR50045) **MOTU 15** *E. pini* (QMOR50707) **MOTU 16** *E. wilsoni* (QMOR50708, QMOR50709, QMOR50710, QMOR50711)

Annexe 4. MOTUs according to GMYC

GMYC (Pons *et al.*, 2006)			
ATP6	***COI***	***EF-1α***	***Gnd***

MOTU 1
E. alyeska (QMOR50670)
MOTU 2
E. californica (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673, QMOR50674, QMOR50675, QMOR50676, QMOR50677, QMOR50678, QMOR50679)
MOTU 3
E. critchfieldi (QMOR50680)
MOTU 4
E. eastopi (QMOR50044, QMOR50681, (QMOR50682, QMOR50683, QMOR50684, QMOR50685)
MOTU 5
E. eastopi (QMOR50686)
MOTU 6
E. essigi (QMOR50687, QMOR50688)
MOTU 7
E. fusca voegtlin (QMOR50689, QMOR50690, QMOR50691)
MOTU 8
E. hillerislambersi (QMOR50692, QMOR50693, QMOR50694)
MOTU 9
E. hoerneri (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698)
MOTU 10
E. kathleenae (QMOR50699, QMOR50700)
MOTU 11
E. kirki (QMOR50701)
MOTU 12
E. knowltoni braggi (QMOR50702, QMOR50703, QMOR50704)
E. knowltoni knowltoni (QMOR50705, QMOR50706)
MOTU 13
E. pini (QMOR50045)
MOTU 14
E. pini (QMOR50707)
MOTU 15
E. wilsoni (QMOR50708, QMOR50709, QMOR50710, QMOR50711)
	MOTU 1
	E. alyeska (QMOR50670)
	MOTU 2-MOTU 7
	E. californica (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672) // *E. californica* (QMOR50673) // *E. californica* (QMOR50674, QMOR50675) // *E. californica* (QMOR50676, QMOR50677) // *E. californica* (QMOR50678) // *E. californica* (QMOR50679)
	MOTU 8
	E. critchfieldi (QMOR50680)
	MOTU 9 - MOTU 12
	E. eastopi (QMOR50044, QMOR50681) //
	E. eastopi (QMOR50682, QMOR50684) //
	E. eastopi (QMOR50683, QMOR50685) //
	E. eastopi (QMOR50686)
	MOTU 13 - MOTU 14
	E. essigi (QMOR50687) // *E. essigi* (QMOR50688)
	MOTU 15
	E. fusca voegtlini (QMOR50689)
	MOTU 16
	E. fusca voegtlini (QMOR50690, QMOR50691)
	MOTU 17
	E. hillerislambersi (QMOR50692, QMOR50693, QMOR50694)
	MOTU 18
	E. hoerneri (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698)
	MOTU 19 - MOTU 20
	E. kathleenae (QMOR50699) // *E. kathleenae* (QMOR50700)
	MOTU 21
	E. kirki (QMOR50701)
	MOTU 22 - MOTU 23
	E. k. braggi (QMOR50702, QMOR50703) // *E. k. braggi* (QMOR50704)
	MOTU 24
	E. k. knowltoni (QMOR50705, QMOR50706)
	MOTU 25 - MOTU 26
	E. pini (QMOR50045) // *E. pini* (QMOR50707)
	MOTU 27 - MOTU 29
	E. wilsoni (QMOR50708, QMOR50709) // *E. wilsoni* (QMOR50710) // *E. wilsoni* (QMOR50711)
		MOTU 1
		E. alyeska (QMOR50670)
		MOTU 2
		E. californica (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673, QMOR50679)
		MOTU 3
		E. californica (QMOR50674, QMOR50675, QMOR50676, QMOR50677, QMOR50678)
		MOTU 4
		E. critchfieldi (QMOR50680)
		MOTU 5
		E. eastopi (QMOR50044, QMOR50681, QMOR50682, QMOR50683, QMOR50684, QMOR50685, QMOR50686)
		MOTU 6
		E. essigi (QMOR50687, QMOR50688)
		MOTU 7
		E. fusca voegtlini (QMOR50689, QMOR50690, QMOR50691)
		MOTU 8
		E. hillerislambersi (QMOR50692, QMOR50693, QMOR50694)
		MOTU 9
		E. hoerneri (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698)
		MOTU 10
		E. kathleenae (QMOR50699, QMOR50700)
		MOTU 11
		E. kirki (QMOR50701)
		MOTU 12
		E. knowltoni braggi (QMOR50702, QMOR50703, QMOR50704)
		MOTU 13
		E. knowltoni knowltoni (QMOR50705, QMOR50706)
		MOTU 14
		E. pini (QMOR50045)
		MOTU 15
		E. pini (QMOR50707)
		MOTU 16
		E. wilsoni (QMOR50708, QMOR50709, QMOR50710, QMOR50711)
			MOTU 1
			E. alyeska (QMOR50670)
			MOTU 2
			E. californica (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673, QMOR50674, QMOR50675, QMOR50679, QMOR50676, QMOR50677, QMOR50678)
			MOTU 3
			E. critchfieldi (QMOR50680)
			MOTU 4
			E. eastopi (QMOR50044, QMOR50681, (QMOR50682, QMOR50683, QMOR50684, QMOR50685, QMOR50686)
			MOTU 5
			E. essigi (QMOR50687, QMOR50688)
			MOTU 6
			E. fusca voegtlini (QMOR50689, QMOR50690, QMOR50691)
			MOTU 7
			E. hillerislambersi (QMOR50692, QMOR50693, QMOR50694)
			MOTU 8
			E. hoerneri (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698)
			MOTU 9
			E. kathleenae (QMOR50699, QMOR50700)
			MOTU 10
			E. kirki (QMOR50701)
			MOTU 11
			E. knowltoni braggi (QMOR50702, QMOR50703, QMOR50704)
			E. knowltoni knowltoni (QMOR50705, QMOR50706)
			MOTU 12
			E. pini (QMOR50045)
			MOTU 13
			E. pini (QMOR50707)
			MOTU 14
			E. wilsoni (QMOR50708, QMOR50709, QMOR50710, QMOR50711)

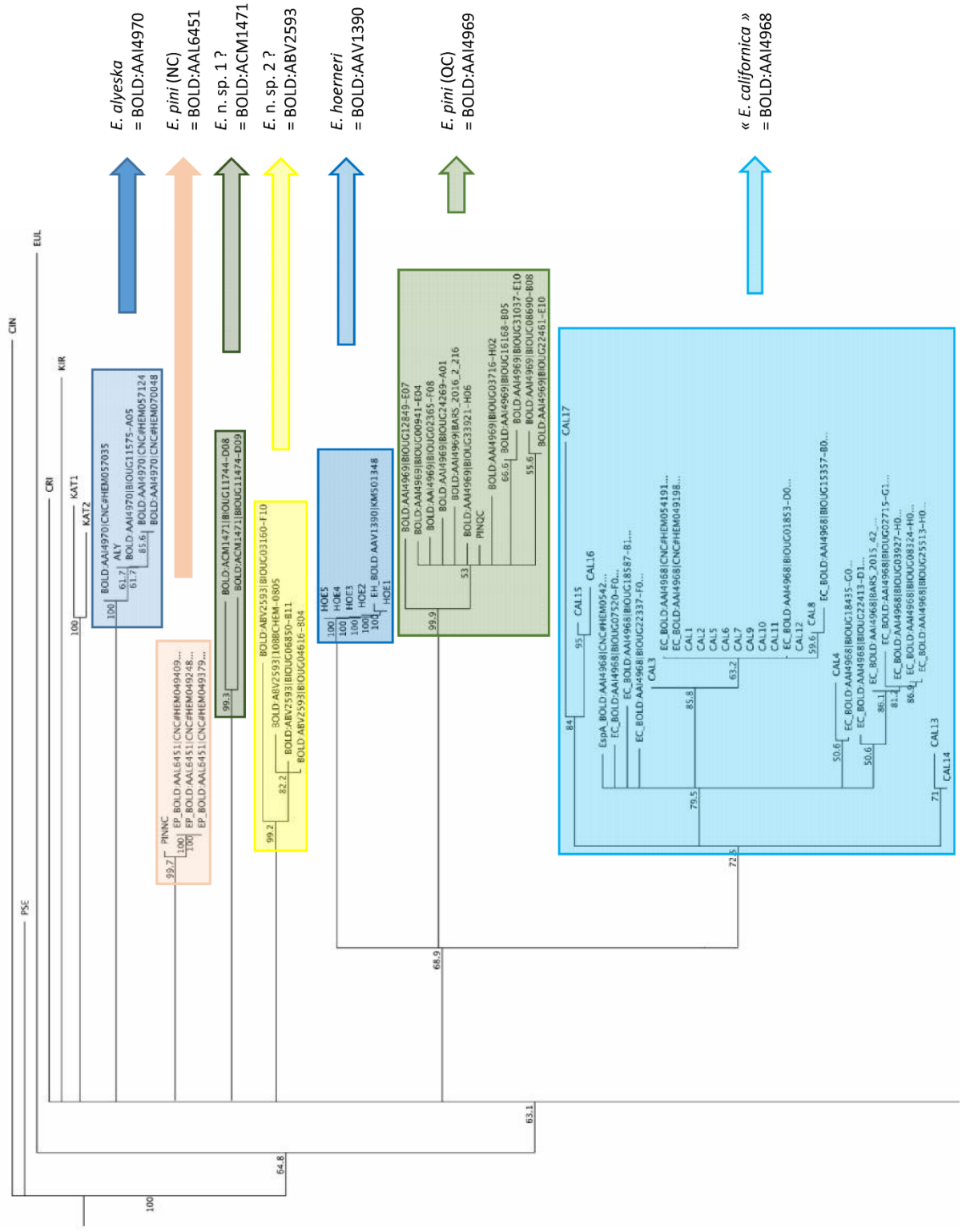
Annexe 5. MOTUs according to bPTP

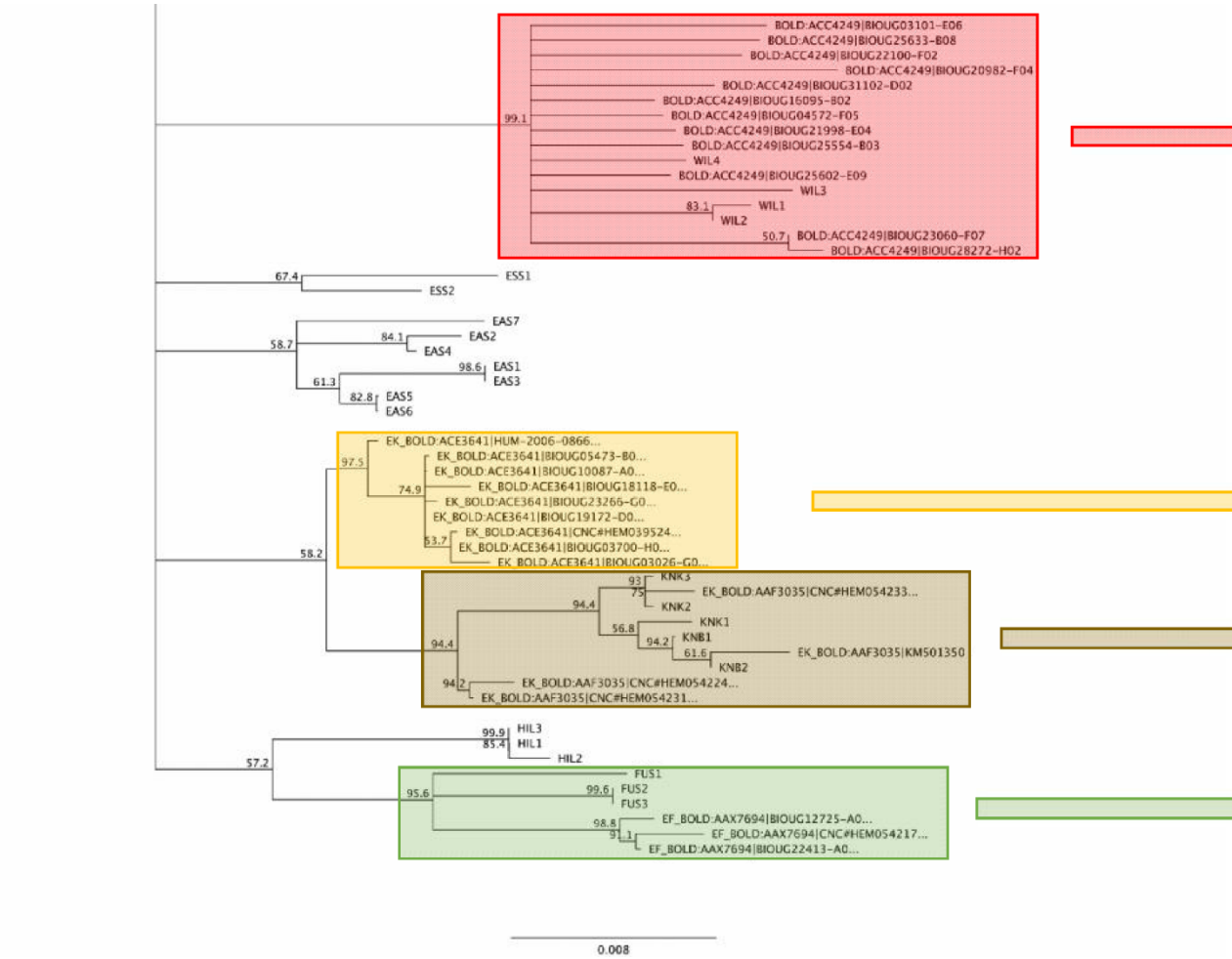
bPTP (Zhang *et al.* , 2013)			
***ATP6* ***	***COI***	***EF-1α*****	***GND***

MOTU 1
E. alyeska (QMOR50670)
MOTU 2-MOTU 5
E. californica (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673, QMOR50679) // *E. californica* (QMOR50674, QMOR50675) // *E. californica* (QMOR50676, QMOR50677) // *E. californica* (QMOR50678)
MOTU 6
E. critchfieldi (QMOR50680)
MOTU 7 - MOTU 10
E. eastopi (QMOR50044, QMOR50681) //
E. eastopi (QMOR50682, QMOR50684) //
E. eastopi (QMOR50683, QMOR50685) //
E. eastopi (QMOR50686)
MOTU 11 - MOTU 12
E. essigi (QMOR50687) // *E. essigi* (QMOR50688)
MOTU 13 - MOTU 14
E. fusca voegtlini (QMOR50689) // *E. fusca voegtlini* (QMOR50690, QMOR50691)
MOTU 15
E. hillerislambersi (QMOR50692, QMOR50693, QMOR50694)
MOTU 16
E. hoerneri (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698)
MOTU 17
E. kathleenae (QMOR50699, QMOR50700)
MOTU 18
E. kirki (QMOR50701)
MOTU 19
E. knowltoni braggi (QMOR50702, QMOR50703, QMOR50704)
E. knowltoni knowltoni (QMOR50705, QMOR50706)
MOTU 20
E. pini (QMOR50045)
MOTU 21
E. pini (QMOR50707)
MOTU 22 - MOTU 25
E. wilsoni (QMOR50708) // *E. wilsoni* (QMOR50709) // *E. wilsoni* (QMOR50710) // *E. wilsoni* (QMOR50711) *
	MOTU 1
	E. alyeska (QMOR50670)
	MOTU 2-MOTU 6
	E. californica (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673) // *E. californica* (QMOR50679) // *E. californica* (QMOR50674, QMOR50675) // *E. californica* (QMOR50676, QMOR50677) // *E. californica* (QMOR50678)
	MOTU 7
	E. critchfieldi (QMOR50680)
	MOTU 8 - MOTU 11
	E. eastopi (QMOR50044, QMOR50681) //
	E. eastopi (QMOR50682, QMOR50684) //
	E. eastopi (QMOR50683, QMOR50685) //
	E. eastopi (QMOR50686)
	MOTU 12 - MOTU 13
	E. essigi (QMOR50687) // *E. essigi* (QMOR50688)
	MOTU 14 - MOTU 15
	E. fusca voegtlini (QMOR50689) // *E. fusca voegtlini* (QMOR50690, QMOR50691)
	MOTU 16
	E. hillerislambersi (QMOR50692, QMOR50693, QMOR50694)
	MOTU 17
	E. hoerneri (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698)
	MOTU 18 - MOTU 19
	E. kathleenae (QMOR50699) // *E. kathleenae* (QMOR50700)
	MOTU 20
	E. kirki (QMOR50701)
	MOTU 21
	E. knowltoni braggi (QMOR50702, QMOR50703, QMOR50704)
	E. knowltoni knowltoni (QMOR50705, QMOR50706)
	MOTU 22
	E. pini (QMOR50045)
	MOTU 23
	E. pini (QMOR50707)
	MOTU 24 - MOTU 26
	E. wilsoni (QMOR50708, QMOR50709) // *E. wilsoni* (QMOR50710) // *E. wilsoni* (QMOR50711)
		MOTU 1
		E. alyeska (QMOR50670)
		MOTU 2-MOTU 5
		E. californica (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673, QMOR50679) // *E. californica* (QMOR50674, QMOR50675) // *E. californica* (QMOR50676, QMOR50677) // *E. californica* (QMOR50678)
		MOTU 6
		E. critchfieldi (QMOR50680)
		MOTU 7
		E. eastopi (QMOR50044, QMOR50681, QMOR50682, QMOR50684, QMOR50683, QMOR50685, QMOR50686)
		MOTU 8
		E. essigi (QMOR50687, QMOR50688)
		MOTU 9 - MOTU 11
		E. fusca voegtlini (QMOR50689) // *E. fusca voegtlini* (QMOR50690) // *E. fusca voegtlini* (QMOR50691)
		MOTU 12- MOTU 14
		E. hillerislambersi (QMOR50692) // E. hillerislambersi (QMOR50693) // *E. hillerislambersi* (QMOR50694)
		MOTU 15
		E. hoerneri (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698)
		MOTU 16
		E. kathleenae (QMOR50699, QMOR50700)
		MOTU 17
		E. kirki (QMOR50701)
		MOTU 18
		E. knowltoni braggi (QMOR50702, QMOR50703, QMOR50704)
		MOTU 19
		E. knowltoni knowltoni (QMOR50705, QMOR50706)
		MOTU 20
		E. pini (QMOR50045)
		MOTU 21
		E. pini (QMOR50707)
		MOTU 22
		E. wilsoni (QMOR50708, QMOR5070, QMOR50710, QMOR50711)
			MOTU 1
			E. alyeska (QMOR50670)
			MOTU 2-MOTU 4
			E. californica (QMOR50043, QMOR50046, QMOR50047, QMOR50048, QMOR50051, QMOR50052, QMOR50054, QMOR50671, QMOR50672, QMOR50673, QMOR50679) // *E. californica* (QMOR50674, QMOR50675) // *E. californica* (QMOR50676, QMOR50677, QMOR50678)
			MOTU 5
			E. critchfieldi (QMOR50680)
			MOTU 6 - MOTU 9
			E. eastopi (QMOR50044, QMOR50681) //
			E. eastopi (QMOR50682, QMOR50684) //
			E. eastopi (QMOR50683, QMOR50685) //
			E. eastopi (QMOR50686)
			MOTU 10 - MOTU 11
			E. essigi (QMOR50687) // *E. essigi* (QMOR50688)
			MOTU 12 - MOTU 13
			E. fusca voegtlini (QMOR50689) // *E. fusca voegtlini* (QMOR50690, QMOR50691)
			MOTU 14
			E. hillerislambersi (QMOR50692, QMOR50693, QMOR50694)
			MOTU 15
			E. hoerneri (QMOR50050, QMOR50695, QMOR50696, QMOR50697, QMOR50698)
			MOTU 16
			E. kathleenae (QMOR50699, QMOR50700)
			MOTU 17
			E. kirki (QMOR50701)
			MOTU 18
			E. knowltoni braggi (QMOR50702, QMOR50703, QMOR50704)
			E. knowltoni knowltoni (QMOR50705, QMOR50706)
			MOTU 19
			E. pini (QMOR50045)
			MOTU 20
			E. pini (QMOR50707)
			MOTU 21
			E. wilsoni (QMOR50708, QMOR50709, QMOR50710, QMOR50711)

* Populations in red indicate difference in BI results. They represent one unique MOTU. ** Only the BI results are indicated. ML results are similar to those

Annexe 6. NJ tree with *Essigella* BOLD sequences





E. wilsoni
= BOLD:ACC4249

E. n. sp. 3 ?
= BOLD:ACE3645

E. knowltoni
= BOLD:AAF3035

E. fusca
= BOLD: AAX7694

Annexe 7. Results of the NJ tree

Barcode Index Number (BIN)	Species name in BOLD	Sample ID in BOLD	Related species after NJ analysis
BOLD:AAF3035	*Essigella knowltoni*	CNC#HEM054233	*Essigella knowltoni*
BOLD:AAF3035	*Essigella knowltoni*	CNC#HEM054224	*Essigella knowltoni*
BOLD:AAF3035	*Essigella knowltoni*	CNC#HEM054231	*Essigella knowltoni*
BOLD:AAF3035	*Essigella knowltoni*	KM501350	*Essigella knowltoni*
BOLD:AAI4968	*Essigella* sp. A rgf-2008	CNC#HEM054228	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	CNC#HEM054191	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	CNC#HEM049198	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG01853-D06	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG02715-G10	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG03927-H04	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG07520-F03	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG08324-H08	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG15357-B09	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG18435-G06	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG22337-F01	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG22413-D11	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BIOUG25513-H09	*Essigella californica*
BOLD:AAI4968	*Essigella californica*	BARS_2015_42_484	*Essigella californica*
BOLD:AAI4969	*Essigella* sp.	BIOUG00941-E04	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG02365-F08	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG03716-H02	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG08690-B08	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG12849-E07	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG16168-B05	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG22461-E10	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG24269-A01	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG31037-E10	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BARS_2016_2_216	*Essigella pini* (QC)
BOLD:AAI4969	*Essigella* sp.	BIOUG33921-H06	*Essigella pini* (QC)
BOLD:AAI4970	*Essigella* sp.	CNC#HEM057035	*Essigella alyeska*
BOLD:AAI4970	*Essigella* sp.	CNC#HEM057124	*Essigella alyeska*
BOLD:AAI4970	*Essigella* sp.	CNC#HEM070048	*Essigella alyeska*
BOLD:AAI4970	*Essigella* sp.	BIOUG11575-A05	*Essigella alyeska*
BOLD:AAL6451	*Essigella pini*	CNC#HEM049248	*Essigella pini* (NC)
BOLD:AAL6451	*Essigella pini*	CNC#HEM049379	*Essigella pini* (NC)
BOLD:AAL6451	*Essigella pini*	CNC#HEM049409	*Essigella pini* (NC)

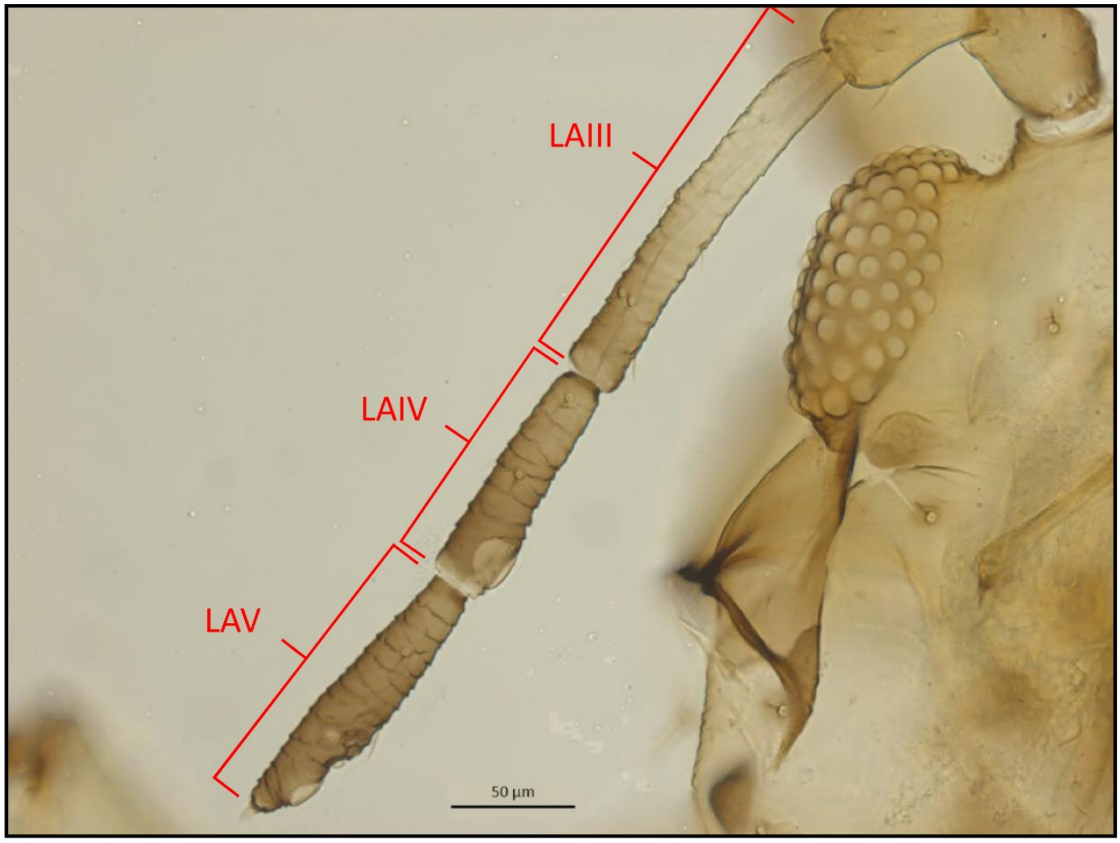
Barcode Index Number (BIN)	Species name in BOLD	Sample ID in BOLD	Related species after NJ analysis
BOLD:AAV1390	*Essigella hoerneri*	KM501348	*Essigella hoerneri*
BOLD:AAX7694	*Essigella fusca*	CNC#HEM054217	*Essigella fusca*
BOLD:AAX7694	*Essigella fusca*	BIOUG12725-A04	*Essigella fusca*
BOLD:AAX7694	*Essigella fusca*	BIOUG22413-A01	*Essigella fusca*
BOLD:ACM1471	*Essigella* sp.	BIOUG11744-D08	*Essigella* n. sp. 1 ?
BOLD:ACM1471	*Essigella* sp.	BIOUG11474-D09	*Essigella* n. sp. 1 ?
BOLD:ABV2593	*Essigella* sp.	10BBCHEM-0805	*Essigella* n. sp. 2 ?
BOLD:ABV2593	*Essigella* sp.	BIOUG03160-F10	*Essigella* n. sp. 2 ?
BOLD:ABV2593	*Essigella* sp.	BIOUG04616-B04	*Essigella* n. sp. 2 ?
BOLD:ABV2593	*Essigella* sp.	BIOUG06850-B11	*Essigella* n. sp. 2 ?
BOLD:ACC4249	*Essigella* sp.	BIOUG03101-E06	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG04572-F05	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG16095-B02	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG20982-F04	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG21998-E04	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG22100-F02	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG23060-F07	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG25554-B03	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG25602-E09	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG25633-B08	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG28272-H02	*Essigella wilsoni*
BOLD:ACC4249	*Essigella* sp.	BIOUG31102-D02	*Essigella wilsoni*
BOLD:ACE3641	*Essigella knowltoni*	HUM-2006-0866	*Essigella* n. sp. 3 ?
BOLD:ACE3641	*Essigella knowltoni*	CNC#HEM039524	*Essigella* n. sp. 3 ?
BOLD:ACE3641	*Essigella knowltoni*	BIOUG03026-G06	*Essigella* n. sp. 3 ?
BOLD:ACE3641	*Essigella knowltoni*	BIOUG03700-H02	*Essigella* n. sp. 3 ?
BOLD:ACE3641	*Essigella knowltoni*	BIOUG05473-B05	*Essigella* n. sp. 3 ?
BOLD:ACE3641	*Essigella knowltoni*	BIOUG10087-A01	*Essigella* n. sp. 3 ?
BOLD:ACE3641	*Essigella knowltoni*	BIOUG18118-E09	*Essigella* n. sp. 3 ?
BOLD:ACE3641	*Essigella knowltoni*	BIOUG19172-D06	*Essigella* n. sp. 3 ?
BOLD:ACE3641	*Essigella knowltoni*	BIOUG23266-G02	*Essigella* n. sp. 3 ?

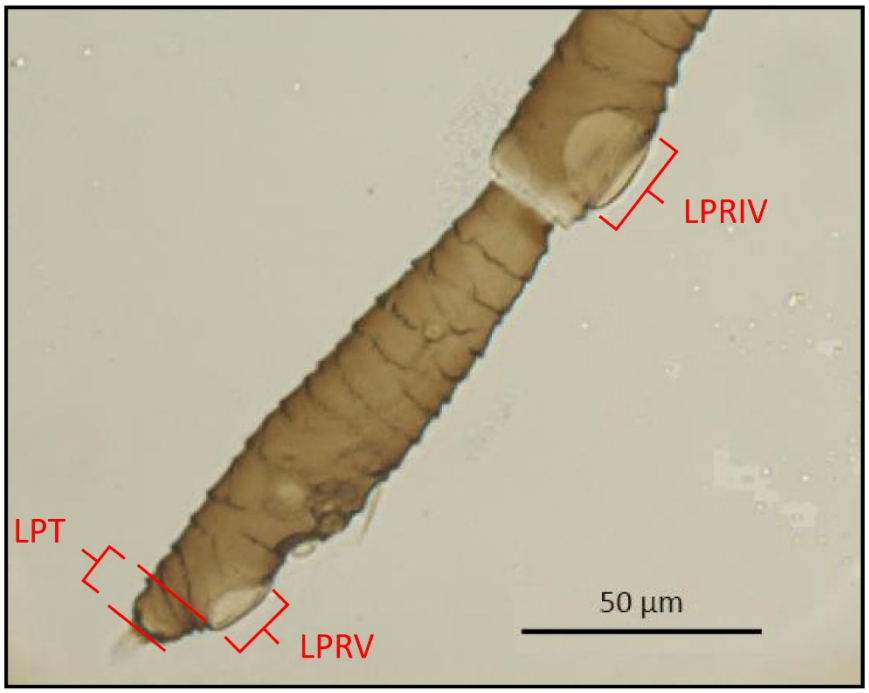
Annexe 8. Results of the BOLD identification tool

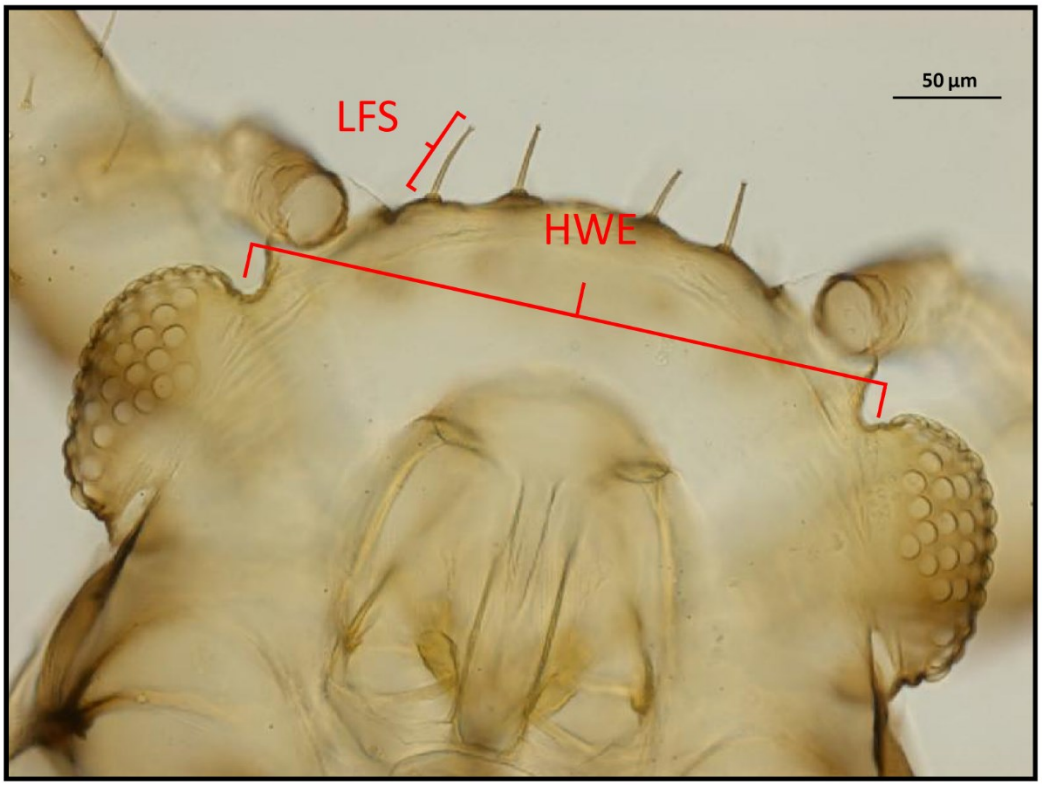
Analyzed population	Morphological identification	Best ID according to BOLD	Best BIN according to BOLD
QMOR50670	*Essigella alyeska*	No match	_
QMOR50043	*E. californica*	*E. californica*	_
QMOR50052	*E. californica*	*E. californica*	_
QMOR50054	*E. californica*	*E. californica*	_
QMOR50046	*E. californica*	*E. californica*	_
QMOR50049	*E. californica*	*E. californica*	_
QMOR50051	*E. californica*	*E. californica*	BOLD:AAI4968
QMOR50047	*E. californica*	*E. californica*	_
QMOR50048	*E. californica*	*E. californica*	_
QMOR50671	*E. californica*	*E. californica*	_
QMOR50672	*E. californica*	*E. californica*	_
QMOR50673	*E. californica*	*E. californica*	_
QMOR50674	*E. californica*	*E. californica*	_
QMOR50675	*E. californica*	*E. californica*	_
QMOR50676	*E. californica*	*E. californica*	_
QMOR50677	*E. californica*	*E. californica*	BOLD:AAI4968
QMOR50678	*E. californica*	*E. californica*	BOLD:AAI4968
QMOR50679	*E. californica*	*E. californica*	BOLD:AAI4968
QMOR50680	*E. critchfieldi*	No match	_
QMOR50044	*E. eastopi*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50681	*E. eastopi*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50682	*E. eastopi*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50683	*E. eastopi*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50684	*E. eastopi*	*E. fusca voegtlini*	_
QMOR50685	*E. eastopi*	*E. fusca voegtlini*	_
QMOR50686	*E. eastopi*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50687	*E. essigi*	*E. knowltoni*	BOLD:ACE3641
QMOR50688	*E. essigi*	*E. knowltoni*	BOLD:ACE3641
QMOR50689	*E. fusca voegtlini*	*E. fusca*	BOLD:AAX7694
QMOR50690	*E. fusca voegtlini*	*E. fusca*	BOLD:AAX7694
QMOR50691	*E. fusca voegtlini*	*E. fusca*	BOLD:AAX7694
QMOR50692	*E. hillerislambersi*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50693	*E. hillerislambersi*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50694	*E. hillerislambersi*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50050	*E. hoerneri*	*E. hoerneri*	BOLD:AAV1390
QMOR50695	*E. hoerneri*	*E. hoerneri*	BOLD:AAV1390
QMOR50696	*E. hoerneri*	*E. hoerneri*	BOLD:AAV1390
QMOR50697	*E. hoerneri*	*E. hoerneri*	BOLD:AAV1390
QMOR50698	*E. hoerneri*	*E. hoerneri*	BOLD:AAV1390
QMOR50699	*E. kathleenae*	No match	_
QMOR50700	*E. kathleenae*	No match	_
QMOR50701	*E. kirki*	No match	_
QMOR50702	*E. knowltoni braggi*	*E. knowltoni*	BOLD:AAF3035
QMOR50703	*E. knowltoni braggi*	*E. knowltoni*	BOLD:AAF3035
QMOR50704	*E. knowltoni braggi*	*E. knowltoni*	BOLD:AAF3035
QMOR50705	*E. knowltoni knowltoni*	*E. knowltoni*	BOLD:AAF3035
QMOR50706	*E. knowltoni knowltoni*	*E. knowltoni*	BOLD:AAF3035
QMOR50045	*E. pini* (NC)	*E. pini*	BOLD:AAL6451
QMOR50707	*E. pini* (QC)	*E. californica*	BOLD:AAI4968
QMOR50708	*E. wilsoni*	*E. fusca voegtlini*	BOLD:ADK4567
QMOR50709	*E. wilsoni*	*E. knowltoni*	BOLD:ACE3641
QMOR50710	*E. wilsoni*	No match	_
QMOR50711	*E. wilsoni*	*E. fusca voegtlini*	BOLD:ADK4567

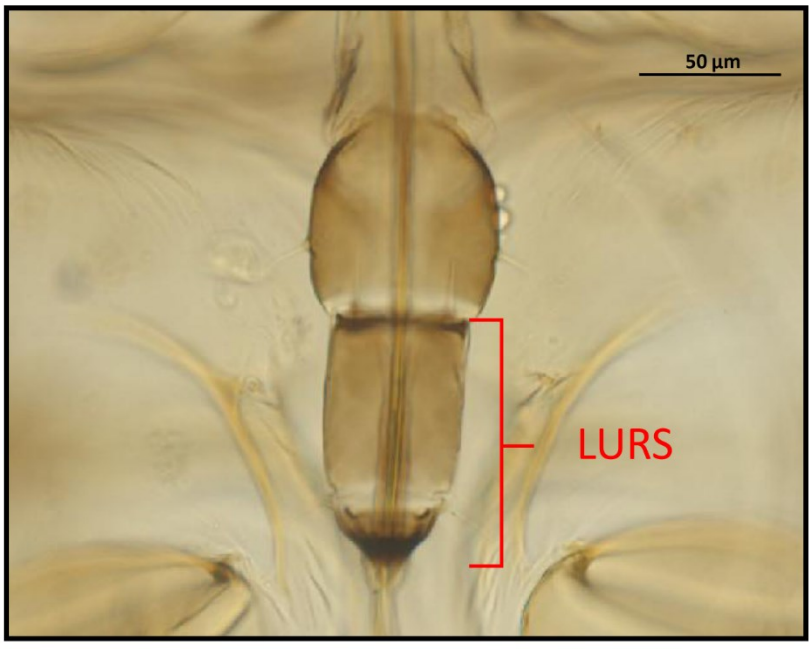
"_" means that no BIN is proposed

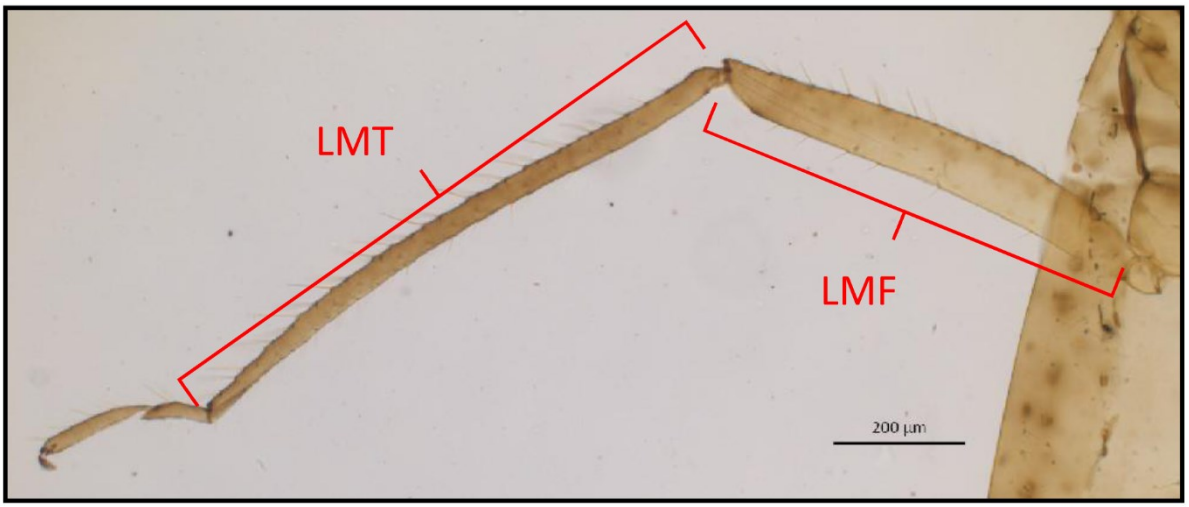
Annexe 9. Details of measurements

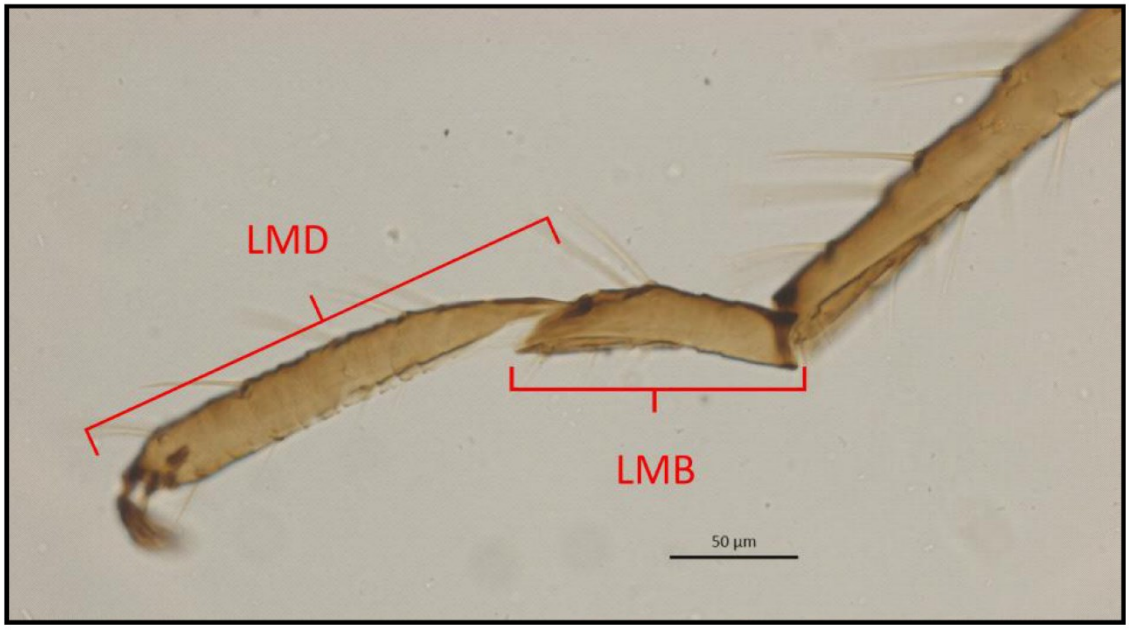


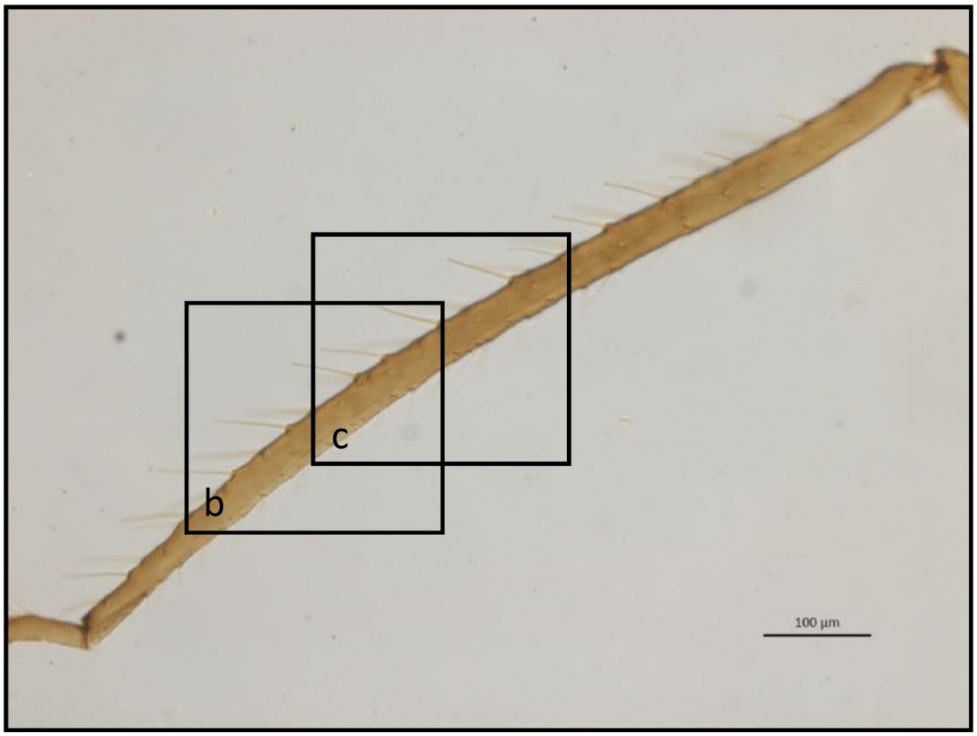


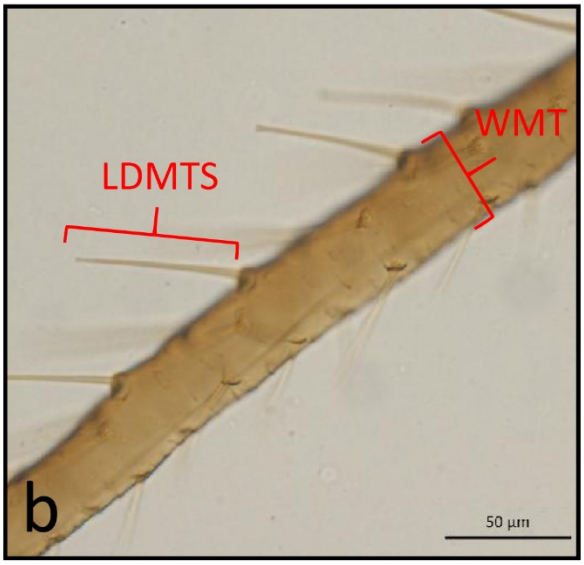

50 µm
LFS
HWE

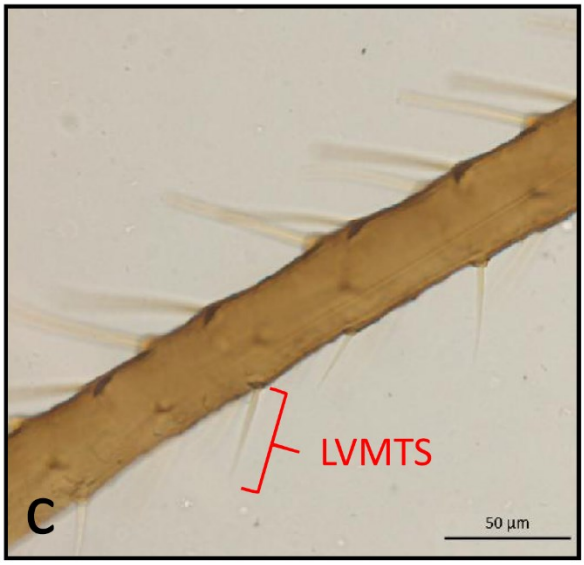

50 µm
LURS

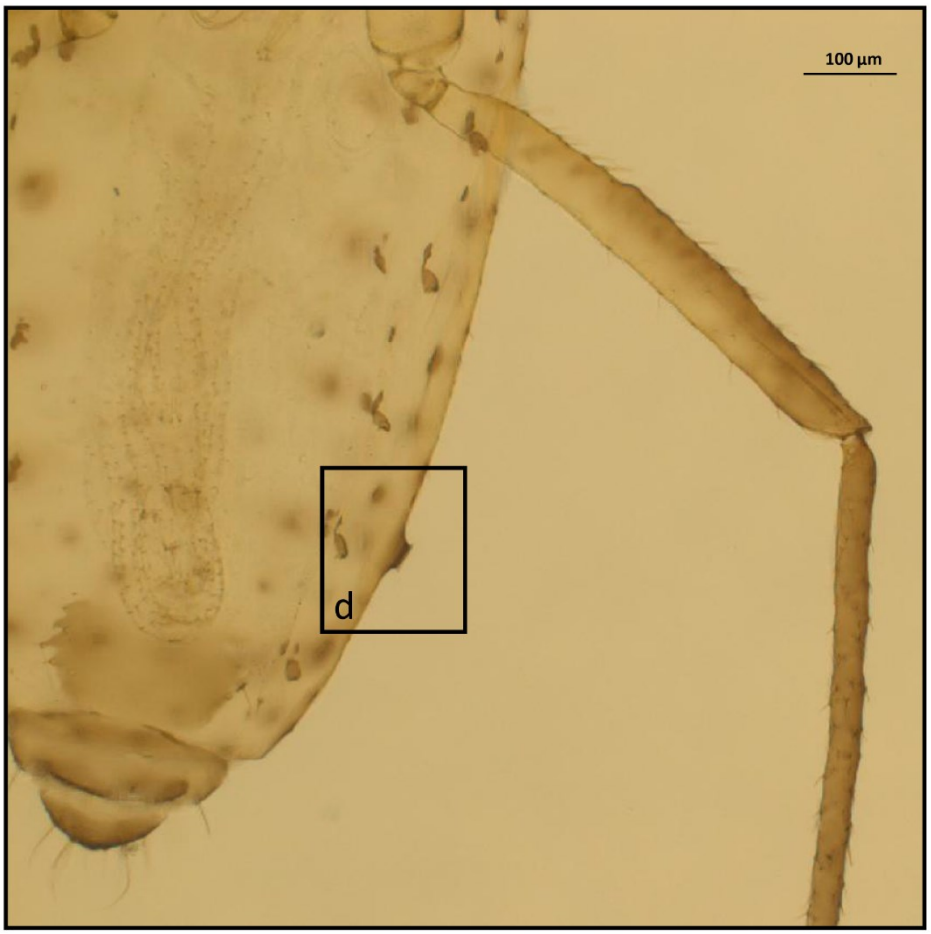

LMT
LMF
200 µm

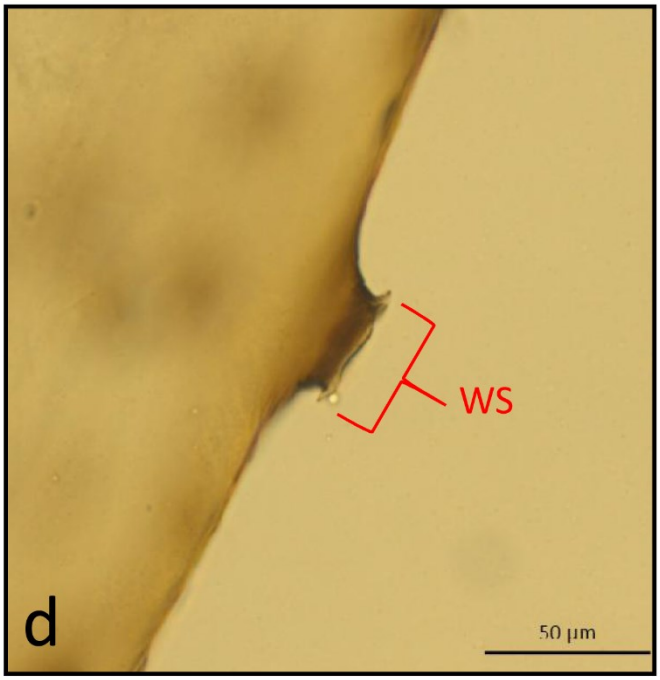

LMD
LMB
50 µm


c
b
100 µm


WMT
LDMTS
b
50 µm


LVMTS
c
50 µm


100 µm
d


WS
d
50 µm

Annexe 10. Measurements used in descriptions

Spécimen	WS (gauche)	WS (droit)	LURS	Antenne gauche						Antenne droite					
				LAIII	LAIV	LAV	LPRIV	LPRV	LPT	LAIII	LAIV	LAV	LPRIV	LPRV	LPT
E. californica (*P. monticola*) 1	–	–	68,987	168,597	91,016	119,304	22,277	21,462	11,022	162,886	90,201	121,006	23,835	20,021	13,891
E. californica (*P. monticola*) 2	–	–	–	–	91,438	–	21,765	19,134	9,875	159,537	90,835	116,023	21,537	20,993	10,555
E. californica (*P. monticola*) 3	–	–	–	–	98,919	121,976	24,663	20,785	12,085	–	97,150	126,085	24,546	22,625	13,145
E. californica (*P. monticola*) 4	38,877	38,354	68,007	–	–	–	–	–	–	178,550	104,190	–	26,732	21,717	14,298
E. californica (*P. monticola*) 5	39,174	–	64,473	163,916	107,831	127,427	24,862	18,456	9,922	–	104,771	125,743	23,314	20,746	7,974
E. californica (*P. monticola*) 6	34,879	37,567	69,769	176,865	96,460	–	25,029	19,975	13,553	–	–	–	–	–	–
E. californica (*P. monticola*) 7	40,440	41,704	76,538	172,949	100,138	119,838	24,550	21,373	11,720	–	100,582	–	–	–	–
E. californica (*P. monticola*) 8	36,733	37,855	70,006	195,290	96,029	–	26,185	21,004	7,648	–	103,365	122,274	26,172	21,465	7,678
E. californica (*P. monticola*) 9	40,332	40,980	70,395	178,108	99,316	130,744	23,204	18,912	16,053	177,078	–	–	–	–	–
E. californica (*P. monticola*) 10	–	–	74,713	–	–	–	–	–	–	–	105,898	129,960	22,953	19,134	13,004
E. californica (*P. monticola*) 11	–	–	69,509	–	–	–	–	–	–	157,641	94,481	–	22,145	–	–
E. californica (*P. monticola*) 12	–	–	–	188,430	–	–	–	–	–	194,491	102,371	–	–	–	–
E. californica (*P. monticola*) 13	–	–	72,693	179,650	–	–	–	–	–	179,955	–	–	–	–	–
E. californica (*P. monticola*) 14	–	–	–	177,546	–	–	–	–	–	173,753	95,466	121,705	23,499	–	14,963
E. californica (*P. monticola*) 15	–	42,622	–	184,888	104,714	130,191	23,854	21,743	10,896	182,062	–	–	–	–	–
E. californica (*P. monticola*) 16	34,433	–	79,784	176,175	–	–	–	–	–	174,808	106,758	130,038	24,399	22,389	–
E. californica (*P. monticola*) 17	38,803	39,208	69,189	–	–	–	–	–	–	166,217	–	–	–	–	–
E. californica (*P. monticola*) 18	–	–	74,468	161,817	92,728	–	–	–	–	–	–	–	–	–	–
E. californica (*P. monticola*) 19	35,080	–	72,106	192,760	110,404	–	–	–	–	197,357	–	–	–	–	–
E. californica (*P. monticola*) 20	–	–	71,469	–	–	–	–	–	–	185,850	104,579	130,252	–	–	–
E. californica (*P. monticola*) 21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E. californica (*P. monticola*) 22	–	–	–	–	–	–	–	–	–	185,776	99,723	120,878	29,101	19,351	12,200
E. californica (*P. monticola*) 23	–	42,475	78,307	–	111,564	141,570	–	–	14,681	–	109,819	–	–	–	–
E. californica (*P. monticola*) 24	–	–	72,817	178,993	103,517	–	–	–	10,975	–	101,762	–	–	–	13,137
E. californica (*P. monticola*) 25	–	40,800	–	–	94,275	127,196	25,296	–	16,814	193,100	96,860	126,381	–	–	11,950
E. californica (*P. monticola*) 26	–	–	73,365	–	91,492	120,010	–	–	–	–	90,478	–	–	–	–

Spécimen	Patte métathoracique gauche													
	LMF	WMF	LDMFS	DMFS (forme)	LVMFS	VMFS (forme)	LMT	WMT	LDMTS	DMTS (forme)	LVMTS	VMTS (forme)	LMB	LMD
E. californica (*P. monticola*) 1	677,491	_	55,451	cupulée	42,932	pointue	952,990	41,162	83,029	cupulée	46,186	pointu	107,154	189,276
E. californica (*P. monticola*) 2	_	_	_	_	_	pointue	_	_	60,873	cupulée	37,917	pointu	_	_
E. californica (*P. monticola*) 3	_	_	42,716	cupulée	38,268	pointue	_	36,733	63,534	cupulée	37,156	pointu	106,348	183,889
E. californica (*P. monticola*) 4	741,472	82,874	70,816	cupulée	50,023	pointue	_	40,704	103,211	pointue	64,036	pointu	_	_
E. californica (*P. monticola*) 5	_	_	71,944	cupulée	38,865	pointue	_	38,470	89,095	cupulée	35,992	pointu	109,411	195,025
E. californica (*P. monticola*) 6	_	71,228	42,014	cupulée	36,305	pointue	876,151	39,501	68,059	cupulée	53,726	pointu	110,267	185,155
E. californica (*P. monticola*) 7	696,645	_	46,145	cupulée	39,834	pointue	997,850	42,378	88,374	cupulée	41,998	pointu	124,320	201,483
E. californica (*P. monticola*) 8	711,836	_	61,531	cupulée	52,619	pointue	981,750	40,234	91,635	cupulée	46,635	pointu	121,074	199,826
E. californica (*P. monticola*) 9	727,324	69,493	72,158	cupulée	44,821	pointue	996,125	37,929	107,697	pointue	66,450	pointu	109,819	200,644
E. californica (*P. monticola*) 10	650,350	71,454	56,559	cupulée	36,756	pointue	951,537	36,775	84,795	presque pointue	42,135	pointu	112,007	189,477
E. californica (*P. monticola*) 11	_	73,851	48,323	cupulée	43,359	pointue	890,023	39,285	73,378	cupulée	42,093	pointu	101,826	189,912
E. californica (*P. monticola*) 12	_	96,205	57,538	cupulée	44,033	pointue	1103,719	47,521	97,790	cupulée	49,617	pointu	130,989	209,953
E. californica (*P. monticola*) 13	_	_	_	_	_	_	_	_	_	_	_	_	_	_
E. californica (*P. monticola*) 14	_	93,251	51,375	cupulée	47,030	pointue	973,345	44,137	86,555	cupulée	55,264	pointu	119,542	207,676
E. californica (*P. monticola*) 15	738,302	94,730	60,804	cupulée	41,314	pointue	_	47,948	83,744	cupulée	60,402	pointu	_	_
E. californica (*P. monticola*) 16	725,329	84,555	59,376	cupulée	44,247	pointue	_	45,345	95,254	presque pointue	52,996	pointu	_	_
E. californica (*P. monticola*) 17	_	_	_	cupulée	_	_	_	_	_	_	_	_	_	_
E. californica (*P. monticola*) 18	699,893	84,456	62,405	cupulée	37,910	pointue	989,658	39,455	80,265	cupulée	46,329	pointu	114,408	192,822
E. californica (*P. monticola*) 19	798,539	101,168	51,460	cupulée	43,812	pointue	_	51,964	88,735	cupulée	65,002	pointu	_	_
E. californica (*P. monticola*) 20	747,327	96,075	53,734	cupulée	42,311	pointue	1055,210	46,923	82,871	cupulée	44,617	pointu	124,998	203,442
E. californica (*P. monticola*) 21	715,272	_	59,775	cupulée	_	pointue	_	_	92,149	presque pointue	52,035	pointu	116,558	201,431
E. californica (*P. monticola*) 22	_	_	74,639	cupulée	48,737	pointue	_	_	95,623	presque pointue	52,744	pointu	_	_
E. californica (*P. monticola*) 23	755,078	101,490	63,782	cupulée	44,591	pointue	1099,303	45,814	81,837	cupulée	50,410	pointu	127,232	204,384
E. californica (*P. monticola*) 24	_	80,531	60,593	cupulée	46,396	pointue	_	42,027	86,453	cupulée	45,884	pointu	118,948	190,109
E. californica (*P. monticola*) 25	_	98,257	69,269	presque pointue	51,672	pointue	1079,712	45,907	97,458	pointue	49,538	pointu	117,033	183,020
E. californica (*P. monticola*) 26	698,398	93,241	78,558	cupulée	46,156	pointue	1009,251	43,702	100,450	pointue	57,891	pointu	113,203	184,411

Spécimen	Patte métathoracique droite														LFS	FS (forme)	BL	HWE	Soies Tergum III	Soies Tergum IV
	LMF	WMF	LDMFS	DMFS (forme)	LVMFS	VMFS (forme)	LMT	WMT	LDMTS	DMTS (forme)	LVMTS	VMTS (forme)	LMB	LMD						
E. californica (*P. monticola*) 1	671,662	_	_	cupulée	_	pointue	973,483	_	70,601	cupulée	49,002	pointue	_	_	48,283	cupulée	1588,514	_	6	6
E. californica (*P. monticola*) 2	651,075	72,072	47,335	cupulée	31,533	pointue	893,342	33,793	68,078	cupulée	30,458	pointue	103,854	184,730	47,719	cupulée	_	_	_	_
E. californica (*P. monticola*) 3	650,956	72,149	50,812	cupulée	35,557	pointue	912,468	39,174	84,637	cupulée	38,263	pointue	107,237	180,342	45,846	cupulée	_	289,070	6	6
E. californica (*P. monticola*) 4	_	_	_	_	_	_	_	_	_	_	_	_	_	_	80,200	cupulée	1687,861	281,206	_	_
E. californica (*P. monticola*) 5	_	_	_	_	_	_	_	_	_	_	_	_	_	_	65,175	cupulée	1795,152	303,254	_	_
E. californica (*P. monticola*) 6	_	_	_	_	_	_	_	_	_	_	_	_	_	_	44,373	cupulée	1594,832	272,623	6	6
E. californica (*P. monticola*) 7	_	75,279	64,329	cupulée	52,622	pointue	1006,015	40,685	90,334	cupulée	74,552	pointue	_	_	59,777	cupulée	1794,650	311,858	_	_
E. californica (*P. monticola*) 8	703,274	_	67,453	cupulée	41,860	pointue	_	40,084	84,702	cupulée	43,817	pointue	118,661	_	57,690	cupulée	1763,645	284,588	6	6
E. californica (*P. monticola*) 9	_	_	73,589	cupulée	47,803	pointue	_	37,823	90,877	presque pointue	49,525	pointue	115,518	202,293	66,366	cupulée	1638,843	280,010	6	6
E. californica (*P. monticola*) 10	664,963	74,207	48,804	cupulée	35,174	pointue	962,951	37,814	83,656	cupulée	46,326	pointue	112,044	190,348	47,349	cupulée	1583,139	270,979	6	_
E. californica (*P. monticola*) 11	_	_	43,333	cupulée	32,573	pointue	_	39,609	68,282	cupulée	36,654	pointue	102,213	187,537	56,477	cupulée	1764,544	285,197	6	6
E. californica (*P. monticola*) 12	_	_	_	_	_	_	_	_	_	_	_	_	_	_	63,345	cupulée	_	_	_	_
E. californica (*P. monticola*) 13	748,387	96,798	59,957	cupulée	45,186	pointue	1053,642	52,473	87,909	cupulée	53,104	pointue	128,100	202,650	62,488	cupulée	_	_	6	_
E. californica (*P. monticola*) 14	_	_	56,530	cupulée	40,362	pointue	_	43,183	84,084	cupulée	40,590	pointue	119,804	201,547	65,165	cupulée	_	_	_	_
E. californica (*P. monticola*) 15	_	_	67,316	cupulée	38,695	pointue	_	_	98,861	pointue	52,247	pointue	124,532	208,495	59,843	cupulée	_	_	_	_
E. californica (*P. monticola*) 16	_	83,083	56,564	cupulée	50,981	pointue	1021,534	44,815	87,632	presque pointue	61,605	pointue	115,101	195,050	67,662	cupulée	1928,395	303,364	_	_
E. californica (*P. monticola*) 17	680,368	_	53,187	cupulée	38,643	pointue	_	43,087	72,620	cupulée	57,880	pointue	_	_	55,279	cupulée	1782,622	_	_	_
E. californica (*P. monticola*) 18	706,709	86,894	58,244	cupulée	41,100	pointue	974,059	37,604	83,651	cupulée	36,938	pointue	116,401	193,764	49,403	cupulée	1818,756	277,680	_	_
E. californica (*P. monticola*) 19	_	104,573	57,805	cupulée	41,151	pointue	1104,168	47,030	82,035	cupulée	54,522	pointue	_	_	61,737	cupulée	1997,879	_	_	_
E. californica (*P. monticola*) 20	_	89,139	56,049	cupulée	44,313	pointue	1070,068	45,244	76,157	cupulée	51,140	pointue	123,575	206,635	66,510	cupulée	1704,015	290,820	6	7
E. californica (*P. monticola*) 21	_	_	_	_	_	_	_	41,416	96,825	presque pointue	68,004	pointue	103,014	_	55,518	cupulée	_	_	_	_
E. californica (*P. monticola*) 22	_	_	82,951	cupulée	44,414	pointue	_	_	104,574	pointue	46,992	pointue	126,360	187,956	59,789	cupulée	1927,963	306,290	6	7
E. californica (*P. monticola*) 23	_	93,390	61,225	cupulée	43,144	pointue	_	43,061	86,974	cupulée	42,649	pointue	127,389	206,801	57,957	cupulée	1988,983	_	6	7
E. californica (*P. monticola*) 24	_	92,232	63,714	cupulée	49,011	pointue	_	42,920	74,390	cupulée	38,810	pointue	120,257	190,642	67,196	cupulée	1850,947	_	6	6
E. californica (*P. monticola*) 25	_	98,665	73,176	presque pointue	52,132	pointue	1068,967	45,527	94,684	presque pointue	48,898	pointue	115,522	185,783	56,393	cupulée	1763,870	_	6	6
E. californica (*P. monticola*) 26	_	92,890	57,651	cupulée	41,738	pointue	_	40,647	89,897	cupulée	55,598	pointue	114,363	192,496	57,917	cupulée	1741,923	_	6	6

Spécimen	WS (gauche)	WS (droit)	LURS	Antenne gauche						Antenne droite					
				LAIII	LAIV	LAV	LPRIV	LPRV	LPT	LAIII	LAIV	LAV	LPRIV	LPRV	LPT
E. californica (*P. albicaulis*) 1	41,465	_	71,376	171,715	97,416	124,432	22,182	18,420	10,710	167,017	97,874	_	23,455	_	_
E. californica (*P. albicaulis*) 2	40,511	41,604	73,650	_	_	_	_	_	_	174,255	104,808	_	23,346	21,911	14,463
E. californica (*P. albicaulis*) 3	_	_	76,297	168,889	97,417	_	20,785	_	_	163,010	96,393	121,218	20,896	17,569	12,119
E. californica (*P. albicaulis*) 4	_	38,334	_	174,761	_	_	_	19,879	12,550	184,605	_	_	_	_	_
E. californica (*P. albicaulis*) 5	42,756	_	76,821	168,693	101,806	122,575	24,550	20,760	12,610	181,379	_	_	_	19,194	14,298
E. californica (*P. albicaulis*) 6	40,641	43,521	79,192	162,204	106,751	119,207	25,804	18,531	11,333	173,331	_	_	_	_	_
E. californica (*P. albicaulis*) 7	38,316	36,681	73,587	174,887	99,819	_	23,443	20,431	10,215	168,432	106,587	113,698	22,718	20,288	8,535
E. californica (*P. muricata*) 1	44,061	43,226	80,161	244,313	117,041	_	27,831	_	_	225,140	113,320	124,632	26,678	20,493	11,616
E. californica (*P. muricata*) 2	_	40,759	80,456	224,970	107,414	125,937	23,324	_	_	_	110,645	_	25,276	_	_
E. californica (*P. muricata*) 3	_	44,388	81,986	254,373	130,000	_	24,172	_	_	_	_	_	_	_	_
E. californica (*P. muricata*) 4	_	_	80,362	231,056	113,601	123,961	24,084	23,443	_	229,355	108,396	125,249	26,049	19,350	12,000
E. californica (*P. muricata*) 5	_	_	75,050	_	113,550	125,931	25,269	20,288	13,290	219,698	110,895	_	24,816	_	_
E. californica (*P. muricata*) 6	40,568	_	78,115	227,275	106,255	122,769	24,746	19,962	14,246	_	_	_	_	_	_
E. californica (*P. muricata*) 7	_	44,584	79,649	229,017	113,634	_	24,926	20,410	13,274	233,252	120,689	120,132	25,343	20,123	13,879
E. californica (*P. muricata*) 8	40,477	_	81,750	214,511	107,690	122,998	24,354	20,217	14,566	_	_	_	_	_	_
E. californica (*P. muricata*) 9	41,972	40,338	80,837	_	109,545	131,204	22,980	19,975	13,991	_	_	_	_	_	_
E. californica (*P. muricata*) 10	40,776	42,049	_	_	_	_	_	_	_	223,044	106,799	124,229	25,797	21,100	12,610
E. californica (*P. muricata*) 11	39,461	39,054	81,278	229,555	105,435	_	24,731	_	_	224,326	105,665	126,305	24,409	17,363	12,895
E. californica (*P. muricata*) 12	42,431	39,819	74,076	_	_	_	24,578	18,681	15,243	_	_	_	_	_	_
E. californica (*P. muricata*) 13	42,500	42,699	76,916	_	_	_	23,443	_	_	_	_	131,597	_	19,306	13,891
E. californica (*P. muricata*) 14	_	_	79,157	_	115,261	131,816	21,963	19,206	14,075	_	_	_	_	_	_
E. californica (*P. muricata*) 15	_	_	84,458	_	_	_	_	_	_	_	_	_	_	_	_
E. californica (*P. muricata*) 16	_	40,172	84,001	_	105,839	128,078	24,823	21,468	14,057	207,523	98,299	125,158	_	_	15,700
E. californica (*P. muricata*) 17	42,258	_	82,541	_	116,528	125,797	21,971	17,383	14,459	_	120,141	130,796	24,862	18,346	14,905
E. californica (*P. muricata*) 18	_	_	75,799	_	100,048	_	22,163	_	_	_	99,291	126,299	22,124	18,327	12,061
E. californica (*P. muricata*) 19	39,150	_	_	_	112,074	128,500	19,917	17,519	14,732	_	113,128	128,764	_	18,314	15,584
E. californica (*P. muricata*) 20	_	_	85,353	242,295	_	_	_	_	_	237,579	117,111	_	_	_	_
E. californica (*P. muricata*) 21	_	39,688	_	_	_	_	_	_	_	256,345	111,114	_	_	_	_
E. californica (*P. muricata*) 22	_	41,269	86,370	254,948	119,513	139,810	_	_	_	252,250	126,534	137,519	20,701	18,390	13,769
E. californica (*P. muricata*) 23	_	_	80,381	222,170	115,233	133,421	_	_	_	_	_	_	_	_	_
E. californica (*P. muricata*) 24	_	_	_	236,865	116,731	_	_	_	_	239,324	109,077	_	_	_	_
E. californica (*P. muricata*) 25	_	_	82,145	241,192	_	_	_	_	_	239,309	119,931	_	24,583	_	_

Spécimen	Patte métathoracique gauche													
	LMF	WMF	LDMFS	DMFS (forme)	LVMFS	VMFS (forme)	LMT	WMT	LDMTS	DMTS (forme)	LVMTS	VMTS (forme)	LMB	LMD
E. californica (*P. albicaulis*) 1	724,249	76,277	36,837	cupulée	44,039	pointue	1056,604	42,306	61,759	cupulée	45,309	pointue	107,235	189,643
E. californica (*P. albicaulis*) 2	_	77,156	33,263	cupulée	31,089	pointue	_	40,521	37,319	cupulée	24,748	pointue	_	_
E. californica (*P. albicaulis*) 3	_	71,335	25,363	cupulée	36,421	pointue	975,083	37,933	41,817	cupulée	40,071	pointue	111,999	205,985
E. californica (*P. albicaulis*) 4	_	_	_	_	_	_	_	_	_	_	_	_	_	_
E. californica (*P. albicaulis*) 5	728,809	75,364	31,494	cupulée	32,685	pointue	1074,402	41,319	48,799	cupulée	48,135	pointue	111,628	190,072
E. californica (*P. albicaulis*) 6	_	75,647	25,900	cupulée	32,687	pointue	1027,831	42,929	41,550	cupulée	34,866	pointue	108,327	189,382
E. californica (*P. albicaulis*) 7	_	76,739	25,221	cupulée	26,061	pointue	_	43,821	33,753	cupulée	34,258	pointue	107,540	189,725
E. californica (*P. muricata*) 1	785,695	93,845	58,761	cupulée	52,420	pointue	1201,528	54,563	96,975	cupulée	76,718	pointue	131,000	197,548
E. californica (*P. muricata*) 2	_	101,544	44,587	cupulée	50,979	pointue	1096,112	52,789	80,615	cupulée	82,835	pointue	120,567	183,715
E. californica (*P. muricata*) 3	922,984	118,737	64,040	cupulée	62,294	pointue	1369,914	59,200	94,189	cupulée	80,633	pointue	139,850	210,946
E. californica (*P. muricata*) 4	_	_	51,500	cupulée	53,779	pointue	_	57,418	80,564	cupulée	73,566	pointue	_	_
E. californica (*P. muricata*) 5	775,312	105,019	51,609	cupulée	53,703	pointue	_	49,525	62,575	cupulée	81,000	pointue	134,799	192,026
E. californica (*P. muricata*) 6	_	_	_	_	_	_	_	_	_	_	_	_	_	_
E. californica (*P. muricata*) 7	850,834	94,684	47,805	cupulée	55,814	pointue	1272,608	54,496	67,728	cupulée	69,779	pointue	133,720	204,003
E. californica (*P. muricata*) 8	793,424	93,053	35,080	cupulée	51,372	pointue	1187,625	53,196	55,996	cupulée	51,073	pointue	130,759	194,981
E. californica (*P. muricata*) 9	804,493	87,505	34,244	cupulée	44,281	pointue	1182,853	51,461	59,220	cupulée	49,935	pointue	129,215	195,891
E. californica (*P. muricata*) 10	793,894	97,673	36,472	cupulée	46,214	pointue	1187,882	53,131	68,327	cupulée	70,114	pointue	133,655	201,915
E. californica (*P. muricata*) 11	_	89,768	42,135	cupulée	53,470	pointue	1163,173	51,937	63,392	cupulée	61,402	pointue	_	_
E. californica (*P. muricata*) 12	_	_	47,548	cupulée	53,129	pointue	_	_	63,065	cupulée	51,356	pointue	129,253	191,445
E. californica (*P. muricata*) 13	_	_	_	_	_	_	_	_	_	_	_	_	_	_
E. californica (*P. muricata*) 14	797,469	90,626	38,038	cupulée	43,433	pointue	1187,333	53,144	49,351	cupulée	58,612	pointue	131,866	199,797
E. californica (*P. muricata*) 15	_	_	52,692	cupulée	53,043	pointue	_	_	89,575	cupulée	75,365	pointue	118,636	189,158
E. californica (*P. muricata*) 16	_	_	54,404	cupulée	56,122	pointue	_	_	87,879	cupulée	72,670	pointue	122,744	186,799
E. californica (*P. muricata*) 17	_	93,682	49,769	cupulée	50,627	pointue	_	53,386	68,340	cupulée	63,345	pointue	138,964	203,325
E. californica (*P. muricata*) 18	_	_	_	_	_	_	_	_	_	_	_	_	_	_
E. californica (*P. muricata*) 19	758,976	101,443	66,823	cupulée	58,667	pointue	_	53,500	94,481	cupulée	65,749	pointue	126,957	192,348
E. californica (*P. muricata*) 20	857,899	122,525	79,367	cupulée	58,299	pointue	1275,354	51,429	100,741	cupulée	79,721	pointue	133,539	192,146
E. californica (*P. muricata*) 21	_	_	64,901	cupulée	60,460	pointue	1398,935	52,265	94,520	cupulée	67,567	pointue	139,052	204,163
E. californica (*P. muricata*) 22	910,381	115,029	67,295	cupulée	62,694	pointue	_	76,021	87,596	cupulée	73,065	pointue	_	_
E. californica (*P. muricata*) 23	_	106,942	56,276	cupulée	54,981	pointue	1284,977	52,201	86,426	cupulée	70,798	pointue	128,930	209,869
E. californica (*P. muricata*) 24	_	112,007	51,335	cupulée	51,910	pointue	1276,749	59,944	85,030	cupulée	72,113	pointue	129,603	200,204
E. californica (*P. muricata*) 25	_	112,985	40,671	cupulée	53,639	pointue	1258,869	61,489	54,078	cupulée	48,699	pointue	131,201	196,175

Spécimen	Patte métathoracique droite														LFS	FS (forme)	BL	HWE	Soies Tergum III	Soies Tergum IV
	LMF	WMF	LDMFS	DMFS (forme)	LVMFS	VMFS (forme)	LMT	WMT	LDMTS	DMTS (forme)	LVMTS	VMTS (forme)	LMB	LMD						
E. californica (*P. albicaulis*) 1	725,233	_	35,511	cupulée	28,310	pointue	1050,693	42,747	54,563	cupulée	44,451	pointue	110,216	206,366	39,539	cupulée	1762,524	293,449	6	6
E. californica (*P. albicaulis*) 2	675,665	77,318	30,902	cupulée	34,781	pointue	987,492	43,900	43,317	cupulée	31,052	pointue	112,358	189,790	38,659	cupulée	1843,518	283,263	6	6
E. californica (*P. albicaulis*) 3	679,431	68,217	25,750	cupulée	29,276	pointue	981,649	36,769	44,790	cupulée	32,288	pointue	111,435	203,206	30,774	cupulée	1667,750	289,084	6	6
E. californica (*P. albicaulis*) 4	716,470	72,689	25,759	cupulée	31,730	pointue	1068,085	40,521	45,930	cupulée	44,333	pointue	113,196	196,045	18,718	cupulée	1627,397	300,702	6	6
E. californica (*P. albicaulis*) 5	_	_	_	_	_	_	_	_	_	_	_	_	_	_	38,977	cupulée	1973,740	326,032	6	6
E. californica (*P. albicaulis*) 6	_	76,821	27,852	cupulée	32,286	pointue	1028,576	41,937	39,397	cupulée	36,100	pointue	114,389	192,747	32,304	cupulée	1802,479	303,660	6	6
E. californica (*P. albicaulis*) 7	_	76,610	32,447	cupulée	31,356	pointue	_	41,558	34,934	cupulée	33,848	pointue	111,780	191,043	29,137	cupulée	2113,296	309,001	_	6
E. californica (*P. muricata*) 1	783,000	92,980	52,724	cupulée	64,036	pointue	1186,202	55,889	85,392	cupulée	76,784	pointue	123,864	200,589	59,696	cupulée	2117,636	364,476	6	6
E. californica (*P. muricata*) 2	_	91,111	50,406	cupulée	60,280	pointue	1064,211	56,825	73,842	cupulée	72,943	pointue	118,762	188,854	46,803	cupulée	2079,355	363,155	6	6
E. californica (*P. muricata*) 3	909,883	112,905	61,548	cupulée	65,981	pointue	1377,663	56,712	91,580	cupulée	78,700	pointue	136,055	208,115	55,734	cupulée	2328,034	_	6	6
E. californica (*P. muricata*) 4	844,027	119,125	50,964	cupulée	54,660	pointue	1297,854	59,283	82,190	cupulée	71,911	pointue	128,558	207,513	54,724	cupulée	2273,282	_	6	6
E. californica (*P. muricata*) 5	_	_	47.0	cupulée	54,290	pointue	_	_	70,225	cupulée	70,837	_	_	_	53,180	cupulée	_	_	6	6
E. californica (*P. muricata*) 6	781,267	92,709	40,030	cupulée	47,423	pointue	1134,166	51,119	47,747	cupulée	53,864	pointue	125,183	191,006	35,090	cupulée	2119,863	361,761	6	6
E. californica (*P. muricata*) 7	_	_	59,665	cupulée	57,161	pointue	_	_	67,846	cupulée	52,508	pointue	_	_	45,001	cupulée	2295,915	374,355	6	6
E. californica (*P. muricata*) 8	796,899	100,328	39,226	cupulée	49,464	pointue	1164,195	57,273	58,618	cupulée	72,835	pointue	123,806	192,007	45,906	cupulée	2165,383	_	6	6
E. californica (*P. muricata*) 9	800,873	92,323	40,692	cupulée	46,475	pointue	1168,913	50,297	54,872	cupulée	51,243	pointue	134,640	_	47,522	cupulée	2144,855	360,156	6	4
E. californica (*P. muricata*) 10	793,173	92,333	42,267	cupulée	52,034	pointue	1203,602	52,777	69,492	cupulée	51,356	pointue	137,617	203,318	47,088	cupulée	2072,462	_	6	7
E. californica (*P. muricata*) 11	780,702	91,390	40,753	cupulée	57,136	pointue	_	51,066	79,785	cupulée	61,161	pointue	_	_	51,571	cupulée	2119,594	376,072	6	6
E. californica (*P. muricata*) 12	_	_	47,064	cupulée	45,277	pointue	_	_	66,188	cupulée	54,196	pointue	129,949	189,883	46,068	cupulée	2031,002	344,729	6	6
E. californica (*P. muricata*) 13	767,746	88,600	44,655	cupulée	51,342	pointue	_	49,879	65,936	cupulée	48,384	pointue	125,259	195,995	45,817	cupulée	1985,927	345,130	6	6
E. californica (*P. muricata*) 14	_	_	_	cupulée	_	_	_	_	51,769	cupulée	60,496	pointue	_	_	39,994	cupulée	2064,228	_	6	6
E. californica (*P. muricata*) 15	714,647	108,373	50,918	cupulée	47,676	pointue	_	66,809	94,004	cupulée	91,076	pointue	119,113	183,359	56,630	cupulée	1913,266	333,583	6	6
E. californica (*P. muricata*) 16	702,344	107,435	53,175	cupulée	60,000	pointue	_	_	90,000	cupulée	92,204	pointue	118,469	_	52,089	cupulée	_	332,589	6	6
E. californica (*P. muricata*) 17	797,121	100,882	41,403	cupulée	49,828	pointue	1197,577	53,197	69,290	cupulée	74,754	pointue	137,267	206,636	53,781	cupulée	_	351,709	6	6
E. californica (*P. muricata*) 18	_	_	44,050	cupulée	_	_	_	_	74,611	cupulée	_	pointue	_	_	49,389	cupulée	_	322,293	6	6
E. californica (*P. muricata*) 19	768,974	99,789	56,322	cupulée	55,972	pointue	1154,978	55,552	92,693	cupulée	70,305	pointue	131,263	200,517	57,527	cupulée	1988,433	_	6	6
E. californica (*P. muricata*) 20	860,826	120,933	76,950	cupulée	64,036	pointue	1323,488	55,860	113,817	cupulée	83,413	pointue	128,678	_	82,699	cupulée	2133,899	350,477	6	6
E. californica (*P. muricata*) 21	_	_	_	_	_	_	_	_	_	_	_	_	_	_	77,460	cupulée	2171,331	_	6	6
E. californica (*P. muricata*) 22	927,846	128,912	68,775	cupulée	53,491	pointue	1450,417	51,240	97,197	cupulée	72,636	pointue	140,297	212,506	69,089	cupulée	2176,070	371,650	6	7
E. californica (*P. muricata*) 23	_	116,190	56,186	cupulée	54,266	pointue	_	50,341	84,948	cupulée	77,442	pointue	132,465	204,604	69,377	cupulée	1953,213	346,153	6	6
E. californica (*P. muricata*) 24	_	110,368	59,591	cupulée	62,889	pointue	_	54,112	81,882	cupulée	63,665	pointue	135,551	_	60,375	cupulée	2100,399	349,500	6	6
E. californica (*P. muricata*) 25	_	106,199	45,969	cupulée	59,874	pointue	_	60,059	61,335	cupulée	45,893	pointue	138,912	202,532	31,896	cupulée	2173,164	364,112	6	6