

Identification, distribution, and genetic diversity of olive lace bugs and olive flea beetles in the Western Cape province of South Africa

By
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The image shows the crest of Stellenbosch University, which is a shield with various symbols, topped with a crown and surrounded by a decorative border.

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Declaration

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Abstract

South Africa is a minor contributor to the olive industry globally. However, that has not stopped the country from making a mark in the industry by achieving several awards for producing the world's finest olive oil. Although the production of olives in South Africa is still new compared to Europe, it has created employment for many and has alleviated poverty in surrounding communities. The production of olives has its fair share of challenges, which include stress from insect pests and diseases. Olive lace bugs and olive flea beetles are considered pests of the olive industry in South Africa, but little is known about these pests and their impact on the production of olives. The similarity between wild African olive trees and cultivated olive suggests that these pests, along with other species associated with olives, jumped from wild olive trees to cultivated olive trees.

The study aimed to contribute to the catalogue of entomofauna associated with wild and cultivated olives in South Africa by identifying olive lace bug and olive flea beetle species using morphological and DNA-based methods in which four olive lace bug species (*Cystechila lineata*, *Neoplerochila paliatseasi*, *Neoplerochila sp.*, and *Plerochila australis*) and two olive flea beetles (*Argopistes capensis* and *Argopistes sexvittatus*) were identified. Phylogenetic analyses and estimates of intra- and inter-specific genetic divergences were determined using novel and publicly available DNA barcodes for the family Tingidae for olive lace bugs, and the tribe Alticini for olive flea beetles. Novel mitochondrial genomes for olive lace bugs and olive flea beetles were generated and the phylogenetic position of olive lace bugs and olive flea beetles within their respective family or tribe. In addition, a survey was conducted for the purpose of identifying olive lace bug and olive flea beetle species distributed in olive orchards farms in the Western Cape.

Phylogenetic analysis and genetic divergence supported the morphological identification of olive lace bugs and olive flea beetles. The complete mitogenomes of olive lace bugs and olive flea beetle species in South Africa were sequenced. The phylogenetic position of olive lace bugs and olive flea beetles was inferred in context of other complete and partial mitogenomes available within their family/tribe. The four olive lace bugs and two olive flea beetles formed clusters of closely related species in their respective phylogenetic trees. These results show that the group of olive lace bugs and the group of olive flea beetles have a recent common mitochondrial ancestor and indicate that adaptation to feeding on *Olea* may have a common ancestral evolutionary origin.

Opsomming

Suid Afrika lewer wêreldwyd 'n geringe bydrae tot die olyfbedryf. Dit het die land egter nie gekeer om 'n merk in die bedryf te maak deur verskeie toekennings te behaal vir die vervaardiging van die beste olyfolie in die wêreld nie. Alhoewel die produksie van olywe in Suid -Afrika nog nuut is in vergelyking met Europa, het dit vir baie werksgeleenthede geskep en armoede in omliggende gemeenskappe verlig. Die produksie van olywe het baie uitdagings wat stres van insekplae en siektes insluit. Olyf kantgoggas en olyf vlooiwers word beskou as plae in die olyfbedryf in Suid Afrika; min is egter bekend oor hierdie plae en die impak daarvan op die produksie van olywe. Die ooreenkoms tussen die wilde Afrika-olyfbome en gekweekte olyf dui daarop dat hierdie plae saam met ander spesies wat met olywe verband hou, van wilde olyfbome na gekweekte olyfbome spring.

Die doel van die studie was om by te dra tot die katalogus van entomofauna wat verband hou met wilde en gekweekte olywe in Suid Afrika deur die identiteit en die bevestiging van die identiteit van die olyf kantgoggas en die olyf vlooiwer te identifiseer en te bevestig met behulp van morfologiese en DNS-gebaseerde metodes waarin vier olyf kantgoggas (*Cystechila lineata*, *Neoplerochila paliatseasi*, *Neoplerochila sp.* en *Plerochila australis*) en twee olyf vlooiere (*Argopistes capensis* en *Argopistes sexvittatus*) geïdentifiseer is. Filogenetiese ontledings en ramings van intra- en interspesifieke genetiese afwykings is bepaal met behulp van nuwe en in die openbaar beskikbare DNS-strepieskodes vir die familie Tingidae vir die olyf kantgogga, en die stam Alticini vir olyf vlooiwers. Nuwe mitochondriale genome vir olyfkantbesies en olyfvlooiwers is gegenereer en die filogenetiese posisie van olyfkantbesies en olyfvlooiwers binne hul onderskeie familie of stam. Daarby, is 'n opname gedoen met die oog op die identifisering van spesies van olyf kantgogga en olyf vlooiwers wat op olyfboorde in die Wes Kaap provinsie versprei word.

Filogenetiese groepering en p-afstande ondersteun die morfologiese identifikasie van olyf kantgogga en olyf vlooiwers. Die volledige mitogenome van olyf kantgogga en spesies olyf vlooiwer in Suid Afrika is opgestel. Die filogenetiese posisie van olyf kantgogga en olyf vlooiwers is afgelei in verband met ander volledige en gedeeltelike mitogenome wat binne hul familie/stam beskikbaar is. Die vier olyf kantgoggas en twee olyf vlooiwers het 'n groep naverwante spesies in hul onderskeie filogenetiese bome gevorm. Hierdie resultate toon aan dat die groep olyf kantgogga en die groep olyf vlooiwers 'n onlangse algemene mitochondriale voorouer het en dui aan dat aanpassing by die voeding van *Olea* 'n gemeenskaplike evolusionêre oorsprong kan hê.

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Abbreviations

% - Percentage

°C – Degrees Celsius

3' - Three-prime

5' - Five-prime

A – Adenine

AA – Amino acid

ATP6 – Adenine triphosphate synthase membrane subunit 6

ATP8 – Adenine triphosphate synthase membrane subunit 8

BBP – Bayesian posterior probabilities

BOLD – Barcode of Life Database

Bp – Base pairs

C – Cytosine

CIPRES – Cyberinfrastructure for Phylogenetic Research

COI / COX1 – Cytochrome oxidase subunit 1

COII / COX2– Cytochrome oxidase subunit 2

COIII / COX3 – Cytochrome oxidase subunit 3

CYTB – Cytochrome b

DHU – dihydrouridine

DNA – Deoxyribonucleic acid

F – Forward primer

G – Gamma distribution

G – Guanine

GTR – General Time-Reversible model

H – haplotype diversity

I – proportion of invariable sites

IF – Infestation rate

IOOC – International Olive Oil Council

ITS – Internal transcribed spacer

k – number of haplotypes

K2P – Kimura 2-parameter

Ka – number of nonsynonymous substitutions

Ka/Ks – ratio of the number of nonsynonymous to the number of synonymous substitutions

Ks – number of synonymous substitutions

MAFFT – Multiple alignment using fast Fourier transform

MEGA X – Molecular Evolutionary Genetic Analysis X

min – minute

ML – Maximum-likelihood

mM – Millimolar

ND1 – NADH dehydrogenase subunit 1

ND2 – NADH dehydrogenase subunit 2

ND3 – NADH dehydrogenase subunit 3

ND4 – NADH dehydrogenase subunit 4

ND4L – NADH dehydrogenase subunit 4L

ND5 – NADH dehydrogenase subunit 5

ND6 – NADH dehydrogenase subunit 6

NGS – Next-generation sequencing

NJ – Neighbour-joining

NUMTs – nuclear mitochondrial DNA origin

PCG12 – Protein-coding genes excluding the 3rd codon position

PCG123 – Protein-coding genes including all codon positions

PCGs – Protein-coding genes

PCR – Polymerase chain reaction

R – Reverse primer

RNA – Ribonucleic acid

rRNA – Ribosomal ribonucleic acid

S – number of polymorphic sites

SD – Standard deviation

SNPS – single nucleotide polymorphisms

T – Thymine

tRNA – Transfer ribonucleic acid

™ – Trademark

μL - Microlitre(s)

μM – Micromolar

π – nucleotide diversity

Chapter 1 Study overview, rationale, and outcomes

1.1 GENERAL INTRODUCTION

The olive (*Olea europaea* L.) is the main cultivated species in the family Oleaceae, which comprises 30 genera and ~600 species (Chiappetta and Muzzalupo, 2012). The olive tree has been widely cultivated for its fruits and the oils expressed from its fruits. The uses of the fruit tree are not limited to being used as food but include their provision of active phenolic compounds and fine wood, and for their ornamental value. The cultivation of olives has been dated back to about six millennia ago, linked with the early emergence of ancient civilizations (Besnard *et al.*, 2018). Its domestication is considered to have occurred in the Near East of the Mediterranean basin and spread to other regions around the Mediterranean basin and beyond (Zohary and Spiegel, 1976). Today, several cultivars are grown for the production of high-quality olive oil and table olives (Bartolini *et al.*, 2005).

The global annual consumption of olive oil is between 3.1 and 3.2 million tons (IOOC, 2019). The largest olive producers in the world include Spain, Italy, and Turkey. The leading African producer is Morocco, followed by Tunisia, Algeria, Libya, and Egypt (Saleh, 2021). The economic importance of the fruit tree is not completely recognized in South Africa as the olive industry in the country is relatively new and a minor contributor globally (Louw, 2019). Even so, it has indirectly contributed to the economy of the country through job creation, skills development, and poverty alleviation. Despite being a small player in the olive industry, South Africa has won several awards for producing some of the world's finest olive oil and has become a major quality olive oil-producing country (Tinline, 2021).

Olive production, much like all other crop production, is challenged by biotic and abiotic stresses. Biotic stresses, particularly insect pests, and diseases cause severe damage to the fruit tree affecting yield and quality. Agricultural losses in olive production as a result of biotic stress have been estimated to be as high as 30%, with half being caused by insect pests (Bueno and Jones, 2002). The largest producers of the world's olives are free of olive pests. Few serious pests of olives are native to South Africa and Australia (Addison *et al.*, 2015; Drake & Ruhoff, 1960; Langley *et al.*, 2020; Powell *et al.*, 2020).

Olive lace bugs (Hemiptera: Tingidae) known as "tingids" are small sap-sucking insects that damage the leaves when they feed on the olive trees (Addison *et al.*, 2015). These insects are known to cause damage to leaves, which manifests as yellow spotting, with loss of vigour, leaf fall, and ultimately reduces fruit yield for about 1-2 years (Addison *et al.*, 2015). Tingids are a poorly studied taxonomic group in South Africa. Previously, one species

(*Plerochila australis*) was reported to be the only species affecting olive orchards in the Western Cape province (Addison *et al.*, 2015; Costa, 1998). Another species, *Neoplerochila paliatseasi* was later identified (Langley *et al.*, 2020). Evidence was gathered that supports the presence of at least two other distinct species (*Cysteochila lineata* and *Neoplerochila* sp.) in the Western Cape province of South Africa.

Olive flea beetles (Coleoptera: Chrysomelidae) are phytophagous, leaf-mining insects that infest olive trees. Olive flea beetles attack young growth and can stunt the growth of young trees (Addison *et al.*, 2015). There are several *Argopistes* species associated with Oleaceae, however, three species (*A. capensis*, *A. sexvittatus*, and *A. oleae*) native to South Africa have been reported to be pests of olive orchards in the Western Cape province (Addison *et al.*, 2015)

This project represents a significant addition to the comprehensive description of the entomofauna associated with olive trees based on morphological, genetic, and infestation data that has been assembled for other olive insects (e.g. olive fruit flies, parasitoids, and seed wasps) (Teixeira da Costa *et al.*, 2019; Powell *et al.*, 2020; Langley *et al.*, 2020). The focus of the study was to contribute to the catalogue of olive entomofauna in South Africa and to provide olive growers in the Western Cape with foundational information on the diversity of phytophagous pests affecting the crop.

1.2 AIMS AND OBJECTIVES

The study aims to contribute to a comprehensive catalogue of the entomofauna associated with wild and cultivated olives in South Africa, primarily focusing on species identification and genetic diversity of some species in the Western Cape. The following objectives were set out to achieve this aim:

1. To conduct a survey of olive lace bug and olive flea beetle infestations in some Western Cape olive orchards.
2. To identify species of olive lace bugs and olive flea beetles affecting wild and cultivated olives in the Western Cape using the integrated approach of morphological identification and DNA barcoding.
3. To determine the genetic diversity and phylogenetic position of South African olive lace bugs and olive flea beetles within their respective family or tribe.

1.3 CHAPTER LAYOUT

This thesis is divided into five chapters: introduction, literature review, two research chapters, and a conclusion. The references of all chapters are given at the end of the document.

Chapter 1: Introduction

A general introduction of the research, aims and objectives, an overview of the layout of the chapters, and the research outputs generated throughout the research.

Chapter 2: Literature review

An overview of the literature on the origin and distribution of olives, the importance of olives, the production of olives globally and in South Africa, threats to the olive industry focusing on pests, and molecular methods.

Chapter 3: Species diversity and phylogenetic relationships of olive lace bugs (Hemiptera: Tingidae) found in South Africa

The combination of morphological and molecular techniques was used to identify olive lace bugs species. The complete mitochondrial genomes of three olive lace bugs species were recovered by NGS and used for phylogenetic reconstruction to infer evolutionary relationships within the Tingidae.

*Chapter 4: Mitogenomics and phylogenetic position of the olive flea beetles *Argopistes capensis* and *Argopistes sexvittatus* (Coleoptera: Chrysomelidae) and an overview of DNA barcoding in the Alticini*

The combination of morphological and molecular techniques was used to identify two olive flea beetle species. The complete mitochondrial genomes of two olive flea beetle species were recovered by NGS and used for phylogenetic reconstruction to infer evolutionary relationships within the tribe Alticini.

Chapter 5: Conclusion

General concluding remarks about the study.

1.4 RESEARCH OUTPUTS

Article in international peer-reviewed journal

Hlaka, V., Guilbert, É., Smit, S.J., van Noort, S., Allsopp, E., Langley, J., van Asch, B. Species diversity and phylogenetic relationships of olive lace bugs (Hemiptera: Tingidae) found in South Africa. *Insects* 2021, 12, 830. doi:10.3390/insects12090830

This publication forms part of Chapter 3. The supplementary data can be accessed online at:

<https://www.mdpi.com/article/10.3390/insects12090830/s1>

Conference contributions

Oral presentation summarising the research performed in Chapter 3, presented by V. Hlaka

Hlaka, P.P.V., Guilbert, É., van Noort, S., Allsopp, E., Langley, J., van Asch, B. Identification, distribution, and genetic diversity of olive lace bugs (Hemiptera: Tingidae) in Western Cape province of South Africa. Entomological Society of Southern Africa 22nd Congress. Tshipise, South Africa. June 28 – July 01, 2021.

Conference proceedings paper summarising the research performed in Chapter 3

Hlaka, V.; van Asch, B. DNA barcoding of olive lace bugs (Hemiptera: Tingidae) reveals previously unknown species diversity in South Africa, in Proceedings of the 1st International Electronic Conference on Entomology, 1–15 July 2021, MDPI: Basel, Switzerland, doi:10.3390/IECE-10610

Chapter 2 Literature Review

2.1 INTRODUCTION

Olive trees, *Olea europaea* L. are evergreen trees that belong to the family Oleaceae which is comprised of 24 genera and 900 species (Sahin and Bilgin, 2017). Within the tribe Oleaeae, *Olea* is the main cultivated species. The olive (*O. europaea* subsp. *europaea*) includes the wild form, *Olea* subsp. *europaea* var. *sylvestris*, and the cultivated form, *Olea europaea* subsp. *europaea* var. *europaea*. *Olea europaea* L. is a complex of species in which six subspecies associated with specific often isolated geographical areas are recognised. These include *Olea europaea* subsp. *cerasiformis*, *O. e. cuspidata*, *O. e. europaea*, *O. e. guanchica*, *O. e. laperrinei*, and *O. e. marocanna* which are composed of different morphotypes associated with specific often isolated geographical areas (Chiappetta and Muzzalupo, 2012) (Figure 2.1)

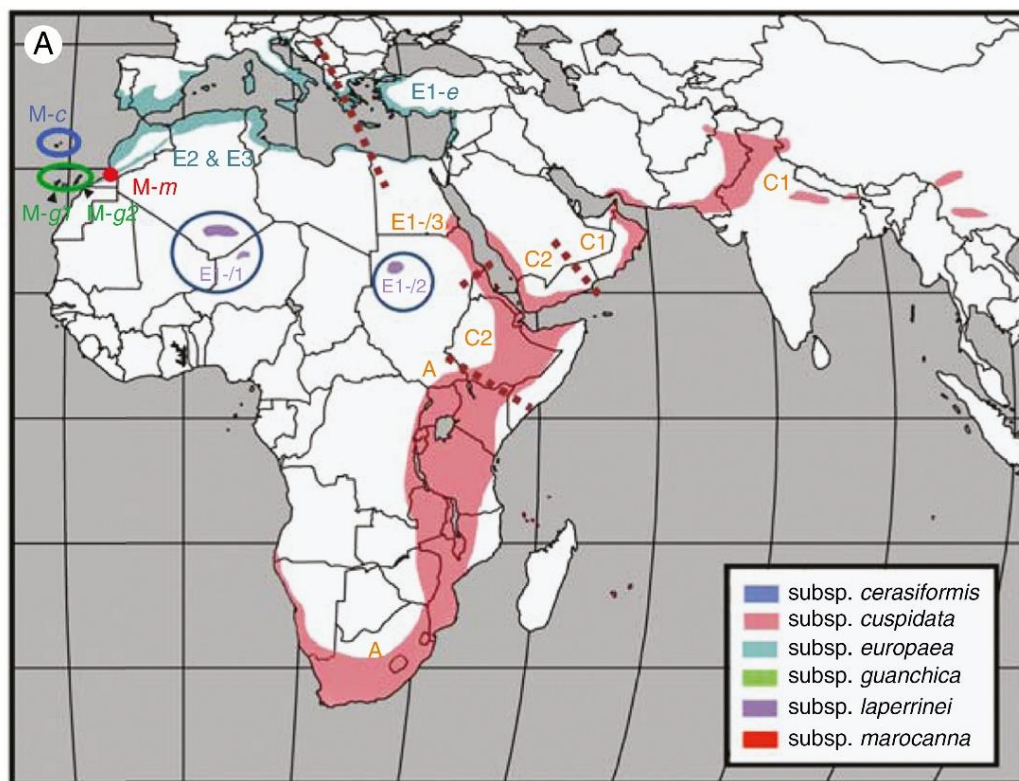


Figure 2.1 Geographical distribution of the olive complex (*Olea europaea* L.) in which six subspecies are currently recognized (according to Rubio de Casas et al., 2006). Reproduced from Besnard et al. 2018.

Olea europaea subsp. *cerasiformis* (Webb & Berth.) Kunk. & Sund. is found in Madeira; *Olea europaea* subsp. *cuspidata*, African wild olive (Wall ex. G. Don) Cif. is distributed in southern and eastern Africa, parts of Asia and China and Arabia; *Olea europaea* subsp. *europaea* is native to the Mediterranean basin; *Olea europaea* subsp. *guanchica* is native to the Canary Islands (Vargas, Hess, Muñoz, & Kadereit); *Olea europaea* subsp. *laperrinei* (Batt. & Trab.) Cif. is distributed in the Saharan mountains; and *Olea europaea* subsp. *maroccana* Greut. & Burd. is distributed in Southwestern Morocco (Besnard *et al.*, 2002). These subspecies are diploid, except for the polyploid *maroccana* and *cerasiformis* (Besnard *et al.*, 2018). The six subspecies are considered to be the primary genetic resource for cultivated olives. Currently, there are several cultivars including Barouni, Frantoio, Kalamata, Manzanilla, and Mission (Costa 1998). Mission is the most common cultivar in the Western Cape because it is suitable for its oil and table olives.

2.2 ORIGIN AND DISTRIBUTION OF THE CULTIVATED OLIVE

The olive has played a vital role in the lives of humankind for many centuries and has been at the core of the development of food and culture. The origin of the olive has been traced back to the Middle East and has been established in countries bordering the Mediterranean Sea and, in many areas, suitable for its cultivation. Over time, the cultivation of the olive spread to the Fertile Crescent then to Israel, Turkey, Egypt, Aegean, Greece, Balkans, Spain, and Italy. Following the discovery of America, the cultivation of olives spread from the south to the north of America in countries including Peru, Argentina, Uruguay, Chile, Mexico, and the United States of America. More recently, the production of olives has been established in other countries without prior traditions of olive oil consumption. Currently, the cultivation of olive is also found in South Africa, Australia, and New Zealand, and China (Figure 2.2) (Cimato 2008). The olive was introduced to South Africa by Jan van Riebeeck, the first Dutch governor of the Cape colony in the 17th century (Karsten, 1955). The current olive industry in South Africa was established in the early 1900s by Ferdinando Costa, an Italian immigrant after observing the natural growth of the wild African olive (*Olea europaea* subsp. *cuspidata*). Ferdinando imported known cultivars from Italy and propagated the cultivars using the indigenous wild olive seedlings as rootstock. He bought a farm in Paarl where he continued to cultivate olives and process olive oil. The greater part of the olive industry is situated in Paarl (Costa, 1998).



Figure 2.2 Geographical distribution of olive-producing regions. Reproduced from Cimato et al. 2011.

2.2 IMPORTANCE OF OLIVES

The olive is one of the oldest crop trees and has been cultivated for many years because of its various uses and benefits. The fruits are economically important in the Mediterranean region and other regions with similar climate conditions for their source of olive oil and table olives (Besnard *et al.*, 2018). The olive is cultivated for its many uses including its use in religious practices, cosmetic and medicinal products. Olives are rich in antioxidants and vitamins and have been used for a variety of health benefits such as lowering cholesterol and blood pressure (Lopez-Lopez *et al.*, 2010). The olive industry represents an important source of income for many families that own these farms and the communities surrounding the farms that are employed directly as farm workers or indirectly in the supply chain of olives.

2.3 MORPHOLOGICAL CHARACTERIZATION AND THE NUTRITIONAL COMPOSITION OF OLIVES

The olive fruit is a single-seeded drupe with a fleshy outer layer composed of three anatomical parts which include the epicarp (protective tissue), mesocarp (pulp), and the endocarp (kernel) containing the seed. The color of the epicarp changes with ripening from a bright green to a purplish-black colour at maturity. The colour change is due to the accumulation of major pigments such as chlorophylls, carotenoids, and anthocyanin (Bianchi 2003). The edible portion of the fruit consist of the epicarp and mesocarp. The mesocarp is the reserve tissue of all constituents including water and fats (Bianchi 2003). The nutritional composition of olives is determined by the ripening stage, processing method, and agronomical factors (Pereira *et al.* 2006).

2.3.1 Ripening stage

The ripening stage in which the fruits of olives are harvested is dependent on the processing method. However, most olives are harvested during mid-autumn when they are green to yellow in colour and firm (Uylaser & Yildiz 2014). As ripening progresses, the fat content increases as the water content decrease. Therefore, green olives will have a lower fat content than black olives. The phenolic compounds and pigmentation change as the fruit ripens. The phenolic compound, oleuropein increases to its maximum level at mature green (Bianchi 2003).

2.3.2 Processing method

Olives are harvested “green ripe” and are regarded inedible because of the phenolic compound oleuropein responsible for the bitter taste. Olives require processing before being released to the market as edible table olives and olive oil. There are three international processing methods used which include the Californian processing method, the Spanish processing method, and the Greek processing method (Pereira *et al.* 2006; Uylaser & Yildiz 2014).

2.3.3 Agronomical factors

The composition of olives is dependent on cultivar type, geographic origin, agricultural practices, agroclimatic condition, and irrigation management. The cultivar type has the most influence on the nutritional composition and varies in size, shape, fat content, and flavor. The phenolic composition differs between cultivars (Ghanbari *et al.* 2012).

2.3.4 Nutritional characteristics of olives

Olives are rich in vitamins, flavonoids, and phenolic substances. Several studies have found in the Mediterranean region a low prevalence of cardiovascular disease due to the intake of olive-based products (Uylaser & Yildiz 2014). A significant amount of phenolic compounds serve as antioxidants and free radical scavengers (Lopez-Lopez *et al.*, 2010). Lipids are a major component of olives. The protein content in olives is low, however, essential amino acids such as glutamic acid and aspartic acid are present. Carbohydrates are absent due to their removal during processing (Lopez-Lopez *et al.*, 2010). Olives are an important source of dietary and fiber. The mineral contents vary within cultivars (Lopez-Lopez *et al.*, 2010).

2.4 THE PRODUCTION OF OLIVES GLOBALLY

The European Union (EU) is the largest contributor to the olive industry worldwide. The EU includes nine olive-producing countries (Spain, Italy, Greece, Portugal, France, Slovenia, Croatia, Cyprus, and Malta) that account for a total of 5 million hectares of olive groves in which the majority is dedicated for the production of olive oil. Spain is the largest producer of olive oil in the EU which accounts for 63% of the EU olive production followed by Italy (17%), Greece (14%), and Portugal (5%) (Figure 2.3)(European Commission 2020). According to the International Olive Oil Council (IOOC, 2021), the production of olive oil worldwide

was estimated at 3,197,000 tonnes with the EU contributing 2,232,500 tonnes (69.8%) (Figure 2.4 A) (IOOC, 2021). The production of table olives worldwide was estimated at 3,134,000 tonnes with the EU producing the most table olives (28.4%) while other countries producing a great amount included Egypt (26.0%), Turkey (14.0%), Algeria (10.0%), Morocco (4.0%) and Syria (3.0%) (Figure 2.4 B) (IOOC, 2021).

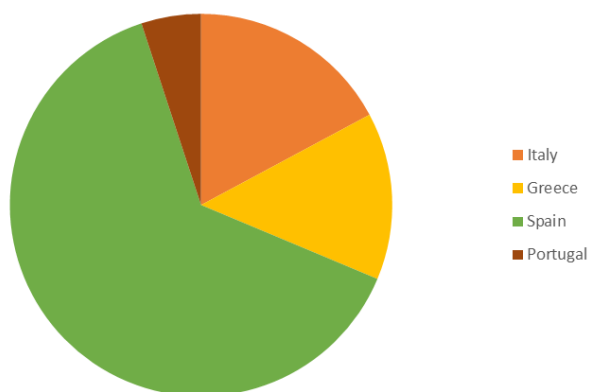


Figure 2.3 The production of olive oil in the European Union

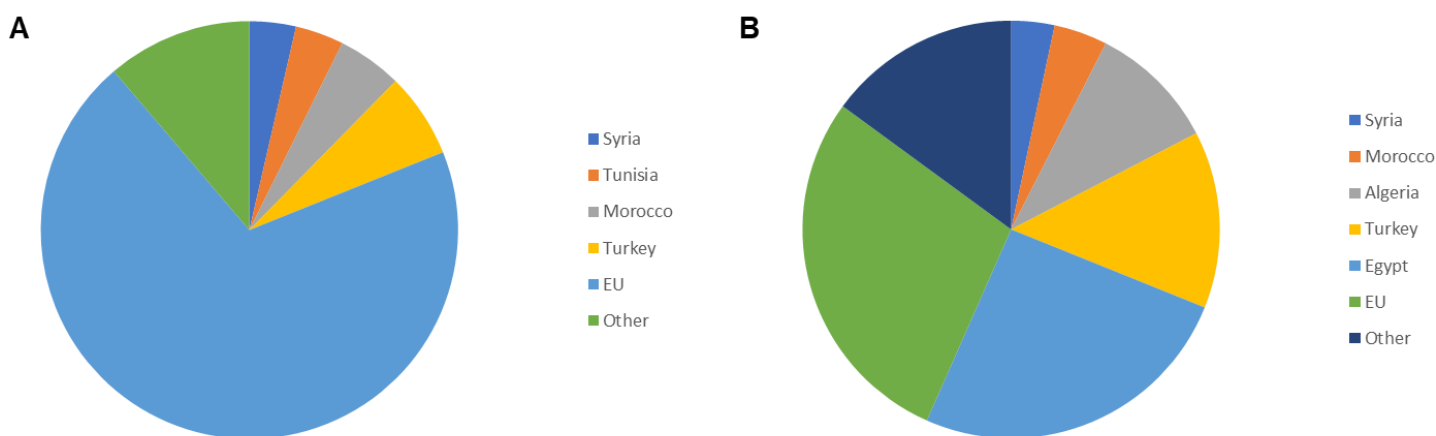


Figure 2.4 Worldwide olive oil and table olive production in 2020/21 (IOOC). **(A)** The production of olive oil worldwide. **(B)** The production of table olives worldwide.

2.5 THE PRODUCTION OF OLIVES IN SOUTH AFRICA

The olive industry has the potential to grow in South Africa, as cultivated olives are suitable crops in the Western Cape because they can be farmed with less water in comparison to other deciduous fruits. Hence, the production of olives is suitable for areas that have fewer amounts of water available for agricultural practices. Despite olives being introduced in the late 17th century in South Africa, the olive industry only started gaining momentum in the 1970s. The rapid growth of the olive industry is due to awareness raised about the health benefits of olives, and increased research on the nutritional value and active compounds present (Costa 1998). Currently, 2 849 hectares of olives are farmed in South Africa. This is a 110% increase over the past ten years from 2008, where previously it was 1 357 hectares. In 2018, there were over 170 olive growers with only 11 growers being the largest with more than 50 hectares each, and the rest farming less than five hectares. The Western Cape is a major contributor to the olive industry in South Africa producing 93% of the country's olive oil. However, South Africa remains a minor contributor to the production of olives globally (Louw 2019). South Africa imports about ~7,000 tonnes of olive oil and 70% of the olive oil are imported from Spain with 19% from Italy. South Africa contributes only 2% of the olive oil imports into the country. The most planted olive cultivar in South Africa is Frantoio (749 hectares), reducing the planting of other cultivars. The second most popular cultivar is Mission (659 hectares), used for the production of both oil and table olive (Figure 2.5) (Louw 2019).



Figure 2.5 Common olive cultivar in South Africa, Mission. Image credit: Hans Braxmeier

2.6 PEST THREATS TO THE OLIVE INDUSTRY

The increased trade and movement of goods, variation in climate conditions, and changes in farming practices have promoted the introduction, re-emergence, and spread of pests and diseases. Several insect pests, diseases, and nematodes affect olives causing moderate to severe damage to olive production (Landa 2019). Various arthropods cause damage to olive trees. The pests are either polyphagous (damaging more than one host species), oligophagous (feeding on a few species), or monophagous (feeding on a single species) to the olive tree, and not all of these pests are equally injurious. Polyphagous pests include the occasional pests on the olives whereas monophagous and oligophagous pests cause the greatest economic losses and pose a threat to olive yield annually (Landa, 2019).

2.6.1 *Insect pests affecting olive orchards overseas*

2.6.1.2 Olive fruit flies

The olive fruit fly, *Bactrocera oleae* (Rossi) is monophagous and one of the most important olive pests responsible for up to 15% of production losses (Figure 1.6 A). The pest is thought to have originated from Africa and has spread to other important olive-producing areas including the Mediterranean basin and south-central Asia. The larvae of fruit flies feed on olive fruits which can cause premature fruit ripening or drop and loss of quality (Mkize *et al.*, 2008).

2.6.1.2 Olive lace bugs

The olive lace bug, *Froggattia olivina* (Horvath) (Hemiptera; Tingidae) is an agricultural pest of cultivated olives in Australia. The species is native to Australia and reported to have moved from its native host, *Notelaea longifolia* to the cultivated olive. *Froggattia olivina* feeds on the underside of leaves olives which results in yellow chlorotic spotting and severe cases can lead to the defoliation of the host (Spooner-Hart 2005).

2.6.2 *Insect pests affecting olive orchards in South Africa*

Olive growers in South Africa are fortunate that there are few local pests of cultivated olives. These include olive fruit flies, seeds wasps, olive lace bugs, and olive flea beetles (Mkize, 2009; Powell *et al.*, 2019; Teixeira da Costa *et al.*, 2019). Other pests include longhorn beetles, moths, and scale insect. These pests are native to South Africa and are thought to have jumped from the wild African olive to the cultivated olive.

2.6.2.1 Olive fruit flies

The olive fruit flies cause less harm to South African olive production in comparison to other olive-producing regions. This is likely due to the presence of parasitoid wasps in southern and eastern Africa (Mkize, 2009). The presence of parasitoid wasp species controls olive fruit fly populations from reaching economically damaging levels in cultivated olives. The diversity of the parasitoid wasps is high in South Africa and has been understudied (Mkize, 2009).

2.6.2.2 Olive seed wasps

Olive seed wasps cause premature fruit drop in olive orchards and are responsible for the production losses. Three olive seed wasps have been confirmed in the Western Cape and include *Eupelmus spermophilus*, *Eurytoma oleae*, and *Sycophila aethiopica* (Figure 2.6 B) (Powell *et al.*, 2020).

2.6.2.3 Olive flea beetles

Olive flea beetles are leaf miners that cause damage to the leaves of olive trees. At least six species of flea beetles associated with wild and cultivated olives have been identified which include *Argopistes capensis* (Bryant), *Argopistes epomistus* nov. sp., *Argopistes lilliputiamus* nov. sp., *Argopistes melanus* nov. sp., *Argopistes oleae* (Bryant), and *Argopistes sexvitattus* (Bryant) (Mkize 2009). However, three olive flea beetle species have been reported to be pests of olives trees including *A. capensis* (Bryant), *A. oleae* (Bryant), and *A. sexvitattus* (Bryant) (Figure 2.6 C – D) (Addison *et al.*, 2015).

2.6.2.4 Olive lace bugs

Neoplerochila paliatseasi and *Plerochila australis* (Hemiptera: Tingidae) are the only olive lace bugs reported infesting wild and cultivated olives in South Africa (Figure 2.6 E - F) (Langley *et al.* 2020). Olive lace bugs feed on the underside of the leaves of olive trees causing chlorotic spotting on the leaves and may result in the reduction of fruit yield.

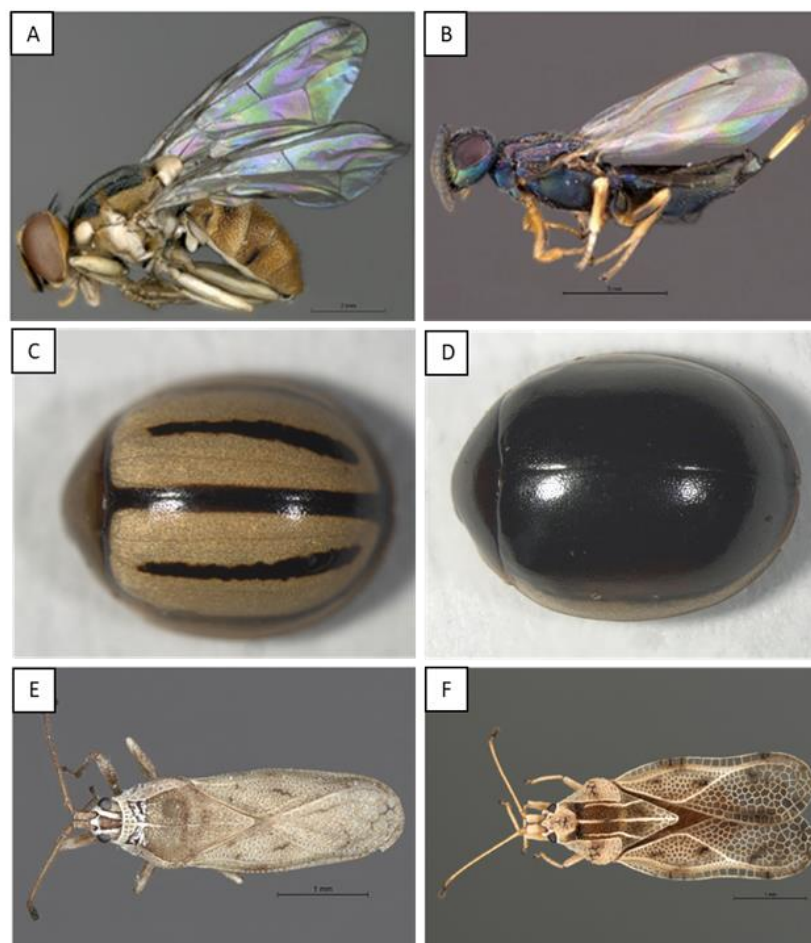


Figure 2.6 Olive pests in South Africa. **(A)** Olive fruit fly, *Bactrocera oleae* by Chante Powell; **(B)** Olive seed wasp, *Eupelmus spermophilus* by Powell et al. (2019); **(C)** Olive flea beetle, *Argopistes sexvitattus* striped morphotype by Beth Grobbelaar; **(D)** Olive flea beetle, *Argopistes sexvitattus* black morphotype by Beth Grobbelaar; **(E)** Olive lace bug, *Plerochila australis* by Hlaka et al., (2021); and **(F)** Olive lace bug, *Neoplerochila paliatseasi* by Langley et al., (2020).

2.7 IMPORTANCE OF SPECIES IDENTIFICATION OF INSECT PESTS OF OLIVES

Insect pests present in olive orchards are often managed using a broad spectrum of insecticides, regardless of whether species have been identified or a proper damage assessment on orchards has been made. The excessive use of chemical pesticides threatens biodiversity and may lead to environmental contamination and health hazards (Landa, 2019). Unnecessary treatments can also lead pests to develop resistance to insecticides. Hence the correct identification of pests is important to ensure that the

appropriate control measure is followed should the pest be known and developed should the pest be unknown. It is necessary to identify pests to adopt an agricultural management practice that gives the best results. Morphological identification is time-consuming and requires trained taxonomists or entomologists for species determination (Barrett and Hebert 2005). However, misidentifications, distinguishing species at immature or developmental stages, damaged samples due to improper handling, cryptic diversity, and species differing by minor morphological characters can make identification challenging and sometimes almost impossible (Ball and Armstrong, 2006; Barrett and Hebert, 2005). Molecular methods, such as DNA barcoding and mitogenomics are available and allow species identification at any developmental stages. These approaches have been successful in identifying a wide range of insects of Arthropoda (Raupach & Radulovici 2015).

2.8 MOLECULAR METHODS FOR SPECIES IDENTIFICATION

For many years, insect identification has been based on traditional taxonomy. The difficulty experienced in morphological identification led to the use of molecular methods. In molecular techniques, molecular markers are routinely used for accurate species identification of organisms. The use of molecular markers in phylogenetic analysis can also infer evolutionary relationships between taxa. Several molecular markers including mitochondrial and nuclear markers are available for the phylogenetic analysis of insects (Raupach & Radulovici 2015).

There are many advantages of using mitochondrial DNA as a molecular marker such as its strict maternal transmission, high mutation rates, high copy number, lack of recombination, and is highly conserved (Park *et al.*, 2010). These properties make mitochondrial DNA a suitable marker for phylogenetic studies. Molecular methods such as DNA barcoding and mitogenomics rely on the use of mitochondrial DNA (Cameron, 2014; Jimbo *et al.*, 2011).

2.7. 7.1 DNA barcoding

DNA barcoding makes use of a short standardized region referred to as “DNA barcodes” used to provide accurate species identification. The molecular approach is dependent on variation within the short standardized region of a genome and is useful for establishing relationships among taxa (Hebert *et al.*, 2003). In insects barcoding, the COI gene has shown high efficiency for species identification (Wilson, 2012). The COI gene is chosen because it has high insertion/deletion events and high substitution rates which makes it suitable for distinguishing among cryptic species (Barrett and Hebert, 2005). It is a suitable

marker because of its low intraspecific variation than interspecific variation. The barcoding method usually does not require taxonomic expertise however, the method is best used as a complement to classic morphological methods. However, there are limitations to the molecular method. The major limitation of DNA barcoding is that it relies upon barcode reference libraries for taxonomic identification of sequences. It is well known that barcode reference libraries can harbour errors such as misidentification and spelling errors and most databases are still incomplete (Pentinsaari *et al.* 2020).

2.7.2 Mitogenomics

Mitochondrial genomes have been widely used in phylogenetic studies, population genetics, and molecular comparative genomics. In insects, the mitochondrial genome is a circular double-stranded molecule between 14 -20 kb in size, comprising 37 genes including 13 protein-coding genes, 2 rRNA, 2 tRNA, and a control region referred to as the AT-rich region (Boore, 1999). The mitochondrial genome is well organized, conserved, and has been used because of its unique properties such as low recombination rates, maternal inheritance, high copy numbers, and evolutionary frequency (Boore 1999). In addition, the complete mitogenome can provide sets of genome-level characters which include the gene order, structural and compositional genomic features which could be useful in phylogenetic analysis (Boore 1999). The complete mitogenome as a marker instead of single-gene markers has been used extensively to construct robust phylogenies. The use of complete mitogenomes can resolve phylogenetic relationships and provide theoretical support in pest control (Ramirez-Rios *et al.* 2017).

2.8 POPULAR ANALYTICAL METHODS IN PHYLOGENETIC STUDIES

There are so many methods to consider reconstructing phylogenetic trees. Neighbour-joining and UPGMA are clustering methods that produce trees quickly but are the least reliable when dealing with deeper divergence (Saitou and Nei, 1987; Sokal and Michener, 1958). Maximum likelihood and Bayesian approaches are the most robust and commonly used. These methods are character-based and can apply a model of sequence evolution which is ideal for reconstructing phylogenies (Yang and Rannala 2012). Both these methods are also more statistical than other available methods. The choice of a method for phylogenetic reconstruction is dependent on the dataset and the parameters set. Bayesian and Maximum likelihood tend to discriminate less in a dataset with low diversity.

Chapter 3 Species diversity and phylogenetic relationships of olive lace bugs (Hemiptera: Tingidae) found in South Africa

3.1 ABSTRACT

Olive lace bugs (Hemiptera: Tingidae) are small sap-sucking insects that feed on wild and cultivated *Olea europaea*. The diversity of olive lace bug species in South Africa, the most important olive producer in the country, has been incompletely surveyed. Adult specimens were collected in the Western Cape province for morphological and DNA-based species identification, and sequencing of complete mitogenomes. *Cysteochila lineata*, *Plerochila australis*, *Neoplerochila paliatseasi*, and *Neoplerochila* sp. were found at 12 sites. Intra- and interspecific genetic divergences and phylogenetic clustering in 30 species in 18 genera of Tingidae using new and publicly available DNA barcodes showed high levels of congruity between taxonomic and genetic data. The phylogenetic position of the four species found in South Africa was inferred using new and available mitogenomes of Tingidae. Notably, olive lace bugs formed a cluster of closely related species. However, *Cysteochila* was non-monophyletic as *C. lineata* was recovered as a sister species to *P. australis* whereas *Cysteochila chiniana*, the other representative of the genus, was grouped with *Trachypeplus jacobsoni* and *Tingis cardui* in a different cluster. This result suggests that feeding on *O. europaea* may have a common origin in Tingidae and warrants future research on potential evolutionary adaptations of olive lace bugs to this plant host.

3.2 INTRODUCTION

Lace bugs (Hemiptera: Tingidae) comprise approximately 2500 species of small phytophagous insects in 300 genera distributed in all tropical and temperate continental and most oceanic regions except for the frigid zones (Guilbert *et al.*, 2014). Lace bug adults and nymphs feed by piercing the abaxial surface of the leaves of living plants to extract sap from cellular tissues (Drake and Ruhoff, 1960). Continuous feeding can result in chlorotic spots that may necrotize with detriment to plant vitality, and heavy infestations may cause premature death of young shoots and defoliation of the host. Lace bugs are generally monophagous, and a species feeds on the same kind of plant or group of closely related plants, including several agricultural crops and ornamentals.

Sub-Saharan Africa has a rich assemblage of native insects associated with Oleaceae, including several species of olive fruit flies and olive flea beetles, and a diversity of parasitoid, hyperparasitoid, and olive seed wasps (Copeland *et al.*, 2004; Mkize *et al.*, 2008; Powell *et al.*, 2020a; b; Teixeira da Costa *et al.*, 2019). Lace bugs feeding on Oleaceae are only found in sub-Saharan Africa: *Catoplatus dilatatus* Jakovlev (on *Olea* sp.), *Cysteochila pallens* Horvath (on *O. chrysophylla*), *Cysteochila sordida* Stål (on *O. verrucosa*), *Olastrida oleae* Schouteden (on *O. europaea*) (Drake and Ruhoff, 1965), *Cysteochila lineata* Duarte Rodrigues (on *O. capensis*), *Cysteochila nervosana* Drake and *Caffrocysta aliwalana* Duarte Rodrigues (on *Olea europaea* subsp. *cuspidata*), and *Plerochila australis* (Distant) and *Neoplerochila paliatseasi* Duarte Rodrigues (on *O. europaea*) (Göllner-Scheiding, 2007; Langley *et al.*, 2020). The exception to this pattern is *Froggattia olivinia* Froggatt, which is native to Australia and feeds not only on *Notelaea longifolia* (Oleaceae) but also on imported *O. europaea* (Drake and Ruhoff, 1965).

In South Africa, cultivated olives are often grown in proximity to African wild olives (*Olea europaea* subsp. *cuspidata*), which may act as a source of both olive pests and their natural enemies. African wild olives and cultivated olives are closely related hence, most insects associated with African wild olives have been found to also occur on cultivated olives (Powell *et al.*, 2020b). Despite the diversity of the native olive-associated entomofauna, South African olive growers face less aggressive threats from phytophagous insects, namely *Bactrocera oleae* Rossi (Diptera: Tephritidae), than their Mediterranean and Californian counterparts. Globally, South Africa is a small producer of boutique olive products mostly sold locally, but the industry also exports table olives and olive oil to neighbouring African countries, the European Union, and the United States of America. Lace bugs affecting wild and cultivated olive trees in the Western Cape province, the most important region for olive production in the country, are commonly referred to as “olive tingids” by local farmers and have been reported to include *P. australis* (Addison *et al.*, 2015; Costa, 1998) and *N. paliatseasi* (Langley *et al.*, 2020). Perceptions on the extent of olive lace bug injury to cultivated olive trees vary from “olive tingids” being a minor pest that does not require management to a threat that impacts olive production and requires insecticide treatment.

The genus *Neoplerochila* was erected by Duarte Rodrigues (Deckert and Göllner-Scheiding, 2006) to hold the species *inflata* Duarte Rodrigues. *Neoplerochila* is only known from Namibia and South Africa and now includes eight species of which *N. millari* Göllner-

Scheiding, *N. dispar* Duarte Rodrigues, *N. weenenana* (Drake), and *N. paliatseasi* are found on Oleaceae. The hosts of *N. inflata* Duarte Rodrigues, *N. katbergana* Drake, *N. uniformis* Duarte Rodrigues and *N. youngai* Duarte Rodrigues are presently unknown (Göllner-Scheiding, 2007). *Neoplerochila paliatseasi* is probably distributed countrywide in South Africa, as it was found in the Limpopo, North West, and Western Cape provinces (Göllner-Scheiding, 2007; Langley *et al.*, 2020), and in Gauteng province in the present study.

The genus *Plerochila* was erected by Drake (1954) to hold *Plerochila australis* Distant and *P. horvathi* Schouteden as close to the genus *Cysteochila* but differing on the shape of paranota and carinae. *Plerochila* currently includes 17 species restricted to Africa, of which *P. australis*, *P. horvathi*, and *P. rutshurica* Schouteden are known to feed on *Olea*. The host plants of most *Plerochila* species are not known (Deckert and Gollner-Scheiding, 2006). *Plerochila australis* has been reported in Ethiopia, the Democratic Republic of Congo, Kenya, Madagascar, Mauritius, Mozambique, Namibia, Sudan, Tanzania, Uganda, and South Africa (Deckert and Gollner-Scheiding, 2006). In South Africa, *Plerochila australis* is probably distributed countrywide as it was reported in the Western Cape, Northern Cape, Gauteng, Limpopo, Mpumalanga, and North West provinces (Deckert and Gollner-Scheiding, 2006).

The Mediterranean Basin and California are currently free from olive lace bugs, but these may become a threat if translocated from their original geographic range. Since a previous report of *N. paliatseasi* in South Africa (Langley *et al.*, 2020), the species is found to be present on Madeira Island (Portugal), where it feeds on cultivated olives (Aukema *et al.*, 2013), but it seems to be restricted to that insular region and has not been found in mainland Europe.

Under the right circumstances, olive lace bugs can become a problem, as is the case of the native Australian *F. olivina*, which moved to cultivated olive trees and African wild olives when imported plants became established in the country starting in the 19th century (Spooner-Hart, 2005). *Froggattia olivina* is now a serious pest of cultivated olives in New South Wales and Queensland, but it has not yet been reported outside Australia.

This work is part of a larger effort to catalogue the diversity of insect species associated with wild and cultivated olives in South Africa. The main objectives were (1) to gain further insights into the diversity of olive lace bugs found in South Africa and (2) to investigate the phylogenetic position of those species within the family Tingidae using new and publicly available mitogenome sequences.

3.3 MATERIALS AND METHODS

3.3.1 Specimen Collection, Morphological Identification, and DNA Extraction

Olive lace bugs were collected in nine areas in the Western Cape (cultivated and wild olive trees) and one site in Pretoria (wild olive trees) between November 2015 and March 2020 (Table S1). Additionally, eight olive farms in the Western Cape were visited between October 2020 and March 2021 during the South African olive growing season when olive lace bugs are likely to be present. Specimens were collected from cultivated olive blocks identified as infested by farm workers. The number of trees surveyed at each farm varied from 10 to 50, and every second tree was sampled in any given block. Specimens were collected directly into individual plastic tubes, euthanized by freezing, and stored in 100% ethanol at -20°C until downstream analyses. DNA was extracted from individual specimens using a standard phenol-chloroform method (Sambrook and Russell, 2001) and stored at -20°C until further use.

Morphological identification of ethanol-preserved specimens was performed by É. Guilbert following original descriptions, photos of type material, and collections available (Deckert and Gollner-Scheiding, 2006; Göllner-Scheiding, 2007). Representative specimens of *C. lineata*, *P. australis*, *N. paliatseasi*, and a nonspecific *Neoplerochila* were imaged and deposited in the entomological collection of the Iziko Museum (Cape Town) for future reference: *C. lineata* SAM-HEM-A012751, *Neoplerochila* sp. SAM-HEM-A012753, *P. australis* SAM-HEM-A010383, and *N. paliatseasi* SAM-HEM-A011647 (SAMC; Curator Simon van Noort). Codens of institutional depositories of voucher specimens follow Evenhuis (2019). Images were acquired with a Leica LAS 4.9 imaging system, comprised of a Leica[®] Z16 microscope (using either a 2x or 5x objective) with a Leica DFC450 Camera and 0.63x video objective attached. The imaging process, using an automated Z-stepper,

was managed using the Leica Application Suite V 4.9 software. Diffused lighting was achieved using a Leica LED5000 HDI dome.

3.3.2 DNA Barcoding

Specimens of *C. lineata* ($n = 25$), *P. australis* ($n = 32$), *N. paliatseasi* ($n = 11$), and *Neoplerochila* sp. ($n = 14$) were sequenced for the standard COI barcoding region (~650 bp) for assessing the congruency between morphological and DNA-based identifications using genetic clustering analysis and estimates of inter- and intraspecific genetic diversity. New species-specific PCR primers were designed for DNA barcoding of *P. australis* and *C. lineata*, based on their mitochondrial genomes (Table S2). *Neoplerochila paliatseasi* and *Neoplerochila* sp. were barcoded using PCR primers specific to *N. paliatseasi* designed in a previous study (Langley *et al.*, 2020). Initial attempts to barcode *C. lineata* were made using the PCR primers specific to *P. australis* and the PCR primers specific to *N. paliatseasi*, but limited success (see Section 3.5) led to the design of species-specific primers for *C. lineata* once the mitogenome of latter species was assembled. All new species-specific PCR primer pairs anneal to the same COI region as the universal DNA barcoding primers, and were designed to be a perfect match to the COI sequence of each species.

PCR amplifications were performed in a total volume of 5 μ L containing 1 \times of KAPA2G Robust HotStart Ready Mix PCR kit (KAPPA Biosystems), 0.5 μ M of each primer, 0.5 μ L of MilliQ H₂O, and 1.0 μ L of template DNA (~100 ng), as follows: 95 °C for 3 min; 35 cycles of 95 °C for 15 s, 15 s at 58 °C for *C. lineata* and 54 °C for *N. paliatseasi* and *P. australis*, 72 °C for 1 min; and a final extension at 72 °C for 1 min. PCR products were sequenced using the reverse PCR primers specific to each species with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) at the Central Analytical Facilities of Stellenbosch University, South Africa.

3.3.3 Intraspecific and Interspecific Genetic Diversity

All DNA barcodes assigned to Tingidae species were downloaded from the Barcode of Life Database (BOLD) Systems v4 (<http://v3.boldsystems.org/>, accessed on 20 October 2020) to provide a broader context for the intra- and interspecific divergence and genetic clustering patterns of the four olive lace bug species found in South Africa. The initial dataset included 1141 sequences that were subsequently filtered for (a) sequences identified to species level, (b) sequences with a minimum length of 500 bp overlapping the standard COI barcoding

region, and (c) species represented by a minimum of three sequences. The final dataset included 367 sequences representing 30 species in 18 genera, including the new sequences ($n = 82$) generated in this study. Multiple sequence alignments were performed with the MAFFT algorithm (Kato and Standley, 2013) in Geneious Prime v2021.1 (<https://www.geneious.com>, accessed on 20 October 2020).

Genetic clustering of the COI sequence dataset of 30 species was assessed using a maximum likelihood (ML) tree constructed in IQ-Tree (Minh *et al.*, 2020), with *Adelphocoris fasciaticollis* (NC_023796.1) (Hemiptera: Miridae) as an outgroup. The best partitioning scheme was determined using the edge-linked greedy strategy (Lanfear *et al.*, 2012) with automatic model selection (Chernomor *et al.*, 2016; Kalyaanamoorthy *et al.*, 2017) commands (`-m MFP + MERGE`). Branch supports were determined using 1000 replicates for both ultrafast bootstrapping and SH-aLRT branch tests (Guindon *et al.*, 2010; Hoang *et al.*, 2018). The final ML tree was drawn using FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/>, accessed on 4 September 2021).

Intra- and interspecific genetic divergences were estimated as p-distances (%) under the Kimura 2-parameter model (Kimura, 1980) in MEGA X (Kumar *et al.*, 2018), with statistical support calculated from 1000 bootstrap replicates. Intraspecific diversity measures (number of haplotypes, number of polymorphic sites, haplotype diversity, and nucleotide diversity) were calculated with Arlequin 3.5 [29]. Median-joining haplotype networks were constructed with Network 10.2, under the default settings (Bandelt *et al.*, 1999). The new sequences COI generated in this study were deposited on GenBank: *C. lineata* (MZ673445 to MZ673468), *N. paliatseasi* (MZ666853 to MZ666863), *Neoplerochila* sp. (MZ673417 to MZ673429), and *P. australis* (MZ676957 to MZ676987) (Table S1).

3.3.4 Sequencing, Assembly, and Annotation of Mitogenomes

The complete mitochondrial genomes for one specimen each of *C. lineata*, *P. australis*, and *Neoplerochila* sp. were sequenced using the Ion Torrent™ S5™ platform (ThermoFisher Scientific, Waltham, MA, USA) available at the Central Analytical Facilities of Stellenbosch University, South Africa. Sequence libraries were prepared using the Ion Xpress™ Plus gDNA Fragment Library Kit (ThermoFisher Scientific, Waltham, MA, USA), according to the

protocol MAN0009847 REV J.0. Libraries were pooled and sequenced using the Ion 540™ Chef Kit (ThermoFisher Scientific). The NGS reads of each species were mapped against the complete mitogenome of *N. paliatseasi* (MN794065) and assembled using Geneious Prime. Open reading frames were identified with Geneious Prime using the invertebrate mitochondrial genetic code. Transfer RNA genes (tRNAs) and their secondary structures were predicted using ARWEN software (<http://130.235.244.92/ARWEN/>, accessed on 15 March 2021) (Laslett and Canback, 2008). The two ribosomal RNA genes (12S rRNA and 16S rRNA) and the large non-coding region presumed to contain the control for transcription and translation (AT-rich region) were manually annotated by comparison with the mitogenomes of other Tingidae available on GenBank. The new complete mitogenomes of *C. lineata*, *Neoplerochila* sp., and *P. australis* were deposited on GenBank under the accession numbers MZ935684, MZ935685, and MZ935686.

3.3.5 Mitogenome Analyses

Nucleotide composition and compositional biases were calculated using Geneious Prime as $AT\ skew = (A - T)/(A + T)$ and $GC\ skew = (G - C)/(G + C)$. Relative synonymous codon usage was calculated in MEGA X. Repeated regions in the AT-rich region were identified using Tandem Repeats Finder v4.09 (Benson, 1999). Start codons and overlapping and intergenic spaces were counted manually. Nonsynonymous (Ka) and synonymous (Ks) substitution rates were calculated using DnaSP6 (Rozas *et al.*, 2017).

3.3.6 Phylogenetic Reconstruction of Tingidae

The phylogenetic position of the olive lace bugs within Tingidae was assessed in the context of the 18 mitogenomes available for the family in GenBank as of October 2020, with *Apolygus lucorum* and *Adelphocoris fasciaticollis* (Hemiptera: Miridae) as outgroups (Table S3). Individual PCGs were extracted from the complete mitogenome sequences and aligned using the translation algorithm in Geneious Prime. Stop codons were removed manually, and individual gene alignments were concatenated to form a single alignment. Poorly aligned regions and gaps in the concatenated alignment were eliminated using GBLOCKS v0.91b (Castresana, 2000). The final alignment was used to generate three sub-datasets: PCG123 (all codon positions), PCG12 (excluding the 3rd codon position), and an amino acid (AA) alignment.

Bayesian analyses were performed on the three datasets under the site-heterogeneous mixture model CAT-GTR in Phylobayes MPI in XSEDE v1.8c (Lartillot *et al.*, 2007; Lartillot & Philippe, 2004) to minimize the effect of mitochondrial compositional heterogeneity on phylogenetic reconstructions (Cai *et al.*, 2020; Timmermans *et al.*, 2016). Constant sites were removed from the alignments and the minimum number of cycles was set to 30,000 with the burn-in set to 1000. The “maxdiff” was set to 0.3, and the minimum effective size was set to 50. Nodal support was estimated as Bayesian posterior probabilities (BPP). PhyloBayes analyses were run on the CIPRES Science Gateway Portal (Miller, Pfeiffer, and Schwartz, 2010). The final trees were drawn using FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/>).

3.4 RESULTS AND DISCUSSION

Olive lace bug infestations are known to affect the development, health, and fruit yield of cultivated olive trees in South Africa, but the diversity of the species present in the region has been incompletely described. A previous study reported the presence of *P. australis* and *N. paliatseasi* in the Western Cape on *Olea europaea*, but definite morphological identification and genetic data were only generated for *N. paliatseasi* at that point (Langley *et al.*, 2020). The present study follows up by confirming the identity of *P. australis* and by reporting the presence of *C. lineata* and one *Neoplerochila* sp. genetically distinct from *N. paliatseasi*. Furthermore, the phylogenetic position of the four species was assessed in the context of mitogenomes publicly available for other Tingidae.

3.4.1 Morphological Identification of Olive Lace Bug Species

Images of representative specimens of *C. lineata*, *P. australis*, *N. paliatseasi*, and *Neoplerochila* sp. analyzed in this study are shown in Figure 3.1. *Cysteochila*, *Neoplerochila*, and *Plerochila* have a similar habitus with slight differences. All of the species analyzed here have wide paranota reflexed onto the pronotum. The paranota are adjoined to the pronotum in *C. lineata* and *P. australis* but not in the two *Neoplerochila* species. *Cysteochila lineata* has paranota reaching and partly covering the lateral carinae. *Plerochila australis* differs from the other species by the paranota being less developed and not reaching the lateral carinae. The paranota of both *Neoplerochila* species reach the lateral carinae but do not cover them. The costal area of these species is uniseriate, but *C. lineata* and *P. australis* have small and round areolae, while *N. paliatseasi* and *Neoplerochila* sp. have larger and subquadrate areolae. *Neoplerochila* sp. is very similar to *N. paliatseasi*: the only morphological difference would be the width of the sutural area, making the width of all the

hemelytra quite uniform similar to *N. youngai*, and not narrowed opposite to the sutural area as in *N. paliatseasi*. Therefore, *N. paliatseasi* and *Neoplerochila* sp. could not be distinguished unambiguously, and comparative morphometric analyses for a full description of *Neoplerochila* sp. will have to be performed in the future.



Figure 3.1 Representative adult specimens of olive lace bug (Hemiptera: Tingidae) species found in South Africa. **(A)** *Cysteochila lineata* Duarte Rodrigues (SAM-HEM-A01275), **(B)** *Neoplerochila paliatseasi* Duarte Rodrigues (SAM-HEM-A011647), **(C)** *Neoplerochila* sp. (SAM-HEM-A012753), and **(D)** *Plerochila australis* (Distant) (SAM-HEM-A010383).

3.4.2 Distribution of *C. lineata*, *P. australis*, *N. paliatseasi*, and *Neoplerochila* sp.

Olive lace bug specimens were collected spanning a period of five years (2015–2020) over the course of other studies, but a survey of olive farms in the Western Cape was only performed during the South African olive-growing season of 2020. In total, 16 sites were visited, and specimens were collected from wild and cultivated ornamental trees in public and private spaces (Figure 3.2A). Most wild and cultivated trees from which specimens were collected showed typical symptoms of olive lace bug infestation, such as chlorotic spots and dried-out leaf tips (Figure 3.2C). Olive lace bugs were found in 12 sites out of the 16 sites visited (75%), including five out of the nine olive farms (56%) (Figure 3.3A). *Plerochila australis* was the most frequently found species (10 sites; 62.5%), *N. paliatseasi* and *C. lineata* were found at four sites (25%), and *Neoplerochila* sp. was only found at one site

(Figure 3.3B). The four species were not found simultaneously at any site, but two sites had three species, two sites had two species, and 50% of the sites had only one species. Formal questionnaires were not performed, but some olive farmers mentioned using insecticides against olive lace bugs, in which case cultivated trees were sprayed twice a year. In cases of heavy infestations, insecticides have been used up to four times a year to significantly reduce populations. As insecticides represent additional economic and environmental costs, it will be interesting to investigate if olive lace bugs have efficient natural enemies that may contribute to managing infestations. In Australia, *F. olivina* is reportedly difficult to manage and control, and low toxicity pyrethrum products are commonly used (Spooner-Hart, 2005).

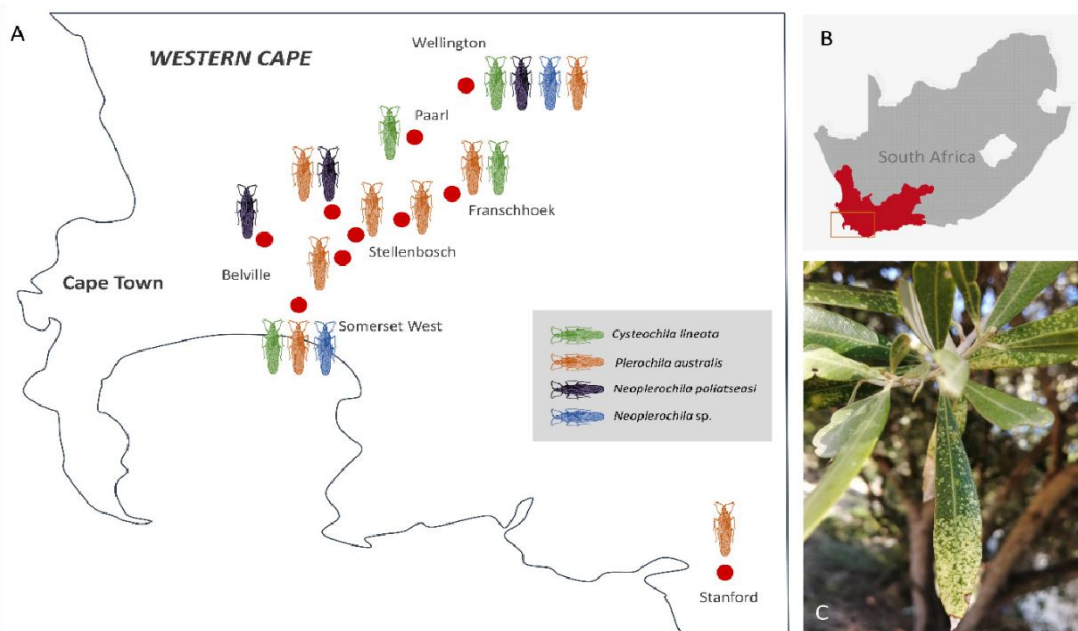


Figure 3.2 (A) Approximate geographic location of broad sampling areas (red dots) of olive lace bugs (*Cystechila lineata*, *Neoplerochila paliatseasi*, *Neoplerochila* sp. and *Plerochila australis*) in the Western Cape province of South Africa. (B) Study area in the Western Cape. (C) Characteristic chlorotic spots on the leaves of a cultivated olive tree caused by feeding activity of olive lace bugs.

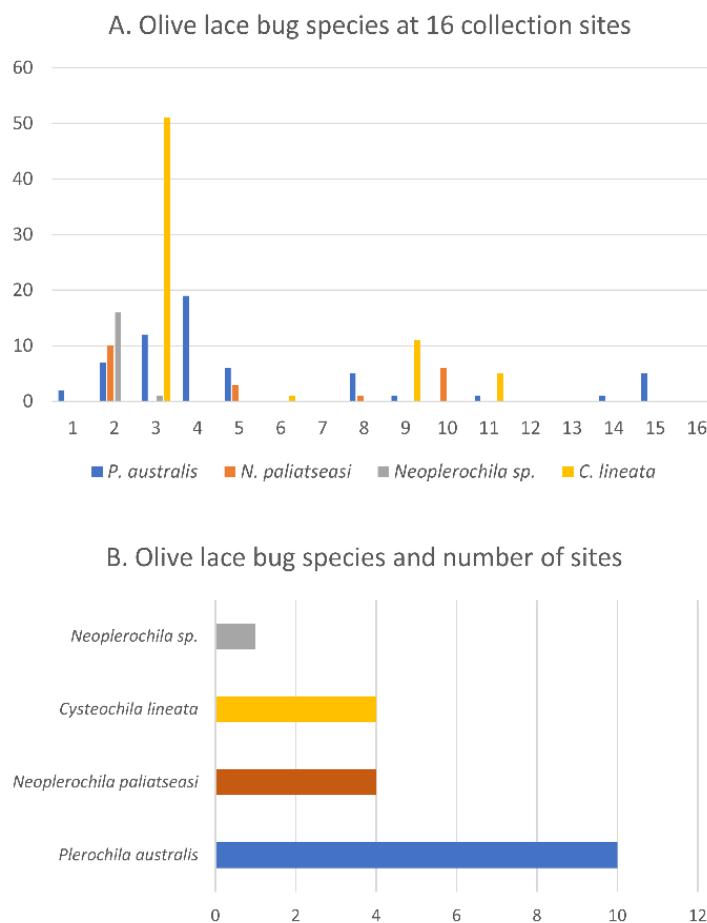


Figure 3.3 (A) Olive lace bug species found at 16 sample collection sites in the Western Cape province of South Africa. **(B)** Olive lace bug species and the number of sites where each species was found.

3.4.3 Haplotype Diversity of *C. lineata*, *P. australis*, *N. paliatseasi*, and *Neoplerochila sp.*

Neoplerochila sp. was the least genetically diverse of the four species as all specimens had the same haplotype. Haplotype diversity is the probability that two randomly selected haplotypes in the sample are different. *Cysteochila lineata*, which was found at four sites, had the highest haplotype diversity ($H = 0.963$), followed by *Plerochila australis*, the most frequently found species ($H = 0.901$) (Table 3.1). The haplotype diversity of *N. paliatseasi* ($H = 0.787$) was lower than that of *C. lineata* and *P. australis* but can still be considered high. Nucleotide diversity is the probability that two randomly chosen homologous nucleotide sites in the sample are different. The nucleotide diversity of the three species was relatively low,

especially the of *N. paliatseasi* ($\pi = 0.005$); therefore, the two *Neoplerochila* were the least diverse species among the four olive lace bugs.

Table 3.1 Genetic diversity estimates for four species of olive lace bugs (Hemiptera: Tingidae) based on the standard COI barcoding region. k—number of haplotypes; S—number of polymorphic sites.

Species	n	k	S	Haplotype Diversity \pm SD	Nucleotide Diversity \pm SD
<i>Cysteochila lineata</i>	25	18	26	0.9633 \pm 0.0235	0.007644 \pm 0.004294
<i>Neoplerochila paliatseasi</i>	17	5	7	0.7868 \pm 0.0590	0.005311 \pm 0.003243
<i>Neoplerochila</i> sp.	14	1	0	n.a.	n.a.
<i>Plerochila australis</i>	32	14	25	0.9012 \pm 0.0324	0.014416 \pm 0.007612

None of the species had high-frequency haplotypes or the classic star-like cluster around a central haplotype that is frequently interpreted as a sign of historical population expansion, and the intraspecific haplotype structure broadly reflected the diversity measures (Figure 3.4). However, the network of *C. lineata* showed several hypothetical haplotypes, and reticulations that were not present in the networks of the other two species. These features often result from the presence of multiple very low-frequency single nucleotide polymorphisms (SNPs) caused by erroneous base-calling due to the presence of double peaks. However, this was not the case as all sequences were of high quality and all SNP positions had single peaks in the electropherograms. It is possible that some of the sequences of *C. lineata* represent NUMTs despite the use of species-specific primers for PCR amplifications (see Section 3.5). If this is the case, the estimates of intraspecific genetic diversity presented here are inflated, but the broad genetic homogeneity and conspecificity of the specimens are not challenged.

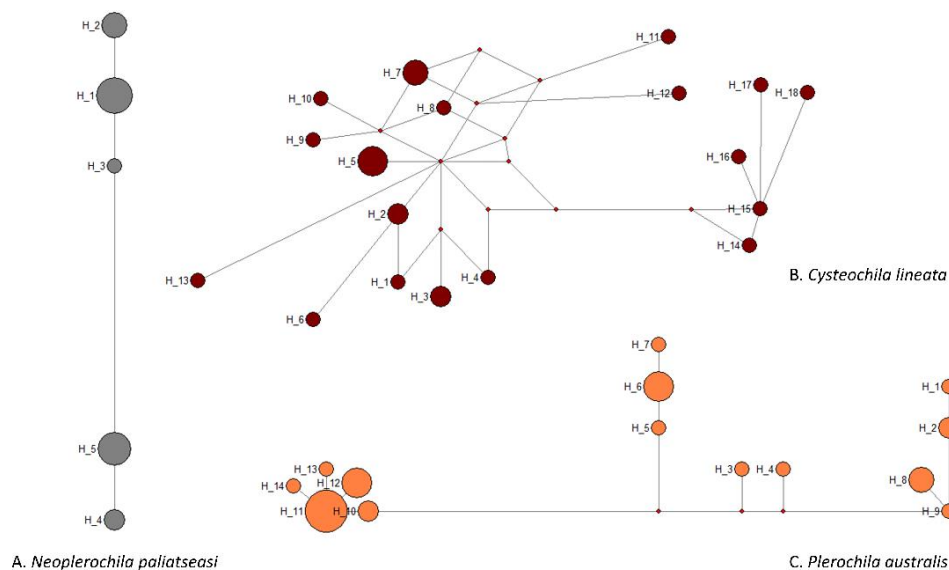


Figure 3.4 Median-joining network of COI haplotypes of three olive lace bug species (Hemiptera: Tingidae) found in South Africa.

3.4.4 Genetic Diversity of 30 Species of Tingidae Based on DNA Barcodes

The intra- and interspecific divergence of *C. lineata*, *Neoplerochila* sp. and *P. australis* were assessed in the context of DNA barcodes for other Tingidae retrieved from BOLD Systems. The genetic clustering analyses based on a ML tree showed that all species in the dataset formed monophyletic clusters with high statistical support (Figure 3.5). Intraspecific genetic divergence was estimated using maximum p-distances (Table S4; Figure 3.6). Most species (83%) had intraspecific maximum p-distances below 2%, and only four species fell in the range between 2% and 3%, indicating a general trend of consistency in specimen identification. The only evident case of potential misidentification or cryptic diversity was *D. foliacea*, which had an intraspecific maximum p-distance of 9.28% due to the presence of a single highly diverged sequence (BOLD Record GMGMM1352-14) (Figure 3.5). Based on the results of a search on BOLD Systems using the “Identification” tool implemented on the platform, GMGMM1352-14 was most similar to a sequence deposited as *D. foliacea foliacea* collected in the Netherlands, but this sequence was not publicly available, as is the case for other *Derephysia*. Therefore, it was not possible to infer the monophyly of *Derephysia*. *Plerochila australis* had an intraspecific maximum p-distance of 3.29%, which may indicate ongoing differentiation in this group. The high divergence of *Neoplerochila paliatseasi* and *Neoplerochila* sp. as a single group supported the hypothesis of two distinct species (max p-distance = 7.12%). The choice of thresholds for intra- and interspecific distances is arbitrary, and no fixed value can be universally applied because variation in intraspecific divergence

can be due to introgression, incomplete lineage sorting and recent speciation (Meier *et al.*, 2006). Nonetheless, estimates of sequence divergence can be useful for inferring patterns of genetic variation that allow for cataloguing of specimens and species into categories and complement morphological and ecological information when observable characters are absent, insufficient, or non-informative. In the case of Tingidae, the data analyzed here indicate that the range of maximum p-distances between 2% and 3% is a reasonable proxy for inferring conspecificity among sequences. Could this notion be extrapolated to congeneric pairs of species, i.e., are congeneric species of Tingidae consistently less diverged than non-congeneric species? Interspecific p-distances between all species pairs ranged from 5.22% to 28.72% (Table S5). Among congeneric species pairs (*Acalypta*, *Corythucha*, *Gargaphia*, *Neoplerochila*, *Stephanitis*, and *Tingis*), these values ranged from 5.21% to 22.83%, and among non-congeneric species pairs, the range was from 13.80% to 28.72%. The least diverged species pairs were indeed congeneric and involved most of the *Corythucha* species pairs, and the pair *N. paliatseasi*/*Neoplerochila* sp. (5.21–12.93%); however, the ranges of genetic divergences between congeneric and non-congeneric species pairs largely overlap, indicating that p-distances are not an adequate proxy for inferring congeners in Tingidae.

3.4.5 Potential Amplification of NUMTs in Cross-Species PCR

The assessments of genetic diversity and haplotype structure in *P. australis*, *C. lineata*, *N. paliatseasi*, and *Neoplerochila* sp. were based on COI sequences obtained using newly designed species-specific PCR primers. However, we first attempted to barcode *C. lineata* using the primers specific to *P. australis* (Ple-F/Ple-R) and *N. paliatseasi* (Neo-F/Neo-R) because the mitogenome of *C. lineata* was the last to be sequenced in the course of this study. These cross-species amplifications of *C. lineata* generated sequences differing by several nucleotides for the same individual and nonspecific PCR products that resulted in low-quality sequences (data not shown). This may have been due to amplifications of nuclear mitochondrial DNA regions (NUMTs). Mitochondrial DNA is undoubtedly useful for assessments of genetic diversity due to its high copy number in the cell, lack of genetic recombination, and high evolutionary rate (Park *et al.*, 2010), but the presence of NUMTs may confound the analyses. The loss of function of NUMTs results in the accumulation of sequence variation over time, which can be detected in protein-coding genes if amino acid codons are converted into internal stop codons and/or indels that generate frameshift mutations. These degraded versions are considered “older” (paleonumts) than “younger” NUMTs that are very similar to true mitochondrial DNA (Song *et al.*, 2014). Spurious

amplification of NUMTs may lead to overestimation of species diversity and erroneous conclusions in phylogenetic and phylogeographic studies. These problems have been pointed out as a major hindrance to DNA barcoding since the early stages of the development and dissemination of the methodology, which often relies on the use of “universal primers” for generating PCR amplicons across wide ranges of taxa (Bensasson *et al.* 2001; Pereira *et al.*, 2021; Song *et al.*, 2014). In our study, the use of species-specific primers eliminated sequence inconsistencies in *C. lineata*, but this solution may not work across all insect taxa. In organisms with large genomes and that are, therefore, more tolerant to the presence of NUMTs, these are likely to be amplified even when species-specific primers are used. For example, a recent study showed that DNA barcoding in Orthoptera is particularly challenging due to the widespread presence of NUMTs, and other mitochondrial markers may have to be employed to infer species diversity correctly (Pereira *et al.*, 2021).

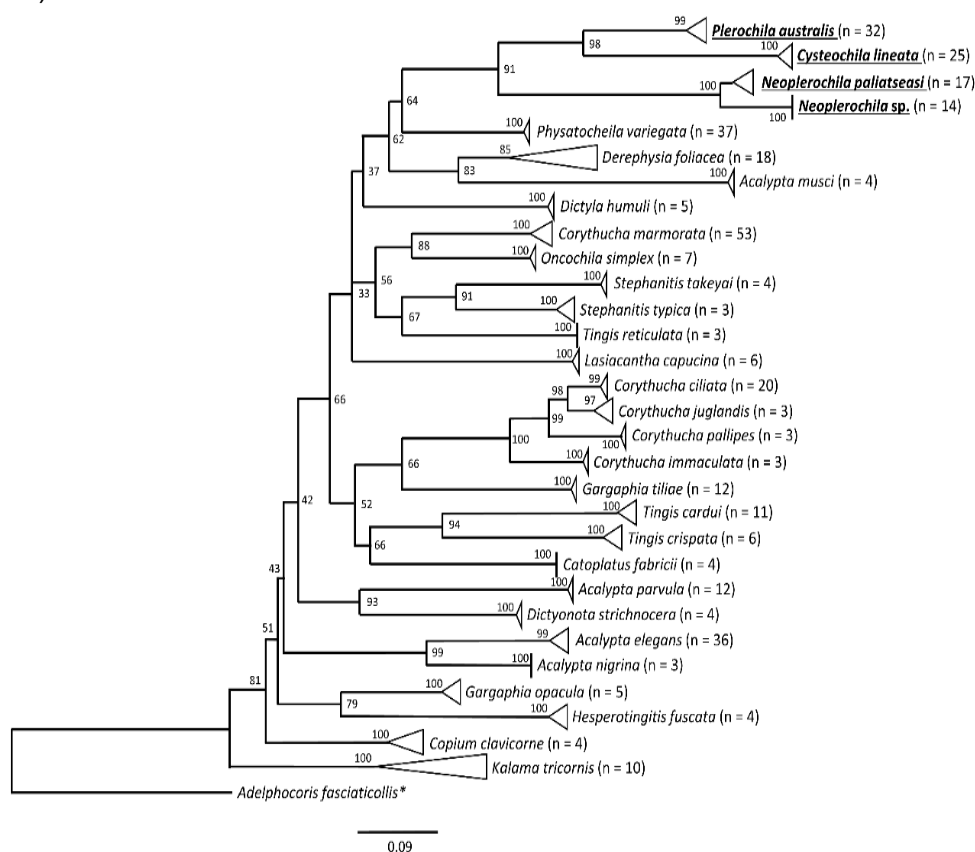


Figure 3.5 Maximum likelihood tree of lace bug species (Hemiptera: Tingidae) based on a 501-bp alignment of standard COI barcoding sequences. The analyses included 349 sequences representing 30 species in 18 genera retrieved from BOLD Systems and the new sequences of the olive lace bugs *Cysteochila lineata*, *Neoplerochila paliatseasi*, *Neoplerochila sp.*, and *Plerochila australis* generated in this study (bold, underlined). Triangles represent collapsed groups of sequences belonging to the same species. * Outgroup (Hemiptera: Miridae).

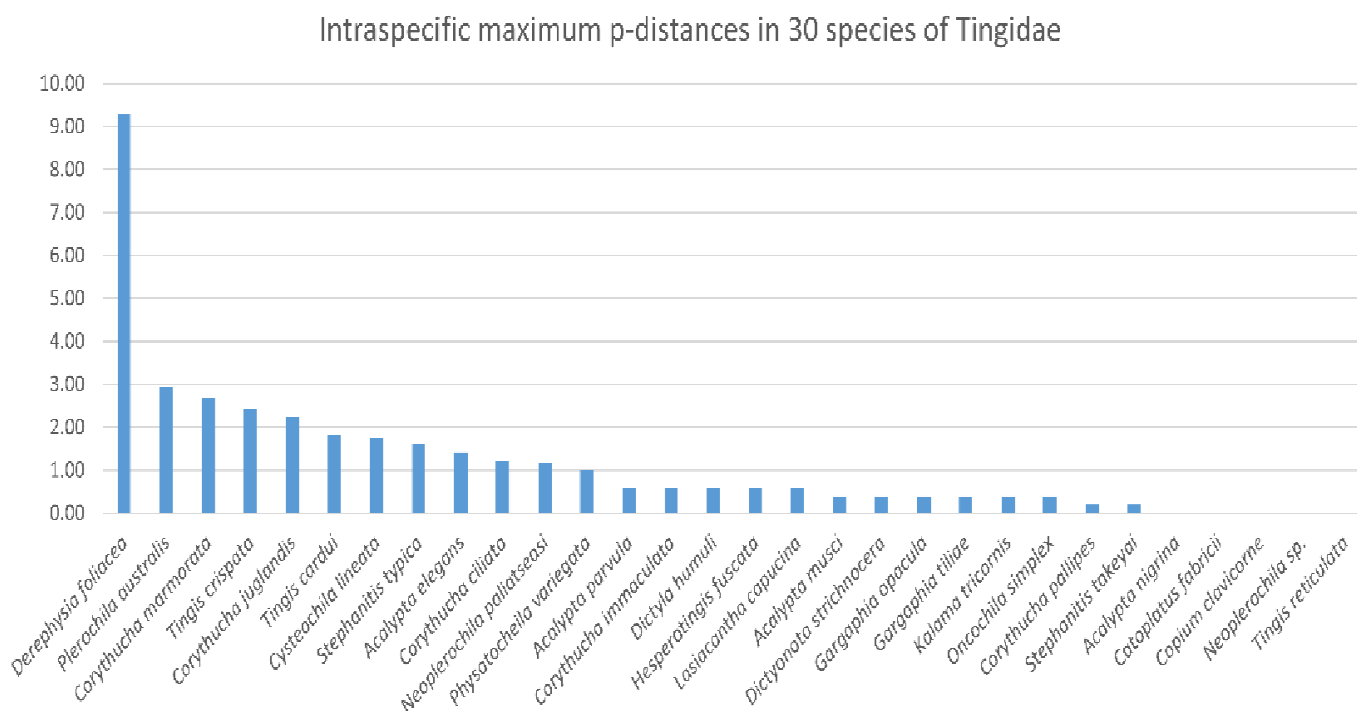


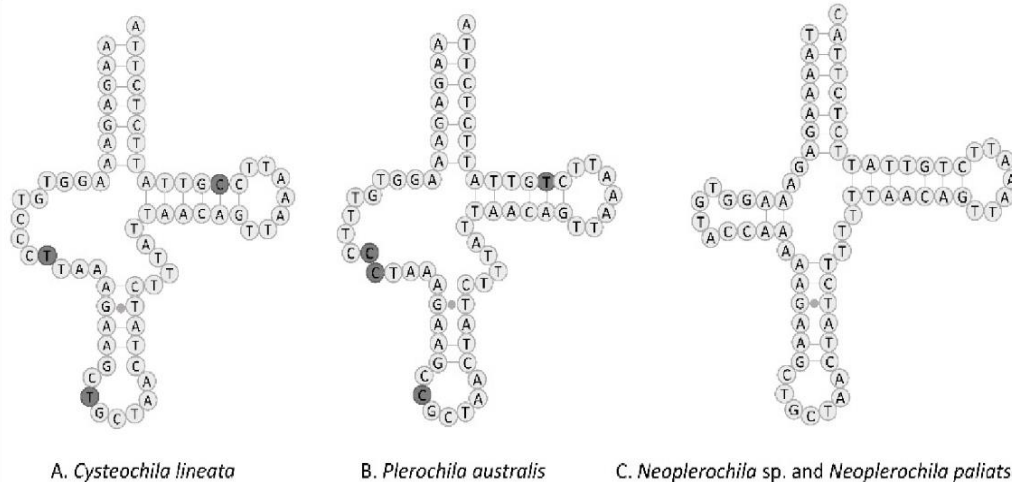
Figure 3.6 Intraspecific maximum p-distances (%) in 30 species of lace bugs (Hemiptera: Tingidae) based on a 501-bp alignment of the standard COI barcoding region (n = 349).

3.4.6 Mitogenomics of Olive Lace Bugs

The NGS runs generated 8,298,222 reads with an average length of 176 bp for *C. lineata*, 13,335,929 reads with an average length of 176 bp for *Neoplerochila* sp., and 15,637,818 reads with an average read length of 168 bp for *P. australis*. A total of 80,411 reads from *C. lineata*, 121,545 reads from *Neoplerochila* sp., and 43,401 reads from *P. australis* were mapped to the reference sequence (*N. palatseasi*, NC_046031). The coverage of the final mitochondrial sequences of *C. lineata* (15,209 bp), *Neoplerochila* sp. (15,339 bp), and *P. australis* (15,208 bp) was 964x, 1384x and 473x, respectively. The quality of mitogenomes is directly associated with read coverage, and low coverage is known to result in sequence gaps and undetermined regions (Ring *et al.*, 2017). The read coverage of each final consensus sequence was high and largely exceeded the minimum required for mitogenomic studies (15x) (Mizuno *et al.*, 2017). The average length of the new mitogenomes (15,252 bp) was in line with other Tingidae (15,355 bp), and the three species had the typical set of mitochondrial genes found in Metazoa: 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal (rRNAs) genes, and a non-coding region presumed to contain the control for replication and transcription (AT-rich region) (Figure 3.7). Twenty-three genes were located on the majority (J) strand, and 14 genes were located on the minority (N) strand (Table S6). The gene arrangement of the PCGs, tRNAs, rRNA and putative control

rRNA (771 bp; between tRNA^{Val} and the AT-rich region) in the four species were in line with the average size of the two genes in other Tingidae (1230 bp and 784 bp, respectively).

Figure 3.8 Predicted structure of tRNA^{Ser1} in the complete mitochondrial genomes of four



olive lace bugs (Hemiptera: Tingidae) found in South Africa, with nucleotide differences highlighted. Inferred canonical Watson–Crick bonds are represented by lines, and non-canonical bonds are represented by dots.

The large non-coding (AT-rich) region believed to contain the control for replication and transcription was located between 12S rRNA and the I-Q-M tRNA cluster, as in other Tingidae. The sizes of the AT-rich region were similar in the four olive lace bugs and ranged from 733 bp in *C. lineata* to 854 bp in *N. paliatseasi*. In other Tingidae, the AT-rich region ranged from 287 bp in *Tingis cardui* to 2215 bp in *Stephanitis chinensis*. Tandem repeats in the control region are common in animals, most likely as a result of slipped-strand mispairing during DNA replication [51]. *Neoplerochila* sp. had two repeats of a 170-bp motif at the 3'-end of the AT-rich region, which were separated by 4 bp and represented 40% of the region, *P. australis* had two repeats of a 166-bp motif separated by 6 bp representing 45% of the region, but no tandem repeats were identified in *C. lineata*. Tandem repeats in the 3'-end of the control region have also been identified in *P. perseae* (six repeats of 36 bp), *C. ciliata* (two repeats of 189 bp), and *N. paliatseasi* (two repeats of 156 bp) (Kocher *et al.*, 2015; Langley *et al.*, 2020).

All PCGs in Tingidae started with a canonical ATN except for ND5 in *Corythucha marmorata* (GTG), and the most frequently used start codon was ATG (Figure 3.9). PCGs in *C. lineata*, *Neoplerochila* sp., *N. paliatseasi* and *P. australis* initiated with ATG in ATP6, COIII, COI, CYTB, ND1, ND4, and ND5; ATT in COII, ND2 and ND6; and ATA in ND4L and ND3, except for ND3 in *C. lineata* (GTG). The alternative start codon GTG has been previously found across a range of insect taxa including some species of Diptera (Zhang *et al.*, 2016), Mecoptera (Beckenbach, 2011), Plecoptera (Stewart and Beckenbach, 2006), and Hemiptera such as *Sogatella furcifera* (Delphacidae) (Zhang *et al.*, 2014) and *Triatoma dimidiata* (Reduviidae) (Dotson and Beard, 2001). Most PCGs in the four olive lace bugs terminated with TAA, except ND4 in *Neoplerochila* sp., ATP6 and ND4 in *P. australis*, and CYTB in *N. paliatseasi*, which terminated with TAG. Incomplete stop codons (TA and T) were present in *C. lineata* (ND5, COII, and ND2), *Neoplerochila* sp. (CYTB and ND5), *N. paliatseasi* (COII, ND3, and ND5), and *P. australis* (COII and ND5). Incomplete stop codons are common in animal mitochondrial genes and are presumed to be completed by posttranscriptional polyadenylation (Ojala *et al.*, 1981).

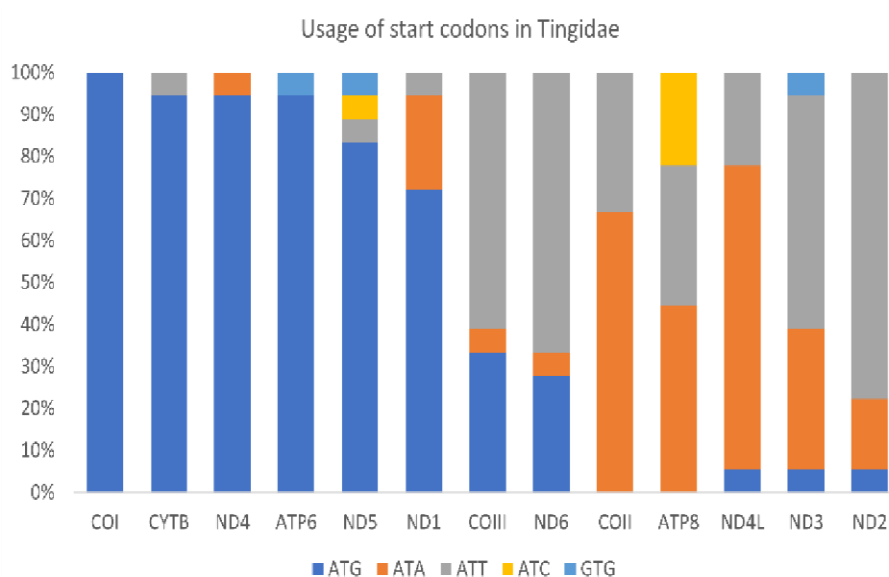


Figure 3.9 Usage of start codons in the complete set of 13 mitochondrial protein-coding genes in 18 species in the family Tingidae (Hemiptera).

The mitogenomes of *C. lineata*, *Neoplerochila* sp., and *P. australis* were highly compact, with an average of 32 bp of intergenic nucleotides at 11 locations. The longest intergenic spacers were located between tRNA^{Thr} and COI in *C. lineata* (9 bp) and *Neoplerochila* sp. (12 bp) and between tRNA^{Gln} and tRNA^{Met} in *P. australis* (10 bp), in line with *N. paliatseasi* and other Tingidae where the total number of intergenic nucleotides ranged from 1 to 48 bp.

Olive lace bugs had an average of 19 gene overlaps, mostly involving tRNAs. The longest overlap (19 bp) was in *C. lineata* between ND4L and tRNA^{Thr}, followed by 14 bp between COIII and ATP6 in *Neoplerochila* sp., 14 bp between ATP6 and COIII in *N. paliatseasi*, and 14 bp between tRNA^{Gln} and tRNA^{Met} in *P. australis*. The total number of gene overlaps varied in other Tingidae and was lower than the average for the four olive lace bugs, ranging from 8 bp in *P. perseae* to 17 bp in *C. ciliata*.

Olive lace bugs had the high A+T content typically found in insect mitogenomes, with an average of 75.1% for the total sequences. The A+T content of the AT-rich regions of the three new mitogenomes (*C. lineata*, 78.2%; *Neoplerochila* sp., 76.9%; *P. australis*, 75.2%) was higher than that of their complete sequences, which was also the case of *N. paliatseasi* and all other Tingidae except *C. ciliata* [49]. Olive lace bugs also had a similar A+T content for the combined tRNAs (average = 77.8%), and combined rRNAs (average = 79.2%). The A+T content of the total PCGs in olive lace bugs varied from 65.8% in *C. lineata* to 81.3% in *Neoplerochila* sp. and *N. paliatseasi*. The A+T content of individual PCGs was lowest in COI in all three species (66.4%), and highest in ATP8 (*C. lineata*, 79.5%) and ND4L (*P. australis*, 80.3%; *Neoplerochila* sp., 81.3%) (Table S7).

The mitochondrial GC content varies among species and is influenced by mutation bias, selection, and DNA repair bias on the complementary DNA strand (Qian *et al.*, 2018). According to the second parity rule, bases in the complementary DNA strand exist at equal frequencies when there are no mutations or selection bias [59]. The presence of AT and GC skews on the same DNA strand may indicate that the species underwent mutations or environmental selection (Qian *et al.*, 2018). *Cysteochila lineata*, *Neoplerochila* sp., and *P. australis* had positive AT skews and negative GC skews in most genes and AT-rich regions, except COI and CYTB. ND6 also did not have a positive AT skew and a negative GC skew in *Neoplerochila* sp. and *P. australis*. In the four olive lace bugs, four of 13 PCGs on the N strand had higher AT skews than PCGs on the J strand. The nucleotide bias towards A and T was reflected in codon usage, with AT-rich codons (UUU, UUA, AUU, AUA, UAU, AAU and AAA) representing an average of 42.3% of all codons. Relative synonymous codon usage (RSCU) for each codon is calculated as the relative frequency of a codon within a mitogenome. An RSCU value higher than 1.0 indicates an over-represented codon, whereas an RSCU value lower than 1.0 indicates an under-represented codon [60]. RSCU was higher than 1.0 in all synonymous codons, indicating that AT-rich codons are favoured (Table S8).

The ratio of non-synonymous to synonymous nucleotide substitutions (Ka/Ks) is generally used as an indicator of selective pressure on protein-coding sequences among different species. A Ka/Ks ratio greater than 1 indicates positive selection, which is assumed to have occurred during the evolution of the sequence. Average Ka/Ks were calculated for individual PCGs across the 18 Tingidae species included in this study (Figure 3.10). All genes had Ka/Ks < 1, which indicates purifying or stabilizing selection, of which ATP8 had the highest Ka/Ks (0.65), and ND1, COI, COII, COIII, and CYTB had the lowest Ka/Ks (0.20).

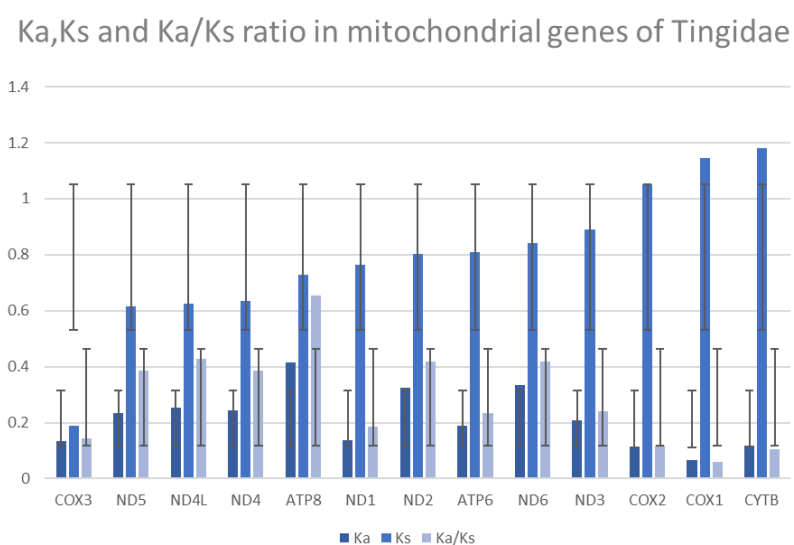


Figure 3.10 Evolutionary rates in 13 mitochondrial protein-coding genes of 18 species of Tingidae (Hemiptera). Ka—number of nonsynonymous substitutions. Ks—number of synonymous substitutions. Ka/Ks—ratio of the number of nonsynonymous to the number of synonymous substitutions.

3.4.7 Phylogenetic Position of Olive Lace Bugs within Tingidae

The mitochondrial phylogeny of Tingidae has been recovered inconsistently across different studies, and this instability may be due to atypical sequence heterogeneity and high levels of mutation rates in the family, and differences in phylogenetic methodological approaches (Kocher *et al.*, 2015; Li *et al.*, 2017; Lin *et al.*, 2017; Liu *et al.*, 2018; Yang *et al.* 2013, 2018). Furthermore, patterns of non-monophyly in mitochondrial phylogenies can result from hybridization and introgression events, and incomplete lineage sorting during speciation, which are more likely to occur among recently diverged species than in older lineages

species (Mutanen *et al.*, 2016). The phylogenetic positions of *C. lineata*, *N. paliatseasi*, *Neoplerochila* sp., and *P. australis* were recovered using the novel sequences and all Tingidae mitogenomes available on GenBank at the time of this study, except *Eteoneus sigillatus* (KU896784; unverified sequence). Phylogenetic analyses were restricted to PCGs because these have the advantage of being translatable and do not generally contain many length variable regions at the genera and species level, and the third codon position is mostly neutral and not constrained by selection (Zardoya, 2020).

The PCG123 and PCG12 data recovered the same tree topology with three unresolved nodes, but PCG12 had slightly higher support for some nodes (Figure 3.11A). The AA tree had only one unresolved node and high statistical support for most nodes (Figure 3.11B). All trees recovered *Phatnoma laciniatum* (Phatnomini) as basal to Tingini, and the same main clades but a different order of deeper nodes. The genera *Stephanitis* and *Corythucha*, which are represented by more than one species, formed monophyletic clades in agreement with previous phylogenies (Langley *et al.*, 2020; Lin *et al.*, 2017). *Ammianus toi* and *Perissonemia borneensis* were also recovered as sister taxa in agreement with previous reconstructions (Langley *et al.*, 2020). The genus *Cysteochila* is represented by two species; however, these did not form a monophyletic clade as *C. lineata* was recovered as a sister species to *P. australis* but *C. chiniana* was placed in a different cluster with *T. jacobsoni* and *T. cardui*. Nevertheless, the four olive lace bugs were placed in the same phylogenetic cluster with high support in both trees. *Cysteochila* is a large genus holding around 136 species closely related to *Plerochila* in which species of *Cysteochila* have been transferred (*C. horvathi* Schouteden, and *C. tzikamana* Drake). In fact, these genera need to be revised, as the morphological characters used to distinguish species show very small differences and many are homoplastic [see (Guilbert *et al.*, 2014)]. This is also the case for *Neoplerochila*, in which species of *Physatocheila* were transferred (*N. katbergana* Drake and *N. weenenana* Drake). Our results show that *C. lineata*, *P. australis*, *N. paliatseasi* and *Neoplerochila* sp. share a mitochondrial ancestor and suggest that feeding on *O. europaea* may have a common evolutionary origin in lace bugs. To test this hypothesis, it will be necessary to confirm which other lace bug species feed on *O. europaea* and to determine their phylogenetic position within a wider range of Tingidae. Candidate species could include species that have been found on *Olea* such as *Neoplerochila millari*; *N. dispar*; *N. weenenana*; *P. horvathi*; *Cysteochila impressa* Horvath; *Physatocheila namibiana* Duarte Rodrigues; and the Australian *F. olivina*, a known pest of cultivated olives.

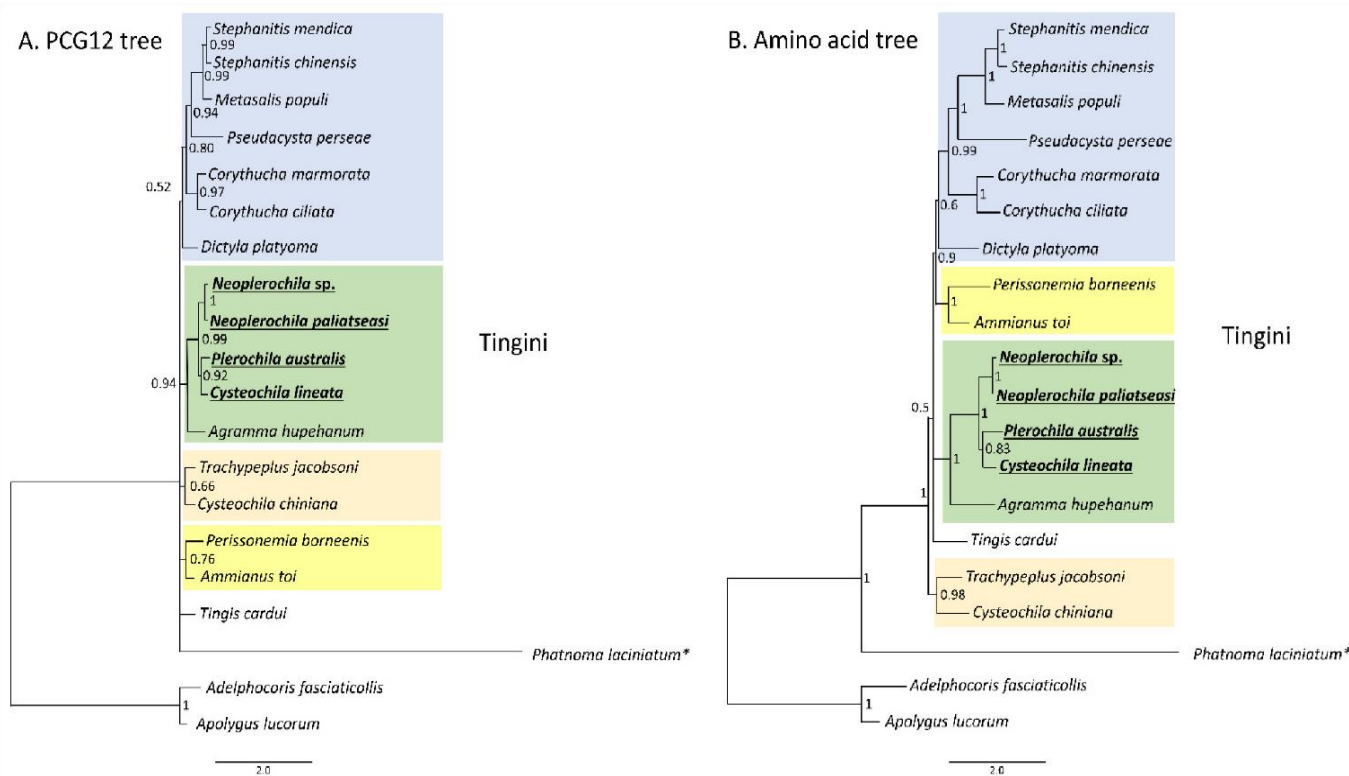


Figure 3.11 Phylogenetic relationships among 18 lace bug species (Hemiptera: Tingidae) based on 13 mitochondrial protein-coding genes. (A) PCG12-only first and second codon positions. (B) Amino acid tree. *Adelphocoris fasciaticollis* and *Apolygus lucorum* (Hemiptera: Miridae) were used as outgroups. Nodal statistical support is given as Bayesian posterior probability. * Phatnomatini.

3.5 CONCLUSION

Cultivated olive trees were introduced to the Western Cape province of South Africa less than 100 years ago, but African wild olives are widely distributed in this and other regions of South Africa. Previous works have shown that the entomofauna affecting *O. europaea* in sub-Saharan Africa most likely co-evolved with African wild olives. We confirm the presence of four species of olive lace bugs in South Africa (*C. lineata*, *P. australis*, *Neoplerochila* sp., and *N. paliatseasi*), of which *P. australis* was the most frequent. The four olive lace bugs have a close phylogenetic relationship among Tingidae, in agreement with their utilization of *O. europaea*. As relatively few olive lace bug species are adapted to *O. europaea*. These results may also suggest highly conserved host preferences and a lack of host-herbivore co-speciation. It will be interesting to gather further evidence for a common origin of this feeding habit.

Chapter 4 Mitogenomics and phylogenetic position of the olive flea beetles *Argopistes capensis* and *Argopistes sexvitattus* (Coleoptera: Chrysomelidae) and an overview of DNA barcoding in the tribe Alticini

4.1 ABSTRACT

Olive flea beetles (Chrysomelidae: Alticini) of the genus *Argopistes* are phytophagous insects that feed on wild and cultivated *Olea europaea* trees in sub-Saharan Africa. Olive flea beetles attack young growth, which ultimately stunts the development of young trees. These olive pests are an understudied group of leaf beetles and currently, there is no genetic data available for these species. The objective of this study was to assess the species diversity of olive flea beetles in the Western Cape province and their phylogenetic position within the tribe Alticini. Adult olive flea beetles were collected from wild and cultivated olive trees in the Western Cape province and morphologically identified as *Argopistes capensis* (Byrant 1944) and *Argopistes sexvitattus* (Byrant 1922). The complete mitochondrial genomes of *A. capensis* and two morphotypes of *A. sexvitattus* (striped and black) were sequenced and annotated. The total length of the mitogenomes ranged between 16,542 bp – 16,566 bp, including 13 PCGs, 2 ribosomal RNAs, 22 transfer RNAs, and a non-coding AT-rich region. The mitogenomes were used for phylogenetic reconstruction using 59 other mitogenomes publicly available for the tribe. The olive flea beetles formed a clade with *Argopistes tsekooni*, suggesting that their host-specificity on Oleaceae may have a common evolutionary origin. Novel and publicly available DNA barcodes for the tribe Alticini were used for phylogenetic analyses and estimates of intra- and inter-specific genetic divergence in 117 species, and most sequences were placed in conspecific clusters; however, non-monophyly affected 14.66% of the total number of species, likely due to taxonomic over-splitting, cryptic diversity, or misidentification among many other issues.

4.2 INTRODUCTION

Flea beetles (Chrysomelidae: Alticini) are small to moderately sized jumping beetles characterized by enlarged hindleg femora that allow the insect to spring into action when disturbed. The tribe Alticini is monophyletic and closely related to the tribe Galerucini, both contained within the subfamily Galerucinae (Bouchard *et al.*, 2011). Alticini includes 4,000 to 8,000 species in approximately 500 genera with worldwide distribution but occurring mainly in the tropical regions of South America, Africa, and Asia (Biondi and D'Alessandro, 2010).

Most flea beetles are specialized feeders, and both adults and larva bore small holes on the external surface of leaves, stems, and, more rarely, flowers of a wide range of higher plants (Feeny *et al.*, 1970). Flea beetle feeding exposes the lower epidermis of the leaves to high levels of sunlight, eventually causing defoliation (Feeny *et al.*, 1970). Leaf damage also leads to a loss of vigour of surrounding tissues which is aggravated in hot and dry conditions. Heavy infestations of flea beetles can be detrimental at the early stages of plant development and will lead to the death of the host if not addressed (Feeny *et al.*, 1970).

Flea beetles affect many agricultural and ornamental plants of economic significance. For example, the crucifer flea beetle (*Phyllotreta cruciferae*) (Goeze 1777), the striped flea beetle (*Phyllotreta striolata*) (Fabricius 1801), Zimmermann's flea beetle (*Phyllotreta zimmermanni*) (Crotch 1873), and the pale-striped flea beetle (*Systema blanda*) (Melsheimer 1847) feed on cruciferous crops (Feeny, 1970), *Epitrix fuscula* (Crotch 1873) feeds on eggplants, and *Epitrix hirtipennis* (Melsheimer 1847) defoliates tobacco. Three *Chaetocnema* species (Stephens 1831), *C. aridula*, *C. hortensis*, and *C. mannerheimi* are pests of cereals, and *C. concinna* is a pest of beetroot, buckwheat, sorrel, and strawberry (Bukejs, 2008). *Luperomorpha xanthodera* (Fairemaire 1888) is a known polyphagous pest of floriculture and ornamental plants, especially roses (Bienkowski and Orlova-Bienkowski, 2018).

Olive flea beetles are found in sub-Saharan Africa feeding on the leaves and stems of the African Wild Olive (*Olea europaea* subs. *cuspidata*) and the European Olive (*Olea europaea* subs. *europaea*). However, it has been reported that some may feed on the actual fruits and have become serious pests of olives (Costa, 1998). Biondi and D'Alessandro (2012) asserted that there are 15 species of *Argopistes* associated with Oleaceae in sub-Saharan Africa. In South Africa, the sixth-largest producer of olives and olive-derived products on the continent, *Argopistes capensis* (Bryant 1944), *Argopistes oleae* (Byrant 1922), and *Argopistes sexvittatus* (Byrant 1922) have been reported as pests of olive orchards in the Western Cape province of South Africa (Addison *et al.*, 2015). A study in the Eastern Cape province of South Africa reported at least three other species of *Argopistes* (*Argopistes epomistus* sp. nov., *Argopistes lilliputiamus* sp. nov., and *Argopistes melanus* sp. nov.) associated with wild and cultivated olives (Mkize, 2009).

Byrant (1922) described and illustrated adults of *A. oleae* and *A. sexvittatus*, and later described *A. capensis*. However, the morphological characters used to describe these species, such as colour and elytra, were not sufficient to accurately differentiate them. As distinguishing the striped form of *A. sexvittatus* from *A. oleae* proved to be challenging, additional morphological characters such as aedeagus shape, the colour and width of the

elytra stripes, and the colour and puncta in the pronotum are now used to identify *A. capensis*, *A. oleae* and *A. sexvitattus* (Mkize, 2009).

This work is part of a larger endeavour to catalogue the rich entomofauna associated with wild and cultivated olives in South Africa. To that purpose, we aimed to identify olive flea beetle species found on wild and cultivated olives in the Western Cape and to assess their phylogenetic position within the tribe Alticini using new complete mitochondrial genomes. The composition of the novel mitogenomes were described. Furthermore, an overview of the potential utility of molecular methods for the identification of Alticini was assessed in 117 species using new and publicly available DNA barcodes.

4.3 MATERIALS & METHODS

4.3.1 Specimen collection, morphological identification, and DNA extraction

Olive flea beetles were collected from wild and cultivated olives at 14 locations in the Western Cape province of South Africa between November 2015 and March 2020 (Table 4.1). In addition, a survey was conducted in eight olive farms during the peak season of the olive flea beetles, between November 2020 and March 2021, where olive flea beetles were collected in potentially infested blocks on the farm, identified by farm workers before field visits. The number of trees surveyed at each farm varied from 10 to 50, and every second tree was sampled at any given block. Olive flea beetles were collected directly from the trees into plastic bags, euthanized by freezing, and individually preserved in 100% ethanol at -4°C for taxonomic identification and DNA analyses. Morphological identifications were initially performed by M. Biondi (University of L'Aquila, Italy) based on high-resolution images and later on representative ethanol-preserved specimens, following the current taxonomic keys. Total DNA was extracted from individual specimens using a standard phenol-chloroform method (Sambrook and Russell, 2001) and stored at -4°C until downstream analyses.

4.3.2 DNA barcoding of *Argopistes* spp.

Adult olive flea beetles were amplified for the standard barcoding region (COI) using the universal arthropod primers LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO 2198 5'-TAACTTCAGGGTGACCAAAAATCA-3' (Folmer *et al.*, 1994). PCR amplifications were performed in a total volume of 5 µL containing 1X of KAPA2G Robust HotStart Ready Mix PCR kit (KAPPA Biosystems), 0.5 µM of each primer, 0.5 µL of MilliQ H₂O, and 1.0 µL of template DNA (~100 ng). The thermal cycling program consisted of 3 min at 95°C; 35 cycles of 15 s at 94°C, Ta of 54°C, 1 min at 72°C; and a final extension of 1 min at 72°C. PCR

products were sequenced using the reverse PCR primers with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) at the Central Analytical Facilities of Stellenbosch University, South Africa.

4.3.3 Sequencing, assembly, and annotation of complete mitogenomes

Two specimens of *A. sexvitattus* (striped and black morphotype) and one specimen of *A. capensis* and were sequenced using the Ion Torrent™ S5™ platform (ThermoFisher Scientific, Waltham, MA, USA) at the Central Analytical Facilities of Stellenbosch University. Sequence libraries were prepared using the Ion Xpress™ Plus gDNA Fragment Library Kit (ThermoFisher Scientific, Waltham, MA, USA), according to the protocol MAN0009847 REV J.0. Libraries were pooled and sequenced using the Ion 540™ Chef Kit (ThermoFisher Scientific). The next-generation sequencing (NGS) reads for each specimen were separately mapped against the complete mitogenome of *Argopistes tsekooni* (Chen 1934) (NC_045929.1) (Long *et al.*, 2019) and assembled using Geneious Prime v2021.1 (<https://www.geneious.com>). under the default settings. Open reading frames were identified with Geneious Prime using the invertebrate mitochondrial genetic code. Transfer RNA genes (tRNAs) and their secondary structures were identified using the ARWEN software (Laslett and Canback, 2008). The two ribosomal RNA genes (12S rRNA and 16S rRNA) and the putative control region (AT-rich region) were manually annotated by comparison with mitogenomes of other Alticini. The DNA barcodes and complete mitogenomes of *Argopistes capensis* and *Argopistes sexvitattus* generated in this study were deposited in GenBank under accession numbers [upon acceptance for publication].

4.3.4 Mitogenome analyses

Start codons and overlapping and intergenic spaces were counted manually. Nucleotide composition and compositional biases were calculated using Geneious Prime as AT-skew = $(A-T)/(A+T)$ and GC-skew = $(G-C)/(G+C)$. Relative synonymous codon usage was calculated using MEGA X (Kumar *et al.*, 2018). The ratio of nonsynonymous (Ka) to synonymous (Ks) substitutions (Ka/Ks) were calculated using DnaSP6 (Rozas *et al.*, 2017).

4.3.5 Phylogenetic analysis within the tribe Alticini

The phylogenetic position of *A. capensis* and *A. sexvitattus* (including morphotypes) within the tribe Alticini was assessed in the context of other complete and partial mitogenomes

available on GenBank as of April 2021, with *Chrysomela vigintipunctata* and *Entomoscelis adonidis* (Chrysomelidae: Chrysomelini) as outgroups (Table S4.1). Individual PCGs were extracted from the mitogenomic sequences and aligned using the translation algorithm in Geneious Prime. Stop codons were removed manually, and individual gene alignments were concatenated to form a single alignment. Poorly aligned regions and gaps in the concatenated alignment were filtered using GBlocks v0.91b (Castresana, 2000). The final alignment was used to generate three sub-datasets: PCG123 (all codon positions), PCG12 (excluding the 3rd codon position), and an amino acid (AA) alignment. The PCG123, PCG12, and amino acid alignments were partitioned with PartitionFinder2 v2.1.1 (Lanfear *et al.*, 2016), using the greedy algorithm for scheme search and the Bayesian Information Criterion for scheme selection. Bayesian inference analysis was performed on MrBayes v3.2.7a (Ronquist and Huelsenbeck, 2003) under the GTR + GAMMA + I substitution model, using two simultaneous runs, with four heated chains of 10,000,000 generations, with a subsampling frequency of 1,000 generations, and a burn-in length of 2,500,000. Confidence in tree topology was estimated as Bayesian posterior probability (BPP). Partitioning schemes and BI were run on the CIPRES Portal v.3.3 (<https://www.phylo.org/>) (Miller *et al.*, 2010).

4.3.6 Intra- and interspecific genetic divergence

All of the barcoding sequences taxonomically assigned to the tribe Alticini were downloaded from GenBank (April 2021). The initial dataset consisted of 5,372 sequences that were subsequently filtered for a) sequences identified to species level, b) sequences with a minimum length of 500 bp overlapping the standard COI barcoding region, and c) species represented by a minimum of six sequences. The final dataset included 2,676 sequences representing 117 species across 22 genera, including the *A. capensis* and *A. sexvittatus* sequences generated in this study. Multiple sequence alignments were performed using the MAFFT algorithm (Kato and Standley, 2013) in Geneious Prime v2021.1 (<https://www.geneious.com/>). Genetic clustering of the COI sequence dataset of 117 species was assessed using a maximum likelihood (ML) tree constructed in IQ-Tree (Minh *et al.*, 2020), with *Entomoscelis adonidis* (KX94349.1) (Chrysomelidae: Chrysomelini) as an outgroup. Branch supports were determined using 1000 replicates for both ultrafast bootstrapping and SH-aLRT branch tests (Guindon *et al.*, 2010; Hoang *et al.*, 2018). The final ML tree was drawn using FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/>). Intraspecific genetic divergences were estimated as a percentage of maximum pairwise distances (p-distance, %) under the K2P model on MEGA X, and interspecific genetic divergence was estimated as mean p-distance (%) under the same model.

4.4 RESULTS AND DISCUSSION

Olive flea beetles are found feeding on wild and cultivated olive trees in sub-Saharan Africa and previous studies have reported a number of *Argopistes* species in South Africa, including *A. capensis*, *A. oleae*, and *A. sexvitattus* in the Western Cape (Addison *et al.*, 2015) and *A. epomistus*, *A. lilliputiamus* and *A. melanus* in the Eastern Cape (Mkize, 2009). However, genetic data for species identification and assessment of their phylogenetic position within the tribe Alticini was not available. To contribute to filling this gap, olive flea beetle specimens were incidentally collected from cultivated and wild olive trees at 14 sites between 2015 and 2020. Additionally, a survey of cultivated olive trees at eight olive farms in the Western Cape and of wild olive trees in the vicinity of the farms was performed during the South African olive growing season of 2020.

4.2.1 Morphological identification of olive flea beetle species

Images of representative specimens of *A. capensis* and *A. sexvitattus* morphotypes analyzed in this study are shown in Figure 4.1. Olive flea beetles are characterized by their oval shape and are usually 4- 5 mm in length. Their heads are usually hidden below the thorax. Species are distinguishable by the colouration and pattern of the elytra (Addison *et al.*, 2015). The colour of the elytra of *A. capensis* and *A. sexvitattus* is uniformly yellowish brown (Figure 4.1 A - B and Figure 4.1 F). The stripes on the elytra in *A. sexvitattus* are generally wider, dark brown, median longitudinal, inner, and lateral margins, while the stripes in *A. capensis* are dark brown, faint, irregular longitudinal stripes. The elytra and thorax in *A. sexvitattus* can be entirely black/brown as shown in (Figure 4.1 C - D) (Addison *et al.*, 2015).

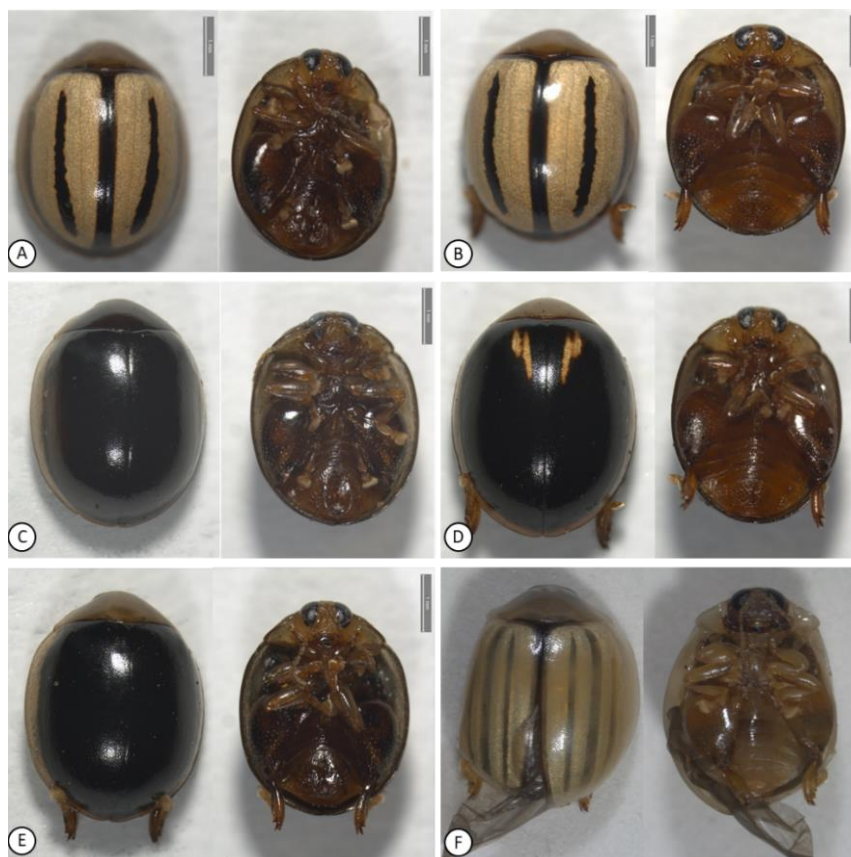


Figure 4.1 Representative adult specimens of olive flea beetles (Chrysomelidae: Alticini) species found in South Africa. (A) *Argopistes sexvitattus* striped morphotype (male), (B) *Argopistes sexvitattus* striped morphotype (female), (C) *Argopistes sexvitattus* black morphotype (male), (D) *Argopistes sexvitattus* black morphotype (female), (E) *Argopistes sexvitattus* black morphotype (male), and (F) *Argopistes capensis* (female). Image credit: Beth Grobbelaar.

4.4.2 Distribution of *A. capensis*, *A. oleae*, and *A. sexvitattus* in wild and cultivated olive trees

Olive flea beetles were collected covering a period of five years (2015 – 2020) over the course of other studies, but a survey of olive farms in the Western Cape (Figure 4.2 B) was only conducted in 2020 during the South African olive-growing season of 2020. A total of 14 sites were visited, and adult olive flea beetle specimens were collected from ornamental wild and cultivated trees in public and private areas, including wild and cultivated trees on olive farms (Figure 4.2 A). Specimens were only collected from wild and cultivated trees which exhibited typical symptoms of olive flea beetle such as holes on the leaves and dried-out leaf tips (Figure 4.2 C).

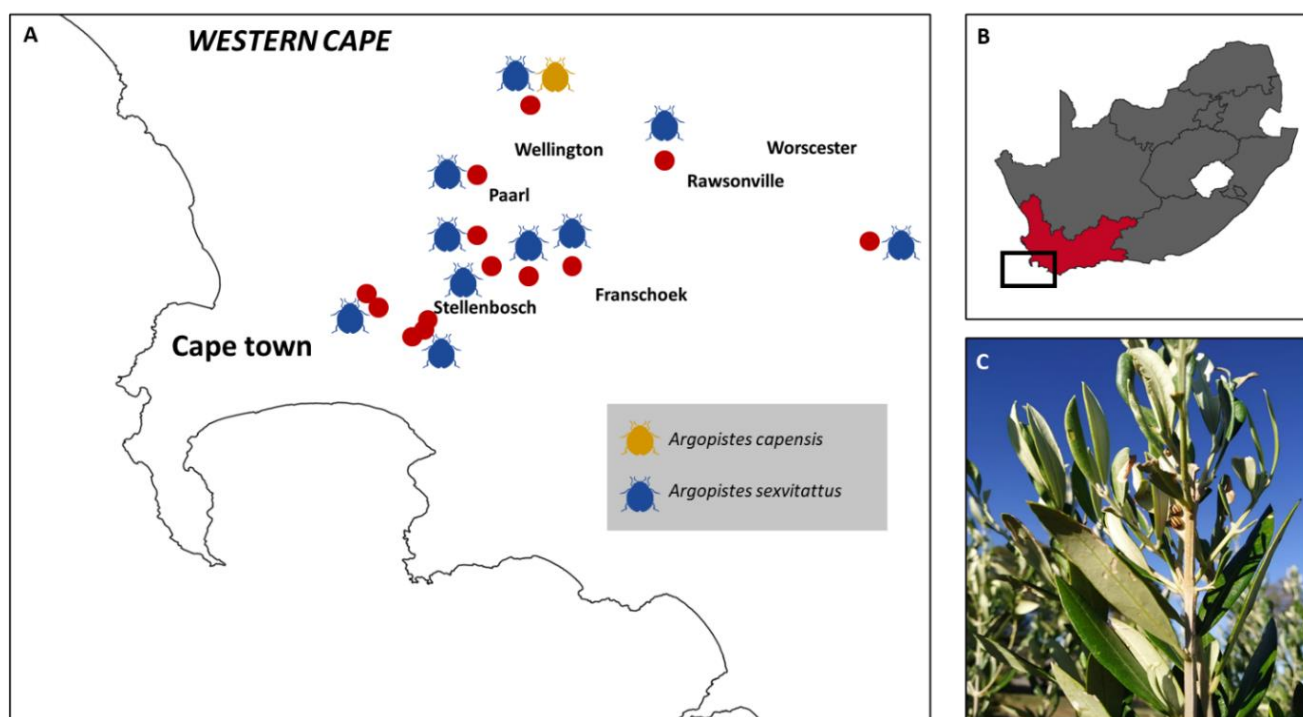


Figure 4.2 (A) Distribution of the olive flea beetle species, *Argopistes capensis*, and *Argopistes sexvitattus* in wild and cultivated olive orchards in the Western Cape province of South Africa. **(B)** Study area in the Western Cape province. **(C)** Characteristic holes on leaves caused by feeding of olive flea beetles.

Argopistes capensis and *A. oleae* were originally described from the Western Cape province and their distribution appeared to be restricted to this particular region of South Africa (Addison *et al.*, 2015). However, a study performed in 2008 confirmed the presence of these and other *Argopistes* species in the Eastern Cape province of South Africa (Mkize, 2009). *Argopistes sexvitattus* may have a wider distribution as it has been reported from various localities in South Africa and Namibia (Addison *et al.*, 2015). *Argopistes capensis*, *A. oleae*, and *A. sexvitattus* have been previously reported in the Western Cape (Addison *et al.*, 2015). However, in the present study *A. sexvitattus* was the dominant species present in numbers (92.86% of sites), only two specimens of *A. capensis* were found at one site (7.14% of sites) and *A. oleae* was never found in over five years (Figure 4.3).

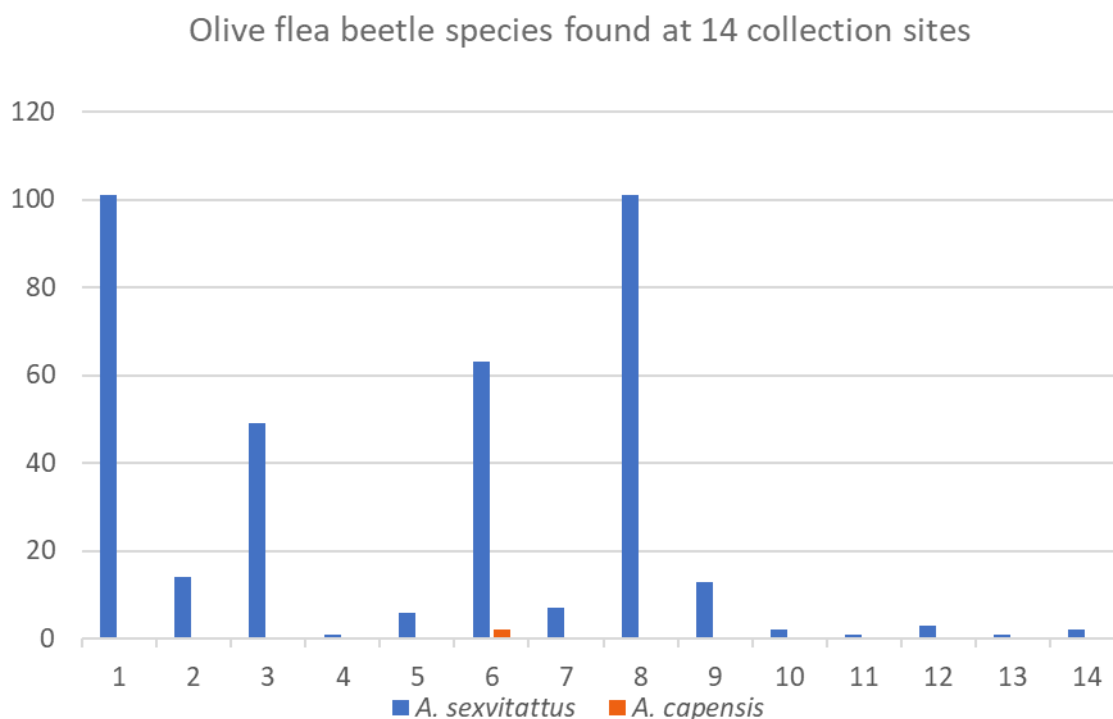


Figure 4.3 Olive flea beetle species found at 14 collection sites in the Western Cape province of South Africa.

In practical terms, *A. sexvitattus* seems to be the only olive flea beetle species currently affecting wild and cultivated olive trees in the Western Cape. Formal questionnaires were not administered, but some olive farmers stated that they use insecticides to combat olive flea beetle infestation, in which cultivated trees are sprayed twice a year. In severe infestations, insecticides have been used up to four times per year to significantly reduce pest populations. Managing olive flea beetle infestations with insecticides is costly and excessive spraying increases the risk of pests developing resistance to the treatment (Landa, 2019). Therefore, other methods of pest combat should be preferred, including the promotion of the presence of natural enemies (Landa, 2019).

A survey of natural enemies of *Argopistes* larvae conducted in the Eastern Cape between 2003-2005 confirmed that the parasitic wasp, *Pseudophanomeris inopinatus* Belokobylskij (Hymenoptera: Braconidae) is the only known enemy to parasitize the larvae of *Argopistes* species in South Africa (Mkize, 2009). However, the rate of parasitism was found to be low, and further assessments of natural enemies of olive flea beetles have not been performed.

4.4.3 The mitogenomes of *Argopistes capensis* and *Argopistes sexvitattus*

The Ion Torrent sequencing runs generated for 8,121,365 reads with an average length of 174 bp for *A. capensis*, 13,755,855 reads with an average read length of 162 bp for *A. sexvitattus* (striped), and 15,919,208 reads with an average read length of 160 bp for *A. sexvitattus* (black). A total of 5,116 reads from *A. capensis*, 113,345 reads from *A. sexvitattus* (striped), and 1,188,759 reads from *A. sexvitattus* (black) were mapped to the reference sequence (*A. tsekooni*). The coverage of the final sequence of *A. capensis* (16,543 bp), *A. sexvitattus* (striped) (16,542 bp), and *A. sexvitattus* (black) (16,566 bp) was 52X, 653X, and 9371X, respectively, exceeding the minimum required (15X) for mitogenomic studies (Ring *et al.*, 2017).

The mitogenome length of *A. capensis* and the *A. sexvitattus* morphotypes were similar to the average length found in the other Alticini species included in this study (16,009 bp), and the species possessed the typical set of 37 mitochondrial genes found in metazoans (Boore, 1999), including 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal (rRNAs) genes, and one control region (AT-rich region) presumed to contain the initiation of transcription and replication (Saito *et al.*, 2005) (Figure 4.4). The majority strand (J-strand) contains most of the genes (23 genes; 9 PCGS and 14 tRNAs), while the remaining 14 genes (PCG's, tRNA, and rRNA) are encoded on the minority strand (N-strand) (Table 4.1). The gene arrangement of the PCGs, tRNAs, rRNA, and putative control region (AT-rich region) seems to be conserved in Alticini and was identical to the hypothetical ancestral organization for Arthropoda in all species included in these analyses (Boore, 1999).

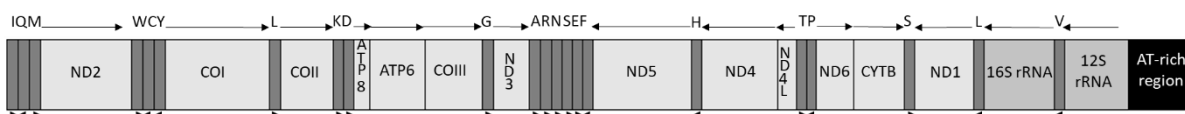


Figure 4.4 Linear map of the complete mitochondrial genomes of *Argopistes capensis* and *Argopistes sexvitattus*. tRNA genes are represented by single-letter abbreviations. Arrows indicate the direction of gene transcription.

Table 4.1 Main features of the complete mitochondrial genome of the olive flea beetles *Argopistes capensis* and *Argopistes sexvittatus* (Coleoptera: Chrysomelidae). J – majority strand; N – minority strand; IGN – number of intergenic nucleotides (negative values indicate overlapping between genes).

Gene/region	Code	Strand	Anticodon	<i>Argopistes capensis</i>				
				Coordinates	Size (bp)	Start	Stop	IGN
tRNA ^{Ile}	I	J	GAT	2-63	61	-	-	-
tRNA ^{Gln}	Q	N	TTG	65-135	71	-	-	0
tRNA ^{Met}	M	J	CAT	134-202	69	-	-	-1
ND2	-	J	-	203-1214	1012	ATT	T--	0
tRNA ^{Trp}	W	J	TCA	1214-1279	66	-	-	-1
tRNA ^{Cys}	C	N	GCA	1270-1335	66	-	-	-10
tRNA ^{Tyr}	Y	N	GTA	1336-1399	64	-	-	0
COI	-	J	-	1401-2934	1534	AAT	T--	1
tRNA ^{Leu2}	L2	J	TAA	2934-3002	69	-	-	-1
COII	-	J	-	3002-3689	688	ATG	T--	-1
tRNA ^{Lys}	K	J	TTT	3690-3761	72	-	-	0
tRNA ^{Asp}	D	J	GTC	3760-3827	68	-	-	-2
ATP8	-	J	-	3828-3983	156	ATT	TAA	0
ATP6	-	J	-	3977-4648	672	ATG	TAA	-7
COIII	-	J	-	4648-5429	782	ATG	TA-	-1
tRNA ^{Gly}	G	J	TCC	5430-5493	64	-	-	0
ND3	-	J	-	5494-5845	352	ATT	T--	-1
tRNA ^{Ala}	A	J	TGC	5845-5911	67	-	-	-1
tRNA ^{Arg}	R	J	TCG	5910-5972	63	-	-	-2
tRNA ^{Asn}	N	J	GTT	5972-6037	66	-	-	-1
tRNA ^{Ser1}	S1	J	TCT	6038-6096	59	-	-	0
tRNA ^{Glu}	E	J	TTC	6096-6161	66	-	-	-1
tRNA ^{Phe}	F	N	GAA	6157-6221	65	-	-	-5
ND5	-	N	-	6221-7919	1699	ATT	T--	-1
tRNA ^{His}	H	N	GTG	7920-7981	62	-	-	0
ND4	-	N	-	7981-9298	1318	ATG	T--	-1
ND4L	-	N	-	9292-9573	282	ATG	TAA	-7
tRNA ^{Thr}	T	J	TGT	9577-9643	67	-	-	3
tRNA ^{Pro}	P	N	TGG	9641-9706	66	-	-	-3
ND6	-	J	-	9708 -10205	498	ATT	TAA	1
CYTB	-	J	-	10205 -11342	1138	ATG	T--	-1
tRNA ^{Ser2}	S2	J	TGA	11343 -11409	67	-	-	0
ND1	-	N	-	11427-12377	951	TTG	TAG	17

tRNA ^{Leu1}	L1	N	TAG	12443-12378	66	-	-	0
16s rRNA	-	N	-	12444-13721	1278	-	-	0
tRNA ^{Val}	-	N	TAC	13722-13790	69	-	-	0
12s rRNA	-	N	-	13791-14528	738	-	-	0
AT-rich region	-	-	-	14529 -16543	2015	-	-	0

				<i>Argopistes sexvitattus</i> (striped morphotype)				
Gene/region	Code	Strand	Anticodon	Coordinates	Size (bp)	Start	Stop	IGN
tRNA ^{Ile}	I	J	GAT	1-63	63	-	-	-
tRNA ^{Gln}	Q	N	TTG	75-145	71	-	-	11
tRNA ^{Met}	M	J	CAT	145-213	69	-	-	-1
ND2	-	J	-	214-1225	1012	ATT	T--	0
tRNA ^{Trp}	W	J	TCA	1225-1290	66	-	-	-1
tRNA ^{Cys}	C	N	GCA	1281-1346	66	-	-	-10
tRNA ^{Tyr}	Y	N	GTA	1347-1410	64	-	-	0
COI	-	J	-	1412-2945	1534	AAT	T--	1
tRNA ^{Leu2}	L2	J	TAA	2945-3013	69	-	-	-1
COII	-	J	-	3013-3700	668	ATG	T--	-1
tRNA ^{Lys}	K	J	TTT	3701-3772	72	-	-	0
tRNA ^{Asp}	D	J	GTC	3771-3835	65	-	-	-2
ATP8	-	J	-	3836-3991	156	ATC	TAA	0
ATP6	-	J	-	3985-4653	669	ATG	TAA	-7
COIII	-	J	-	4653-5434	782	ATG	TA-	-1
tRNA ^{Gly}	G	J	TCC	5435-5499	65	-	-	0
ND3	-	J	-	5502-5835	352	ATT	T--	0
tRNA ^{Ala}	A	J	TGC	5851-5916	66	-	-	-1
tRNA ^{Arg}	R	J	TCG	5915-5979	65	-	-	-2
tRNA ^{Asn}	N	J	GTT	5979-6043	65	-	-	-1
tRNA ^{Ser1}	S1	J	TCT	6044 -6101	58	-	-	0
tRNA ^{Glu}	E	J	TTC	6101-6165	66	-	-	-1
tRNA ^{Phe}	F	N	GAA	6162-6226	65	-	-	-4
ND5	-	N	-	6226-7924	1699	ATT	T--	-1
tRNA ^{His}	H	N	GTG	7925-7986	62	-	-	0
ND4	-	N	-	7987-9304	1318	ATG	T--	-1
ND4L	-	N	-	9298-9579	282	ATG	TAA	-7
tRNA ^{Thr}	T	J	TGT	9583-9649	67	-	-	3
tRNA ^{Pro}	P	N	TGG	9647-9712	66	-	-	-3
ND6	-	J	-	9714 - 10211	498	ATT	TAA	1
CYTB	-	J	-	10211-11348	1138	ATG	T--	-1
tRNA ^{Ser2}	S2	J	TGA	11349 - 11415	67	-	-	0

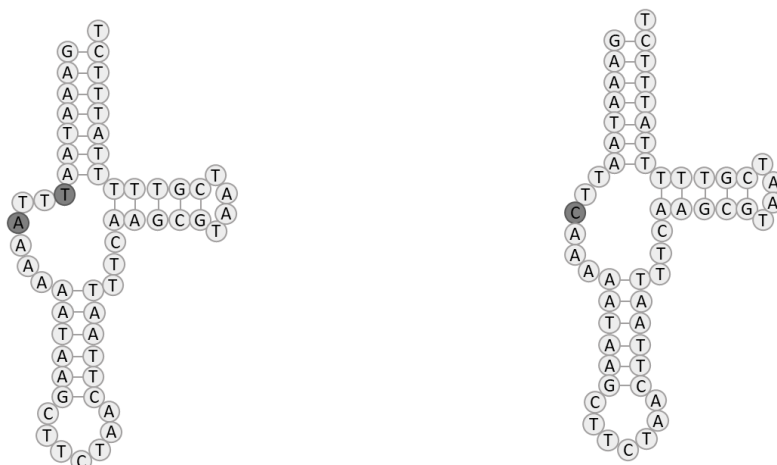
ND1	-	N	-	11433-12383	951	TTG	TAG	17
tRNA ^{Leu1}	L1	N	TAG	12384-12449	66	-	-	0
16s rRNA	-	N	-	12450-13727	1278	-	-	0
tRNA ^{Val}	-	N	TAC	13728-13795	68	-	-	0
12s rRNA	-	N	-	13796-14533	738	-	-	0
AT-rich region	-	-	-	14534-16542	2009	-	-	0

				<i>Argopistes sexvitattus</i> (black morphotype)				
Gene/region	Code	Strand	Anticodon	Coordinates	Size (bp)	Start	Stop	IGN
tRNA ^{Ile}	I	J	GAT	1-63	63	-	-	-
tRNA ^{Gln}	Q	N	TTG	75-145	71	-	-	11
tRNA ^{Met}	M	J	CAT	145-213	69	-	-	-1
ND2	-	J	-	214-1225	1012	ATT	T--	0
tRNA ^{Trp}	W	J	TCA	1225-1290	66	-	-	-1
tRNA ^{Cys}	C	N	GCA	1281-1346	66	-	-	-10
tRNA ^{Tyr}	Y	N	GTA	1346-1410	65	-	-	-1
COI	-	J	-	1412-2945	1534	AAT	T--	1
tRNA ^{Leu2}	L2	J	TAA	2945-3013	69	-	-	-1
COII	-	J	-	3013-3700	688	ATG	T--	-1
tRNA ^{Lys}	K	J	TTT	3701-3772	72	-	-	0
tRNA ^{Asp}	D	J	GTC	3771-3836	66	-	-	-2
ATP8	-	J	-	3837-3992	156	ATC	TAA	0
ATP6	-	J	-	3986-4654	669	ATG	TAA	-7
COIII	-	J	-	4654-5435	782	ATG	TA-	-1
tRNA ^{Gly}	G	J	TCC	5436-5499	64	-	-	0
ND3	-	J	-	5500-5851	352	ATT	T--	0
tRNA ^{Ala}	A	J	TGC	5851-5916	66	-	-	-1
tRNA ^{Arg}	R	J	TCG	5915-5979	65	-	-	-2
tRNA ^{Asn}	N	J	GTT	5979-6043	65	-	-	-1
tRNA ^{Ser1}	S1	J	TCT	6044-6101	58	-	-	0
tRNA ^{Glu}	E	J	TTC	6101-6166	66	-	-	-1
tRNA ^{Phe}	F	N	GAA	6162-6226	65	-	-	-5
ND5	-	N	-	6226-7924	1699	ATT	T--	-1
tRNA ^{His}	H	N	GTG	7925-7986	62	-	-	0
ND4	-	N	-	7987-9304	1318	ATG	T--	0
ND4L	-	N	-	9298-9579	282	ATG	TAA	-7
tRNA ^{Thr}	T	J	TGT	9583-9649	67	-	-	3
tRNA ^{Pro}	P	N	TGG	9647-9712	66	-	-	-3
ND6	-	J	-	9714 -10211	498	ATT	TAA	1
CYTB	-	J	-	10211 -11348	1138	ATG	T--	-1

tRNA ^{Ser2}	S2	J	TGA	11349 -11415	67	-	-	0
ND1	-	N	-	11433-12383	951	TTG	TAG	17
tRNA ^{Leu1}	L1	N	TAG	12449-12384	66	-	-	0
16s rRNA	-	N	-	12450-13728	1279	-	-	0
tRNA ^{Val}	-	N	TAC	13729-13796	68	-	-	0
12s rRNA	-	N	-	13797-14534	738	-	-	0
AT-rich region	-	-	-	14535 -16566	2032	-	-	0

4.4.4 transfer RNAs, ribosomal RNAs, and AT-rich region

The set of tRNA genes identified with the ARWEN software was manually compared to those identified in other Alticini, and the most probable 22 tRNAs were annotated. Typical cloverleaf-like structures were predicted for all genes except tRNA^{Ser1}, which had an identical structure in both species (including morphotypes), but two differences in the nucleotide sequence, including an additional nucleotide in the tRNA^{Ser1} of *A. capensis* (Figure 4.5). The dihydrouridine (DHU) arm of tRNA^{Ser1} (TCT) was reduced and replaced by a simple loop in both species, as is commonly observed in many metazoan mitogenomes (Bernt *et al.*, 2013). This feature was also observed in other Alticini species such as *Agasicles hygrophila* (Selman and Vogt 1971) (Li *et al.*, 2015).



A. *Argopistes capensis*

B. *Argopistes sexvittatus* morphotypes (striped and black)

Figure 4.5 Predicted structure of tRNA^{Ser1} in the complete mitochondrial genomes of *Argopistes capensis* and *Argopistes sexvittatus* (Chrysomelidae: Alticini). The differences between the two tRNAs are highlighted in grey. Inferred canonical Watson-Crick bonds are represented by lines.

The length of tRNAs ranged from 59 bp (tRNA^{Ser1}) to 72 bp (tRNA^{Lys}) in *A. capensis*, and 58 bp (tRNA^{Ser1}) to 72 bp (tRNA^{Lys}) in *A. sexvittatus* morphotypes. The 16S rRNA gene was located between tRNA^{Leu1} and tRNA^{Val}, and the 12S rRNA was located between tRNA^{Val} and the AT-rich region. The length of the large ribosomal RNA (16S rRNA) gene was 1,278 bp for *A. capensis* and *A. sexvittatus* (striped), and 1,279 bp for *A. sexvittatus* (black) which is slightly higher than the average length of other Alticini species (1,220 bp), while the small ribosomal RNA (12S rRNA) gene was 738 bp for *A. capensis* and *A. sexvittatus* morphotypes which is slightly lower than the average length of other Alticini species (756 bp). The control region was located between the 12S rRNA and I-Q-M tRNA cluster and was annotated as the AT-rich region in comparison to other Alticini mitogenomes. The size of the AT-rich region was similar in *A. capensis* (2,015 bp), *A. sexvittatus* (striped) (2,009 bp), and *A. sexvittatus* (black) (2,032 bp), and similar to the AT-rich region in *A. tsekooni* (2,020 bp). The AT-rich region of *A. capensis* and *A. sexvittatus* (striped) was within the wide range found in other Alticini species, which varies from 1,309 bp in *Macrohaltica subplicata* (Leconte 1859) to 2,020 bp in *A. tsekooni*.

4.4.5 Start codons and stop codons

Most PCGs in Alticini started with ATN, except ND1 in 14 species, COI in four species, ND5 in three species, COII and ND2 in two species, and ATP8 and ND4L in one species, all of which used alternative start codons (GTG and AAT). The most frequently used start codon was ATG and the least frequently used was AAT (Figure 4.6).

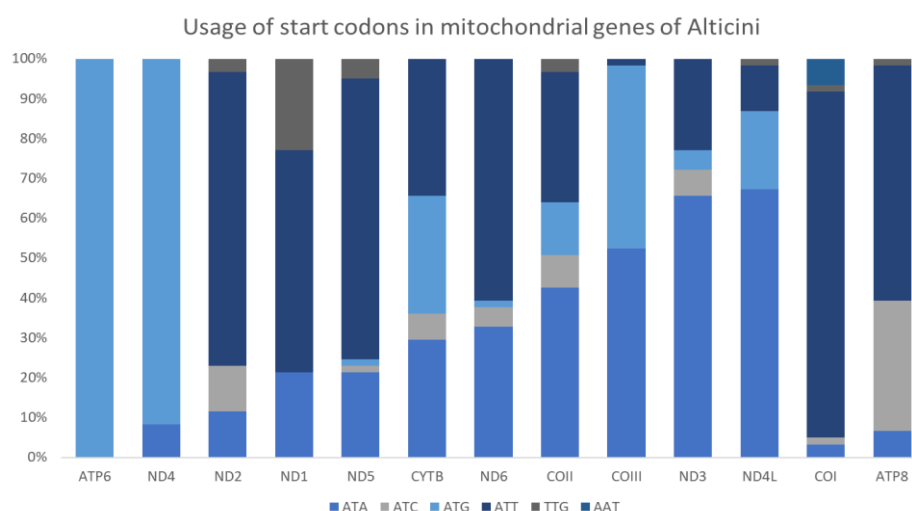


Figure 4.6 Usage of start codons found in the complete set of mitochondrial protein-coding genes in 62 flea beetle species within the tribe Alticini.

All PCGs in *A. capensis* and the *A. sexvitattus* morphotypes also used ATN start codons: ATG in ATP6, COII, COIII, CYTB, ND4, and ND4L, ATT in ND2, ND3, ND4, ND5, and ND6, and ATC in ATP8, except for COI (AAT) and ND1 (TTG). TTG has been previously found as a start codon for other coleopteran mitogenomes including *Altica cirsiicola* (Ohno 1960), *Altica fragariae* (Nakane 1955), and *Altica viridicyanea* (Baly 1874) (Nie *et al.*, 2019), and *A. tsekooni* (Long *et al.*, 2019). Most PCGs in both species terminated with the typical complete stop codon TAA, except ND1 which terminated with TAG. Incomplete stop codons (TA and T) were present in *A. capensis* and *A. sexvitattus* (COI, COII, COIII, CYTB, ND2, ND3, ND4, and ND5), and are generally presumed to be completed by posttranscriptional polyadenylation (Ojala *et al.*, 1981; Sheffield *et al.*, 2008).

4.4.6 Intergenic regions and spacers

Both species had highly compact mitogenomes with only short intergenic spaces at four locations in a total of 22 bp in *A. capensis*, and five locations (total of 33 bp) in *A. sexvitattus* morphotypes. Intergenic spacers ranged between 1 and 17 bp, with the longest located between ND1 and tRNA^{Ser2} in both species (17 bp) similar to *A. tsekooni* (17 bp) and other Alticini where the total number of intergenic nucleotides range from 1 to 61 bp. *Argopistes capensis* had a total of 19 gene overlaps and the *A. sexvitattus* morphotypes had 18, mostly involving tRNAs. The longest overlap in both species was between tRNA^{Cys} and tRNA^{Trp} (10 bp) in opposite directions. ND4L-ND4 and ATP8-ATP6 overlap by 7 bp, as in *A. tsekooni*.

4.4.7 Nucleotide composition and codon usage

Argopistes capensis and the two *A. sexvitattus* morphotypes had the high A+T content typically found in insect mitogenomes. The A+T content of the AT-rich regions of the mitogenomes (*A. capensis*, 90.5%; *A. sexvitattus* (striped), 85.7%; *A. sexvitattus* (black), 86.9%) was higher than that of their complete sequences (average, 80%). The A+T content of the combined tRNAs in *A. sexvitattus* (black) (81.0%) was higher than that of *A. capensis* (80.2%) and *A. sexvitattus* (striped) (80.8%). The A+T content of the combined rRNAs in *A. capensis* (82.9%) and the *A. sexvitattus* morphotypes (82.8%) were similar. The total PCGs of *A. capensis* and the *A. sexvitattus* morphotypes had an A+T content of 78.2% and 78.5% respectively. The A+T content in all genes ranged from COI (71.6 %) to ATP8 (90.3%) in *A. capensis*, from COI (71.6%) to ATP8 (87.0%) in *A. sexvitattus* (striped), and from COI (71.6%) to ATP8 (87.4%) in *A. sexvitattus* (black). The PCG with the highest A+T content in both species was ATP8 (Table 4.2).

Mitochondrial G+C content differs between species and is influenced by mutation bias, selection, and DNA repair bias (Qian *et al.*, 2018). When there are no mutations or selection bias, bases in the complementary DNA strand exist at equal frequencies, according to the second parity rule (Chen *et al.* 2019). The occurrence of AT- and GC-skews on the same DNA strand could mean that the species underwent mutations or environmental selection (Qian *et al.*, 2018).

The three mitogenomes had negative AT-skew and negative GC-skew in most genes (PCGs, tRNAs, rRNA, and AT-rich region) except ATP8, ND1, ND4, ND4L, and ND5, as well as COII in *A. sexvitattus*. In both species, four of the 13 PCGs encoded by the minority strand (N) had higher AT-skew values than PCGs encoded on the majority strand (J). The nucleotide bias towards A and T was reflected in codon usage, with the AT-rich codons (UUU, UUA, AUU, AUA, UAU, AAU, and AAA) representing 42.3% of all codons in *A. capensis*, 51.5% in *A. sexvitattus* (striped) and 52.1% in *A. sexvitattus* (black).

Relative synonymous codon usage (RSCU) for each codon is calculated as the relative frequency of a codon within a mitogenome. An RSCU value higher than 1.0 indicates an over-represented codon, whereas an RSCU value lower than one indicates an under-represented codon (Sharp *et al.*, 1986). RSCU was higher than 1.0 among all synonymous codons, indicating that AT-rich codons are favored among synonymous codons (Table 4.3).

4.4.8 Synonymous and nonsynonymous rates of protein-coding genes of *Alticini*

Average Ka/Ks was calculated for individual PCGs across the 62 *Alticini* species included in this study (Figure 4.7). The Ka/Ks ratio of non-synonymous (Ka) nucleotide substitutions to synonymous nucleotide substitutions (Ks) is used as an indicator of selective pressure on protein-coding sequences among different sequences (Wang *et al.*, 2009). A Ka/Ks ratio greater than 1 indicates positive selection, which is assumed to have occurred during the evolution of the sequence. ATP8 had the highest Ka/Ks (0.61), followed by ND4L and ND6 (0.39) and COX1 had the lowest Ka/Ks ratio (0.07). All genes had Ka/Ks < 1, which indicates that all PCGs are evolving under purifying selection.

Table 4.2 Nucleotide compositions of the complete mitochondrial sequences of the olive flea beetles, *Argopistes capensis* and *Argopistes sexvitattus*. AT-skew = $(A - T)/(A + T)$; CG-skew = $(G - C)/(G + C)$.

	<i>Argopistes capensis</i>									
Gene/region	A%	C%	G%	T%	A+T%	G+C%	AT-skew	GC-skew	Size (bp)	% (size)
COI	33.0	14.3	14.1	38.6	71.6	28.4	-0.08	-0.01	1534	9.3
COII	37.1	13.4	9.6	40.0	77.1	23.0	-0.04	-0.17	688	4.2
COIII	33.4	13.8	11.8	40.2	73.6	25.6	-0.09	-0.08	782	4.7
CYTB	35.9	13.2	10.9	40.0	75.9	24.1	-0.05	-0.10	1138	6.9
ATP6	37.9	13.7	8.8	39.6	77.5	22.5	-0.02	-0.22	672	4.1
ATP8	47.4	7.7	1.9	42.9	90.3	9.6	0.05	-0.60	156	0.9
ND1	49.0	13.7	7.8	29.5	78.5	21.5	0.25	-0.27	951	5.7
ND2	39.1	12.0	6.5	42.4	81.5	18.5	-0.04	-0.30	1012	6.1
ND3	36.6	13.4	7.4	42.6	79.2	20.8	-0.08	-0.29	352	2.1
ND4	51.1	12.3	7.2	29.4	80.5	19.5	0.27	-0.26	1318	8.0
ND4L	53.2	11.7	4.6	30.5	83.7	16.3	0.27	-0.44	282	1.7
ND5	48.1	11.8	8.0	32.0	80.1	19.8	0.20	-0.19	1699	10.3
ND6	39.8	10.4	5.2	44.6	84.4	15.6	-0.06	-0.33	498	3.0
16s rRNA	44.6	11.4	5.6	38.3	82.9	17.0	0.08	-0.34	1278	7.7
12s rRNA	44.2	11.2	5.8	38.8	83	17.0	0.07	-0.32	738	4.5
Total PCGs	41.5	12.8	9.0	36.7	78.2	21.8	0.06	-0.17	11082	67.0
Total tRNAs	41.6	11.3	8.5	38.6	80.2	19.8	0.04	-0.14	1383	8.4
Total rRNAs	44.4	11.4	5.7	38.5	82.9	17.1	0.07	-0.33	2016	12.2
AT-rich region	49.2	4.0	5.4	41.3	90.5	9.4	0.09	0.15	2015	12.2
Complete mtDNA	42.5	11.8	8.2	37.5	80	20.0	0.06	-0.18	16543	100.0

	<i>Argopistes sexvitattus</i> (striped morphotype)									
Gene/region	A%	C%	G%	T%	A+T%	G+C%	AT-skew	GC-skew	Size (bp)	% (size)
COI	32.7	14.4	14.0	38.9	71.6	28.4	-0.09	-0.01	1534	9.3
COII	38.1	14.8	9.6	37.5	75.6	24.4	0.01	-0.21	688	0.0
COIII	34.0	14.1	11.8	40.2	74.2	25.9	-0.08	-0.09	782	4.7
CYTB	35.7	12.5	10.6	41.2	76.9	23.1	-0.07	-0.08	1138	6.9
ATP6	37.3	13.2	9.6	40.0	77.3	22.8	-0.03	-0.16	669	4.0
ATP8	44.8	10.4	2.6	42.2	87.0	13.0	0.03	-0.60	156	0.9
ND1	49.3	11.9	8.0	30.8	80.1	19.9	0.23	-0.20	951	5.7
ND2	39.1	11.7	6.8	42.4	81.5	18.5	-0.04	-0.26	1012	6.1
ND3	37.5	11.1	8.2	43.2	80.7	19.3	-0.07	-0.15	352	2.1
ND4	51.3	12.1	7.6	29.1	80.4	19.7	0.28	-0.23	1318	8.0
ND4L	53.9	10.3	4.3	31.6	85.5	14.6	0.26	-0.41	282	1.7
ND5	49.4	11.5	7.8	31.3	80.7	19.3	0.22	-0.19	1699	10.3
ND6	40.4	8.0	5.2	46.3	86.7	13.2	-0.07	-0.21	497	3.0

16s rRNA	44.6	11.6	5.6	38.4	83.0	17.2	0.07	-0.35	1278	7.7
12s rRNA	43.2	11.5	6.0	39.3	82.5	17.5	0.05	-0.31	738	4.5
Total PCGs	41.7	12.4	9.1	36.8	78.5	21.5	0.06	-0.15	11078	67.0
Total tRNAs	41.8	11.0	8.2	39.0	80.8	19.2	0.03	-0.15	1382	8.4
Total rRNAs	44.1	11.4	5.6	38.7	82.8	17.0	0.07	-0.34	2016	12.2
AT-rich region	46.1	8.2	6.1	39.6	85.7	14.3	0.08	-0.15	2009	12.1
Complete mtDNA	42.4	11.7	8.3	37.5	79.9	20.0	0.06	-0.17	16542	100.0

<i>Argopistes sexvitattus</i> (black morphotype)										
Gene/region	A%	C%	G%	T%	A+T%	G+C%	AT-skew	GC-skew	Size (bp)	% (size)
COI	32.7	14.4	14.0	38.9	71.6	28.4	-0.09	-0.01	1534	9.3
COII	37.9	14.8	9.7	37.5	75.4	24.5	0.01	-0.21	688	4.2
COIII	33.9	14.1	11.8	40.3	74.2	25.9	-0.09	-0.09	782	4.7
CYTB	35.6	12.7	10.6	41.0	76.6	23.3	-0.07	-0.09	1138	6.9
ATP6	37.4	13.0	9.6	40.1	77.5	22.6	-0.03	-0.15	669	4.0
ATP8	45.0	9.7	2.6	42.4	87.4	12.3	0.03	-0.58	156	0.9
ND1	49.3	11.9	8.0	30.8	80.1	19.9	0.23	-0.20	951	5.7
ND2	38.9	11.7	7.0	42.4	81.3	18.7	-0.04	-0.25	1012	6.1
ND3	37.5	11.1	8.2	43.2	80.7	19.3	-0.07	-0.15	352	2.1
ND4	51.3	12.1	7.6	29.1	80.4	19.7	0.28	-0.23	1318	8.0
ND4L	53.9	10.3	4.3	31.6	85.5	14.6	0.26	-0.41	282	1.7
ND5	49.6	11.5	7.7	31.3	80.9	19.2	0.23	-0.20	1699	10.3
ND6	40.4	8.0	5.2	46.3	86.7	13.2	-0.07	-0.21	498	3.0
16s rRNA	44.6	11.3	5.6	38.4	83.0	16.9	0.07	-0.34	1279	7.7
12s rRNA	43.2	11.5	6.0	39.3	82.5	17.5	0.05	-0.31	738	4.5
Total PCGs	41.7	12.4	9.1	36.8	78.5	21.5	0.06	-0.15	11079	66.9
Total tRNAs	41.8	10.9	8.1	39.2	81.0	19.0	0.03	-0.15	1452	8.8
Total rRNAs	44.1	11.4	5.8	38.7	82.8	17.2	0.07	-0.33	2017	12.2
AT-rich region	45.8	7.5	5.6	41.1	86.9	13.1	0.05	-0.15	2032	12.3
Complete mtDNA	42.4	11.6	8.2	37.7	80.1	19.8	0.06	-0.17	16566	100.0

Table 4.3 Codon usage in the complete mitochondrial genomes of the olive flea beetles *Argopistes capensis* and *Argopistes sexvittatus*. Amino acids are labeled according to the IUPAC-IUB single-letter codes. N - the total number of occurrences in all protein-coding genes, RSCU - relative synonymous codon usage.

<i>Argopistes capensis</i>							
Amino acid	Codon	N	RSCU	Amino acid	Codon	N	RSCU
F	UUU	352	1.56	Y	UAU	252	1.54
	UUC	99	0.44		UAC	75	0.46
L	UUA	304	2.95	H	CAU	80	1.58
	UUG	64	0.62		CAC	21	0.42
	CUU	101	0.98	Q	CAA	87	1.47
	CUC	25	0.24		CAG	31	0.53
	CUA	88	0.85	N	AAU	428	1.62
	CUG	36	0.35		AAC	99	0.38
I	AUU	380	1.65	K	AAA	501	1.76
	AUC	80	0.35		AAG	69	0.24
M	AUA	302	1.72	D	GAU	64	1.51
	AUG	50	0.28		GAC	21	0.49
V	GUU	36	1.48	E	GAA	109	1.63
	GUC	13	0.54		GAG	25	0.37
	GUA	36	1.48	C	UGU	26	0.95
	GUG	12	0.49		UGC	29	1.05
S	UCU	72	1.66	W	UGA	62	1.48
	UCC	32	0.74		UGG	22	0.52
	UCA	82	1.89	R	CGU	7	0.93
	UCG	14	0.32		CGC	3	0.4
P	CCU	51	1.51		CGA	19	2.53
	CCC	36	1.07		CGG	1	0.13
	CCA	41	1.21	S	AGU	34	0.78
	CCG	7	0.21		AGC	24	0.55
T	ACU	70	1.48		AGA	53	1.22
	ACC	44	0.93		AGG	37	0.85
	ACA	64	1.35	G	GGU	23	1.35
	ACG	11	0.23		GGC	9	0.53
A	GCU	27	1.48		GGA	26	1.53
	GCC	16	0.88		GGG	10	0.59
	GCA	28	1.53				
	GCG	2	0.11				

<i>Argopistes sexvitattus</i> (striped morphotype)							
Amino acid	Codon	N	RSCU	Amino acid	Codon	N	RSCU
F	UUU	331	1.57	Y	UAU	269	1.58
	UUC	91	0.43		UAC	72	0.42
L	UUA	327	3.3	H	CAU	76	1.6
	UUG	57	0.58		CAC	19	0.4
	CUU	79	0.8	Q	CAA	105	1.72
	CUC	31	0.31		CAG	17	0.28
	CUA	75	0.76	N	AAU	403	1.67
	CUG	25	0.25		AAC	81	0.33
I	AUU	415	1.62	K	AAA	431	1.75
	AUC	96	0.38		AAG	62	0.25
M	AUA	329	1.77	D	GAU	63	1.54
	AUG	43	0.23		GAC	19	0.46
V	GUU	42	1.49	E	GAA	67	1.79
	GUC	9	0.32		GAG	8	0.21
	GUA	54	1.91	C	UGU	23	1.1
	GUG	8	0.28		UGC	19	0.9
S	UCU	74	1.33	W	UGA	77	1.5
	UCC	38	0.68		UGG	26	0.5
	UCA	100	1.8	R	CGU	6	0.83
	UCG	14	0.25		CGC	0	0
P	CCU	42	1.3		CGA	20	2.76
	CCC	33	1.02		CGG	3	0.41
	CCA	49	1.52	S	AGU	35	0.63
	CCG	5	0.16		AGC	53	0.95
T	ACU	76	1.34		AGA	102	1.84
	ACC	39	0.69		AGG	28	0.5
	ACA	93	1.64	G	GGU	25	1.04
	ACG	19	0.33		GGC	5	0.21
A	GCU	40	1.82		GGA	57	2.38
	GCC	17	0.77		GGG	9	0.38
	GCA	30	1.36				
	GCG	1	0.05				

<i>Argopistes sexvitattus</i> (black morphotype)							
Amino acid	Codon	N	RSCU	Amino acid	Codon	N	RSCU
F	UUU	344	1.55	Y	UAU	301	1.64
	UUC	100	0.45		UAC	67	0.36
L	UUA	363	3.47	H	CAU	80	1.65
	UUG	48	0.46		CAC	17	0.35
	CUU	86	0.82	Q	CAA	84	1.57
	CUC	22	0.21		CAG	23	0.43
	CUA	93	0.89	N	AAU	396	1.54
	CUG	15	0.14		AAC	119	0.46
I	AUU	398	1.62	K	AAA	453	1.63
	AUC	92	0.38		AAG	104	0.37
M	AUA	321	1.72	D	GAU	50	1.43
	AUG	53	0.28		GAC	20	0.57
V	GUU	31	1.57	E	GAA	84	1.62
	GUC	9	0.46		GAG	20	0.38
	GUA	34	1.72	C	UGU	33	0.97
	GUG	5	0.25		UGC	35	1.03
S	UCU	73	1.35	W	UGA	71	1.22
	UCC	49	0.91		UGG	45	0.78
	UCA	99	1.83	R	CGU	10	0.89
	UCG	14	0.26		CGC	5	0.44
P	CCU	30	1.05		CGA	24	2.13
	CCC	29	1.02		CGG	6	0.53
	CCA	50	1.75	S	AGU	45	0.83
	CCG	5	0.18		AGC	44	0.81
T	ACU	74	1.44		AGA	63	1.17
	ACC	40	0.78		AGG	45	0.83
	ACA	80	1.55	G	GGU	17	1.05
	ACG	12	0.23		GGC	8	0.49
A	GCU	25	1.47		GGA	36	2.22
	GCC	14	0.82		GGG	4	0.25
	GCA	26	1.53				
	GCG	3	0.18				

Ka, Ks and Ka/Ks ratio in mitochondrial genes of the tribe Alticini

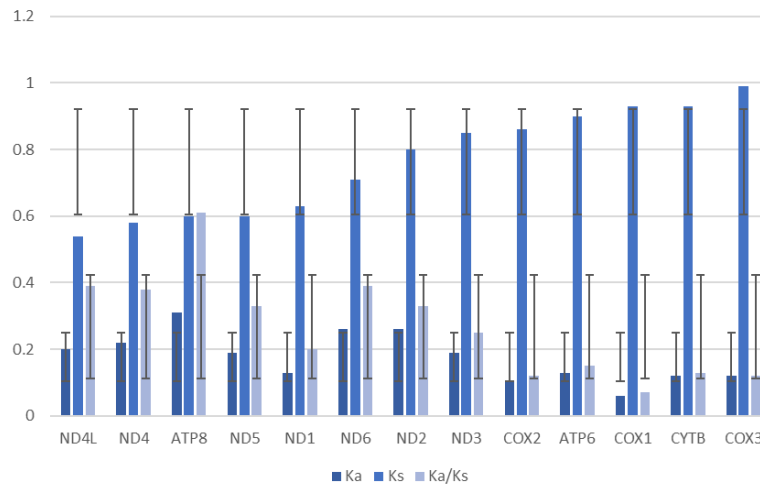


Figure 4.7 Evolutionary rates of 13 protein-coding genes in the mitogenomes of 62 Alticini species. The rate of nonsynonymous substitutions (Ka), the rate of synonymous substitutions (Ks), and the ratio of the rate of nonsynonymous substitutions to synonymous substitutions (Ka/Ks) were estimated for each protein-coding gene.

4.4.9 Phylogenetic position of *A. capensis* and *A. sexvitattus* within Alticini

The phylogenetic position of *A. capensis* and *A. sexvitattus* morphotypes within the tribe Alticini was recovered using the novel mitogenome sequences and all available mitogenomes available on GenBank as of April 2021 (Table S10). Three datasets were used in the phylogenetic reconstruction: PCG123 (all codon positions) with each codon position partitioned, PCG12 (except 3rd position), and PCGAA (amino acid). The phylogenetic trees recovered different topologies (Figure 4.8). The PCGAA tree had higher statistical support for the majority of nodes (BPP = 1) and the positions of all the species were completely resolved. The positions of all the species in PCG123 were also completely resolved. However, this was not the case for the PCG12 tree as the position of *Mantura chrysanthemii* (Koch 1803), and the position of the sister species *Neocrepidodera brevicollis* (Daniel 1904) and *Orestia punctipennis* (Lucas 1849) were unresolved. *Agasicles hygrophila* occupied the basal position in PCG123, but not in PCG12 and PCGAA, the species formed a clade with the genus *Altica* and *Macrohaltica subplicata*. In all the trees, *Argopistes* clade was sister to the *Apteropeda* Chevrolat 1836 clade. The position of *Batophila aerata* (Marsham 1802) differed in the three trees with low statistical support in PCG123 and PCG12. *Batophila aerata* was recovered closely related to genera *Argopistes* and *Apteropeda* in PCG123. In contrast, *B. aerata* was recovered closely related to a clade formed between *Altica* and *M. subplicata* in PCG12. In PCGAA, *B. aerata* formed a clade

with the genus *Phyllotreta* with high statistical support (BPP = 1). *Neocrepidodera brevicollis* formed a clade with *Orestia punctipennis* in all three trees with high statistical support. The position of *Mantura chrysanthemi* differed in all phylogenetic analyses. *Mantura chrysanthemi* formed a clade with the genus *Psylliodes* in PCG123 whereas in PCG12 the position of *M. chrysanthemi* is unresolved. In PCGAA, *M. chrysanthemi* formed a clade with *N. brevicollis* and *O. punctipennis* with high statistical support. All genera represented by multiple species formed monophyletic clades with high statistical support in all phylogenetic analyses. *Longitarsus* (Latreille 1829) and *Aphthona* (Chevrolat 1836) were recovered as sister genera in agreement with previous phylogenies (Nie *et al.*, 2018). The arrangements of *Longitarsus* species were identical in PCG 123 and PCG12 but differed in PCGAA. The genus *Altica* (Geoffroy 1762) and *M. subplicata* were also recovered as sister taxa, as in previous reconstructions (Nie *et al.*, 2018). *Chaetocnema* (Stephens 1831) and *Phyllotreta* (Chevrolat 1836) were recovered as sister genera in disagreement with previous phylogenies. Previously, *Chaetocnema* was recovered as closely related to *Psylliodes* (Latreille 1829) and *Crepidodera* (Chevrolat 1836) (Nie *et al.*, 2018). The two olive flea beetles were placed in the same phylogenetic clade with *A. tsekooni* with high statistical support (BPP = 1) in all the trees. This result show adaptation of olive flea beetles, *Argopistes* spp. feeding on *Oleaceae*. As shown here, mitogenome data is useful for inferring phylogenetic patterns and relationships among individual organisms, provided that the morphology is congruent with the genetic data. Mitogenome data are very informative for the phylogenetic placement of individual organisms at tribe level.

4.4.10 Variation in mitochondrial genomes of *A. sexvittatus* morphotypes

A. sexvittatus (striped) and *A. sexvittatus* (black) had similar mitochondrial genome sequences with 96.7% identical sites. A total of 19 nucleotide substitutions were identified in the 13 PCGs in the alignment of *A. sexvittatus* (striped) and *A. sexvittatus* (black) mitochondrial genome sequences. ATP6, COX1, COX2, and COX3 had only a single nucleotide substitution, followed by ATP8, ND2, and ND5 with two nucleotide substitutions, ND4 with four nucleotide substitutions, and CYTB with five nucleotide substitutions. No nucleotide substitutions were identified in the genes ND1, ND3, ND4L, and ND6. These types of nucleotide substitutions are referred to as single nucleotide polymorphisms (SNP), which is usually a variation at a single position in DNA among individuals (Khlestkina and Salina, 2005). A gene is described as having more than one allele if SNPs occur within it. Some SNPs may lead to variations in the amino acid sequence; however, this is not the case in

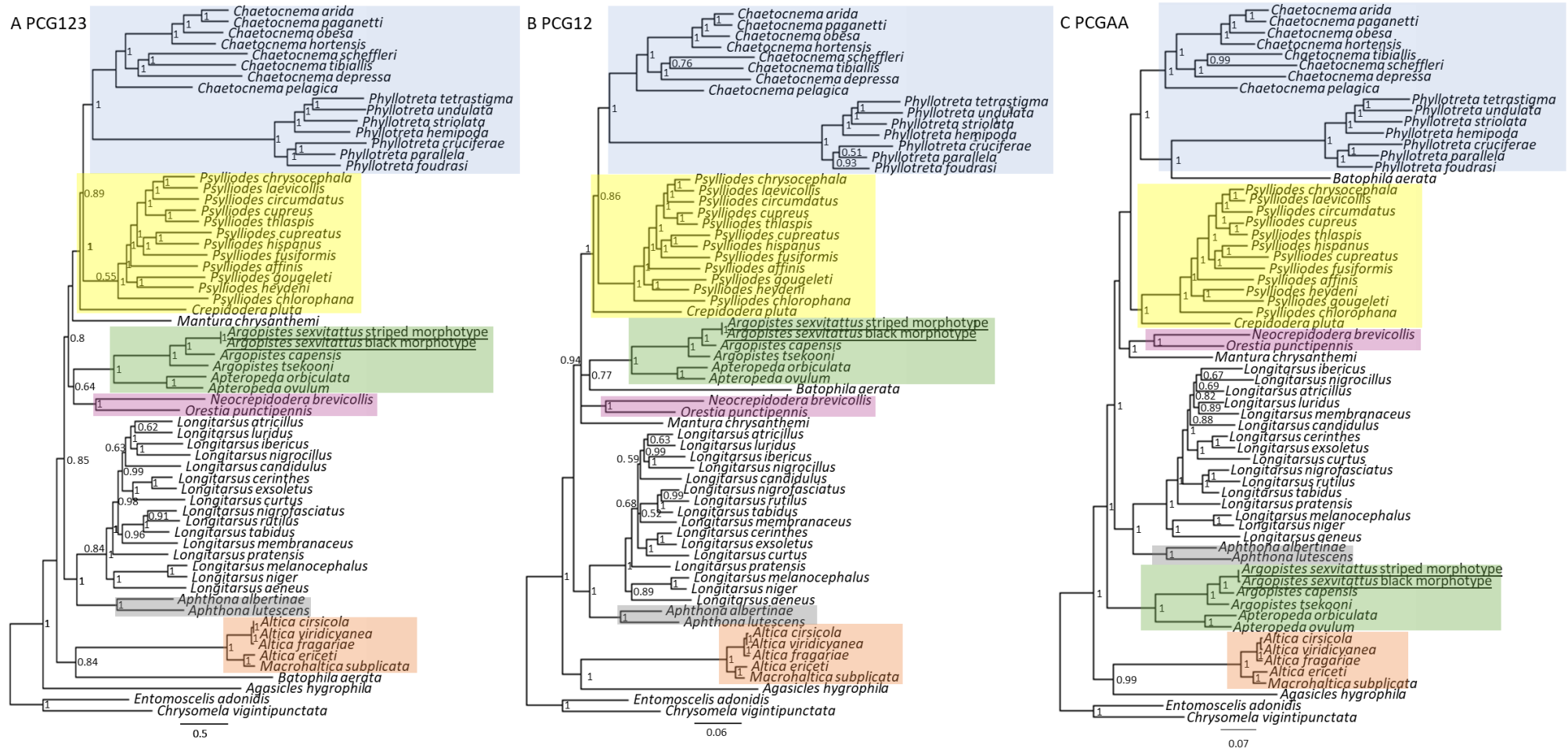


Figure 4.8. Phylogenetic relationships among 62 flea beetle species (Chrysomelidae: Alticini) based on 13 mitochondrial protein-coding genes using Bayesian Inference. (A) PCG123 - all codon positions; (B) PCG12 - only 1st and 2nd codon positions and (C) PCGAA- amino acid. *Chrysomela vigintipunctata* and *Entomoscelis adonidis* (Chrysomelidae: Chrysomelini) were used as outgroups. Nodal statistical support is given as Bayesian posterior probability.

these two *A. sexvitattus* specimens. CYTB had the most nucleotide substitutions. In previous studies, CYTB has been proven to be more variable when sequences belonging to the same species are considered (Ndong *et al.*, 2015). In this study, the COI was used as a DNA barcode marker for *A. sexvitattus*, but there were no significant variations in the sequences of the different morphotypes barcoded. Therefore, CYTB should be considered a DNA marker for *A. sexvitattus* morphotypes and other species that have more than one morphotype.

4.4.11 Intraspecific divergence of *A. capensis* and *A. sexvitattus*

DNA-based species identification was consistent with morphological identification, and different morphotypes of *A. sexvitattus* were not associated with specific COI haplotypes (Table S4.2, Figure 4.9). The intraspecific genetic divergence of *A. sexvitattus* based on DNA barcodes for 31 individuals (intraspecific max p-distances = 0.40%) was similar to the majority of Alticini species (67%), which had maximum intraspecific p-distances below 3%. As only one specimen of *A. capensis* was sequenced it was not possible to gain insights into the intraspecific genetic divergence for this species. Therefore, only the intraspecific genetic divergence of *A. sexvitattus* was assessed in the context of 115 other species of Alticini.

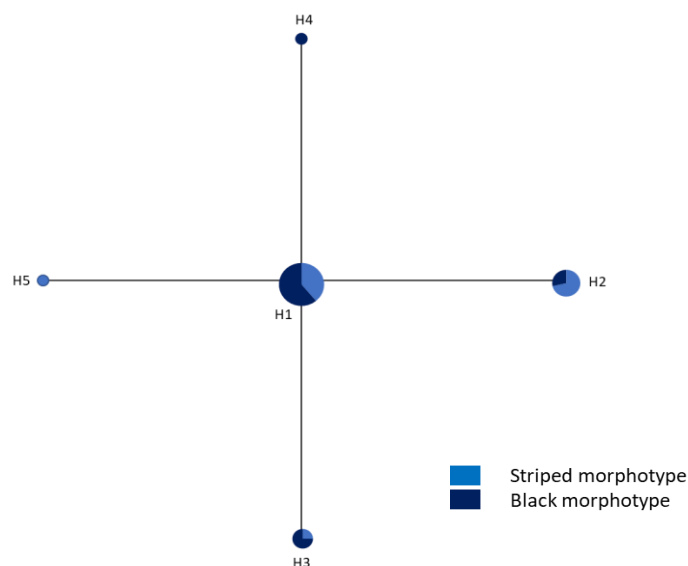


Figure 4.9 Median-joining network of COI haplotypes of the two morphotypes of *A. sexvitattus*.

4.4.12 Alticini species have a high incidence of non-monophyly

Intraspecific maximum p-distances in Alticini ranged from 0.00% to 25.28%, and were lowest for *Andersonaltica villabarrancoli*, *Longitarsus dorsalis*, *Longitarsus symphyti*, *Phyllotreta albionica*, and *Psylliodes crambicola* (0.00%), and highest for *Altica cirsicola* (25.28%) (TABLE S4.3, Figure 4.10). Genetic clustering showed that 99 species (85.34%) formed monophyletic clades with high statistical support, but 17 species (14.66%) were non-monophyletic (Figure S4.1). A significant amount of the monophyletic species (24 out of the 99) had high maximum intraspecific p-distances (3.09% - 25.28%).

There are several reasons for species appearing non-monophyletic in phylogenetic analyses which include incomplete lineage sorting, introgression, and hybridization; likely to occur in recently diverged species than older lineages. Other reasons include misidentifications, clerical errors, presence of NUMTs, contamination, and methodological issues in phylogenetic inference and taxonomic inconsistencies that result from over-splitting of species, lumping of species that are usually cryptic (Mutanen *et al.*, 2016). Overall, these results showed the incidence of non-monophyly due to potential misidentifications, cryptic diversity, and instances of possible taxonomic over-splitting.

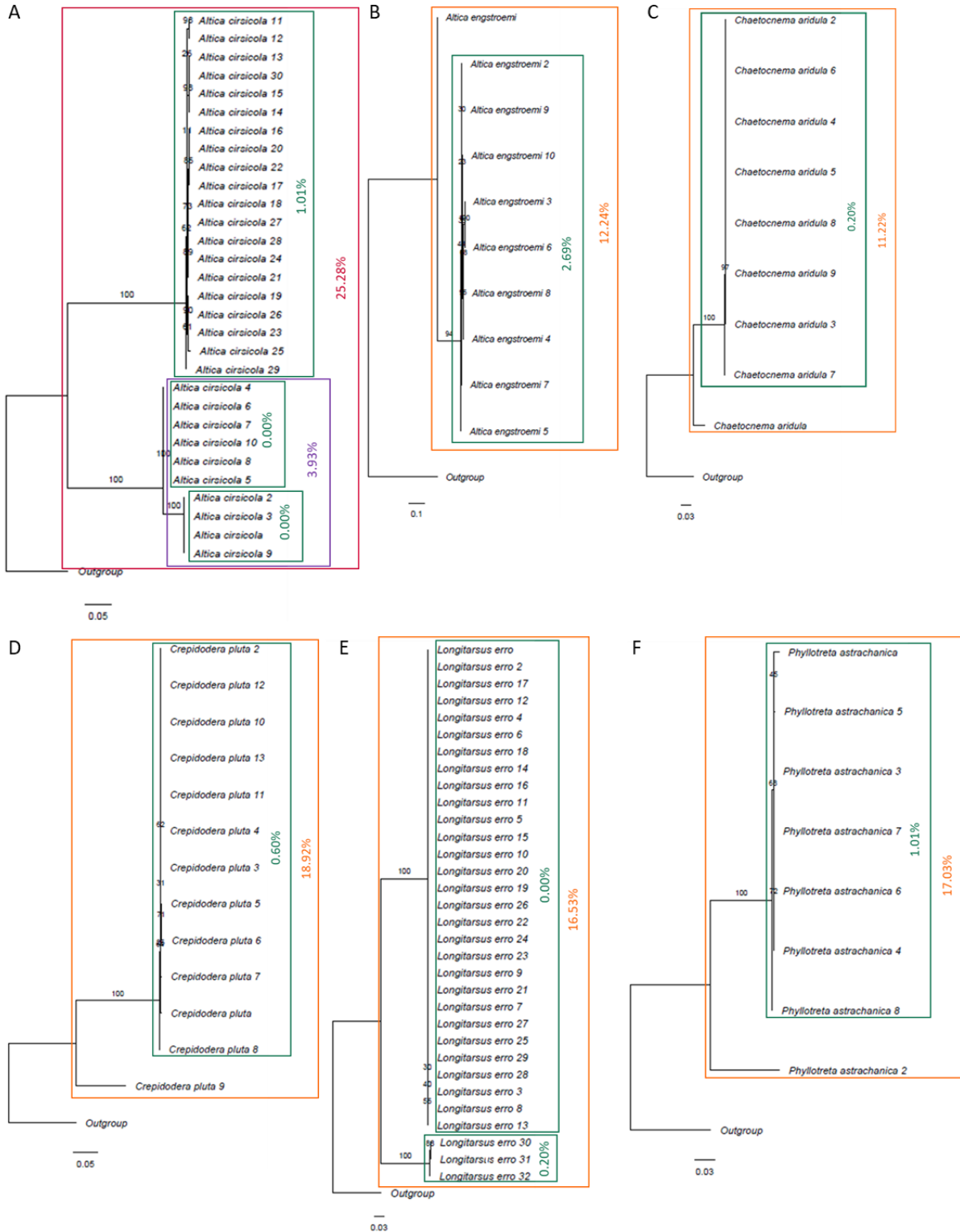
Possible cases of cryptic diversity

Twenty-four species (*Aphthona cyparissiae*, *Aphthona lutescens*, *Aphthona nonstriata*, *Batophila rubi*, *Chaetocnema concinna*, *Derocrepis rufipes*, *Hermaeophaga mercurialis*, *Longitarsus lycopi*, *Longitarsus melanocephalus*, *Longitarsus obliterates*, *Longitarsus parvulus*, *Longitarsus pellucidus*, *Longitarsus suturellus*, *Longitarsus tabidus*, *Mandarella flaviventris*, *Mandarella tsoui*, *Mandarella uenoi*, *Neocrepidodera femorata*, *Neocrepidodera ferruginea*, *Neocrepidodera transversa*, *Phyllotreta nigripes*, *Phyllotreta striolata*, *Podagrica fuscicornis* and *Psylliodes cucullatus*) were monophyletic but had high intraspecific maximum p-distances, including well supported multiple lineages, suggesting that these could represent cryptic diversity (Figure S4.2). In the case of *Mandarella* spp., the multiple lineages are very common and may indicate local adaptation or an even more complicated evolutionary process (Lee *et al.*, 2011). *Phyllotreta nigripes* recovered two clades which have been observed previously (Şahin *et al.* 2019).

Possible cases of misidentification or over-splitting

Altica cirsicola, *Altica engstroemi*, *Chaetocnema aridula*, *Chaetocnema pluta*, *Longitarsus erro*, and *Phyllotreta astrachanica* were polyphyletic with high max p-distances as a result of one or more sequences that were highly diverged (Figure S4.3). The polyphyly of *L. erro* is

common and has been observed in a previous study (Salvi *et al.*, 2020). This may be likely to misidentification or contamination. A highly diverged sequence belonging to *P. astrachanica* grouped with *P. atra* (intraspecific max p-distance = 1.01%) indicating that the sequence was misidentified (Figure S4.3 G).



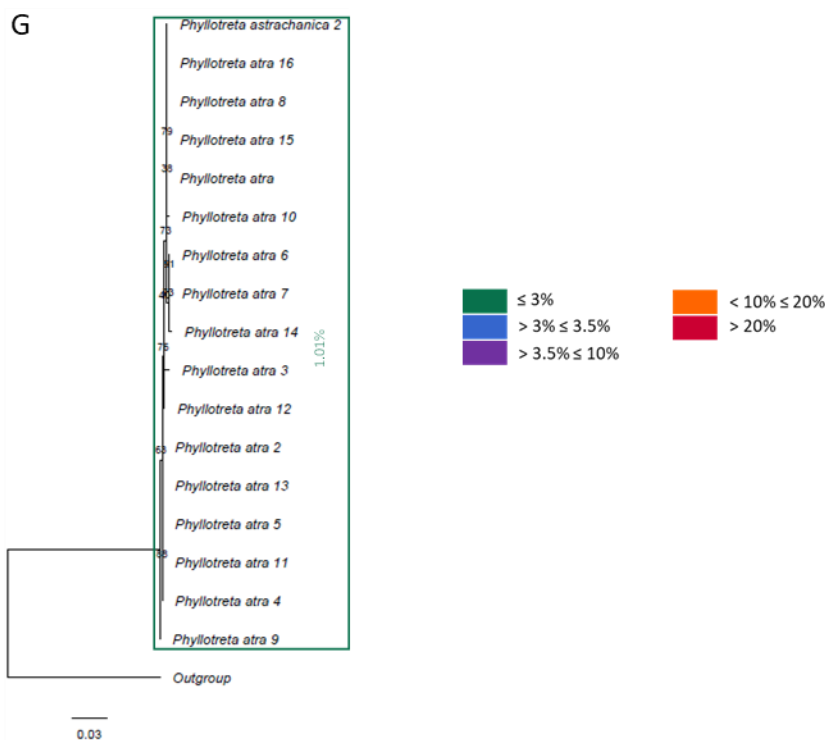


Figure 4.10 Maximum likelihood trees of flea beetle species (Hemiptera: Tingidae) based on 501-bp alignments of standard COI barcoding sequences that showed polyphyly possible due to misidentification.

Altica aenescens (intraspecific max p-distance = 2.45%) and *Altica chamaenerii* (intraspecific max p-distance 2.04%) had maximum p-distances lower than 3%; however, *A. chamaenerii* was paraphyletic. The maximum intraspecific p-distance of both species was 2.46% and indicated that these are a single species, which may be a result of taxonomic over-splitting or recent speciation (Figure 4.11 A). *Altica chamaenerii* also formed group with *Altica engstroemi* (intraspecific max p-distance = 3.31%) (Figure 4.11 B). These suggest that they may be difficult to distinguish morphologically between *A. chamaenerii*, *A. chamaenerii*, and *A. engstroemi*.

Altica cirsiicola, *A. fragariae*, and *A. viridicyanea* appeared paraphyletic and had high maximum intraspecific p-distances of 25.28%, 4.59%, and 3.51%, respectively; indicating heterospecificity of the sequences, which may be due to misidentification or contamination, NUMTs, and hybridization. *Altica fragariae*, *A. cirsiicola*, and *A. viridicyanea* formed a group of highly similar sequences (intraspecific max p-distance 1.41%), which indicated that

the group may be conspecific (Figure 4.11 C). *Altica fragariae* and *A. viridicyanea* also formed a group of similar sequences (intraspecific max p-distance 1.42%) indicating that the group may also be conspecific. *Altica cirsiicola*, *Altica fragariae*, and *Altica viridicyanea* are highly similar morphologically and require additional characters to differentiate them (Yu *et al.*, 1996)(Nie *et al.*, 2019; Yu *et al.*, 1996).

Crepidodera aurata (intraspecific max p-distance = 17.94%), *Crepidodera aurea* (intraspecific max p-distance = 18.77%), and *Crepidodera fulvicornis* (intraspecific max p-distance = 18.71%) were also paraphyletic and had high maximum intraspecific p-distances likely due to misidentification. *Crepidodera aurea* and a single sequence *C. aurata* also formed a group of similar sequences (intraspecific p-max 0.81%) (Figure 4.11 D).

Crepidodera aurata and a single sequence of *C. fulvicornis* formed a group of highly similar sequences (intraspecific max p-distance 0.81%), which indicates that the group may be conspecific (Figure 4.11 E). *Crepidodera aurea* formed another group with *C. fulvicornis* (intraspecific p-max 1.42%) (Figure 4.11 F). *Crepidodera aurata*, *C. aurea*, and *C. fulvicornis* may be morphologically similar, and therefore difficult to distinguish by non-expert expert taxonomists.

Phyllotreta albionica (intraspecific max p-distance = 0.00%) and *Phyllotreta cruciferae* (intraspecific max p-distance 0.20%) formed a group of similar sequences. The intraspecific max p-distance between both species (0.20%) suggests that the sequences represented conspecific individuals, which may be a result of taxonomic over-splitting or recent speciation (Figure 4.11 G). The grouping of sequences belonging to these species was also observed in a previous study (Şahin *et al.*, 2019). Despite molecular data suggesting that these may be a single species, morphologically *P. cruciferae* and *P. albionica* are similar with the only significant difference being the colour of the lustre which is blue in *P. cruciferae*, and bronze in *P. albionica* (Capinera, 2001). *Phyllotreta cruciferae* and *P. albionica* may require taxonomic revision and additional morphological characters apart from colour to confirm or dispute that these species may be oversplit.

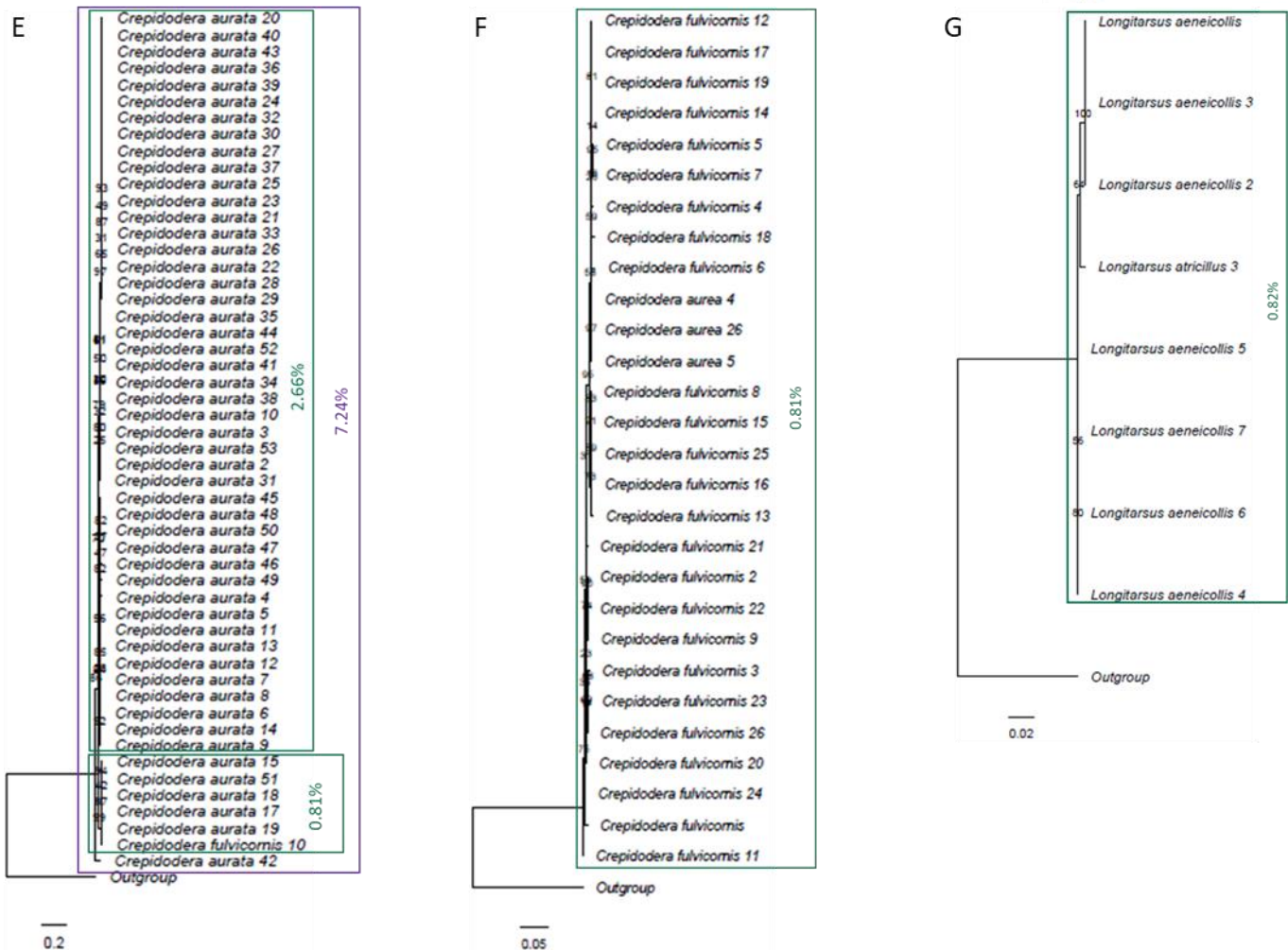
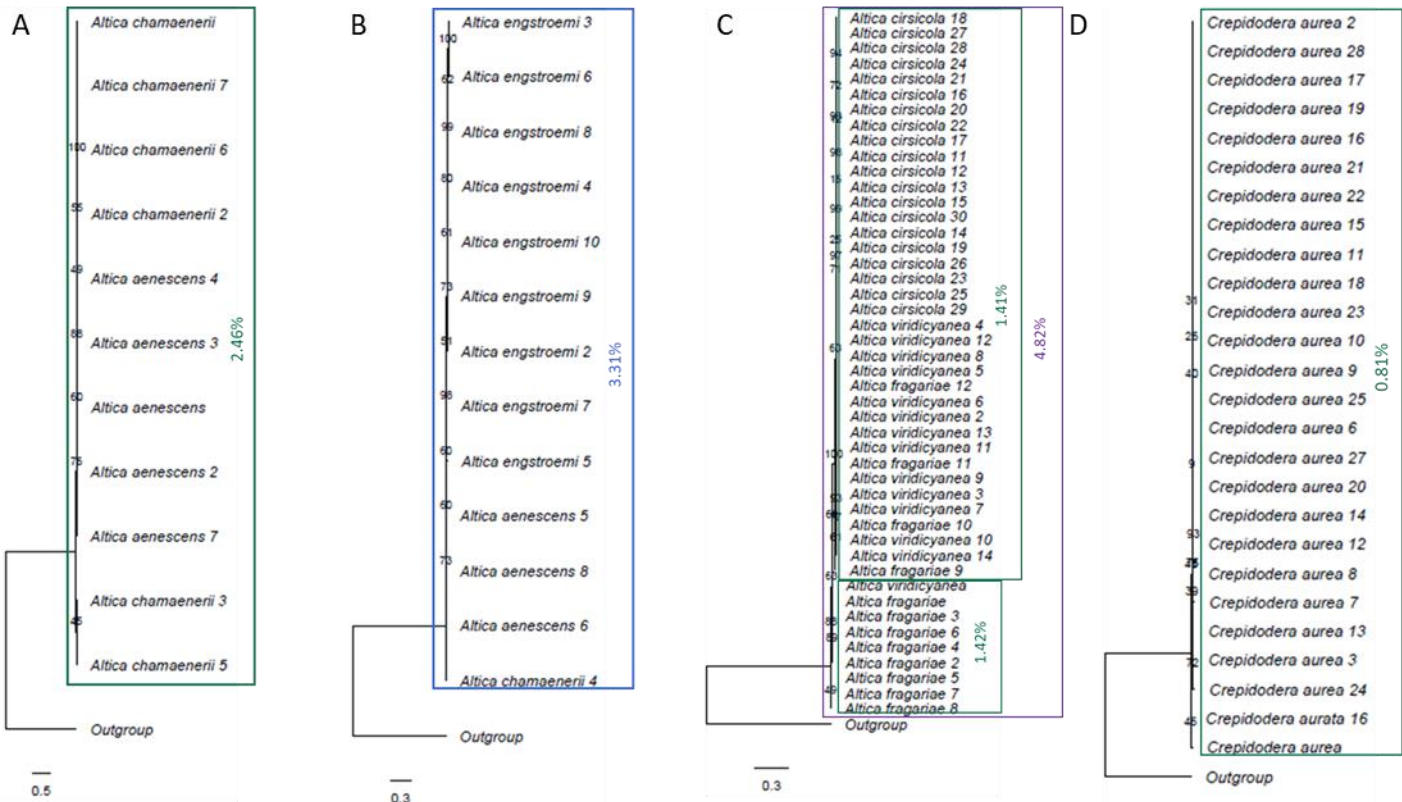
Longitarsus atricillus had a high maximum p-distance (4.61%) when including a single sequence that grouped with the *Longitarsus aeneicollis* cluster in the ML tree (FIGURE S1). The maximum intraspecific p-distance of the *L. aeneicollis* cluster, including the outlying *L. atricillus* sequence, was 0.82%, which is significantly lower than the threshold suggesting

that the sequences are conspecific and the single sequence of *L. atricillus* may be misidentified (Figure 4.11 H).

Longitarsus luridus (intraspecific max p-distance = 2.88%) and *Longitarsus testaceus* (intraspecific max p-distance 0.60%) had low maximum p-distances; however, *L. luridus* (was paraphyletic. The maximum intraspecific p-distance of both species was 3.07% and indicated that these are a single species, which may be due to taxonomic over-splitting or recent speciation(Figure 4.11 I).

Longitarsus pratensis had a high intraspecific max p-distance intraspecific max p-distance (6.31%) when including a single highly diverged sequence. The identity of this sequence remains unknown, based on the results of a search on BOLD Systems. *Longitarsus scutellaris* (intraspecific max p-distance 0.00%) and *L. pratensis* (intraspecific max p-distance 2.87%) excluding the misidentified *L. pratensis* sequence formed a group of similar sequences. The intraspecific max p-distance between both species was determined (3.08%) and indicated that the sequences represented conspecific individuals, suggesting that this is a case of taxonomic over-splitting (Figure 4.11 J). The species were morphologically similar making it difficult to distinguish from one another and are genetically undifferentiated as observed as well in a previous study (Salvi *et al.*, 2020). The taxonomic revision of these species is necessary.

Despite the low maximum intraspecific p-distance, *Psylliodes isatidis* (2.88%) appeared to be paraphyletic, as a single sequence *P. isatidis* grouped with the *P. crambicola* cluster. The interspecific distance between both species (2.88%) was below 3%, which indicates that they belong to the same species (Figure 4.11 K).



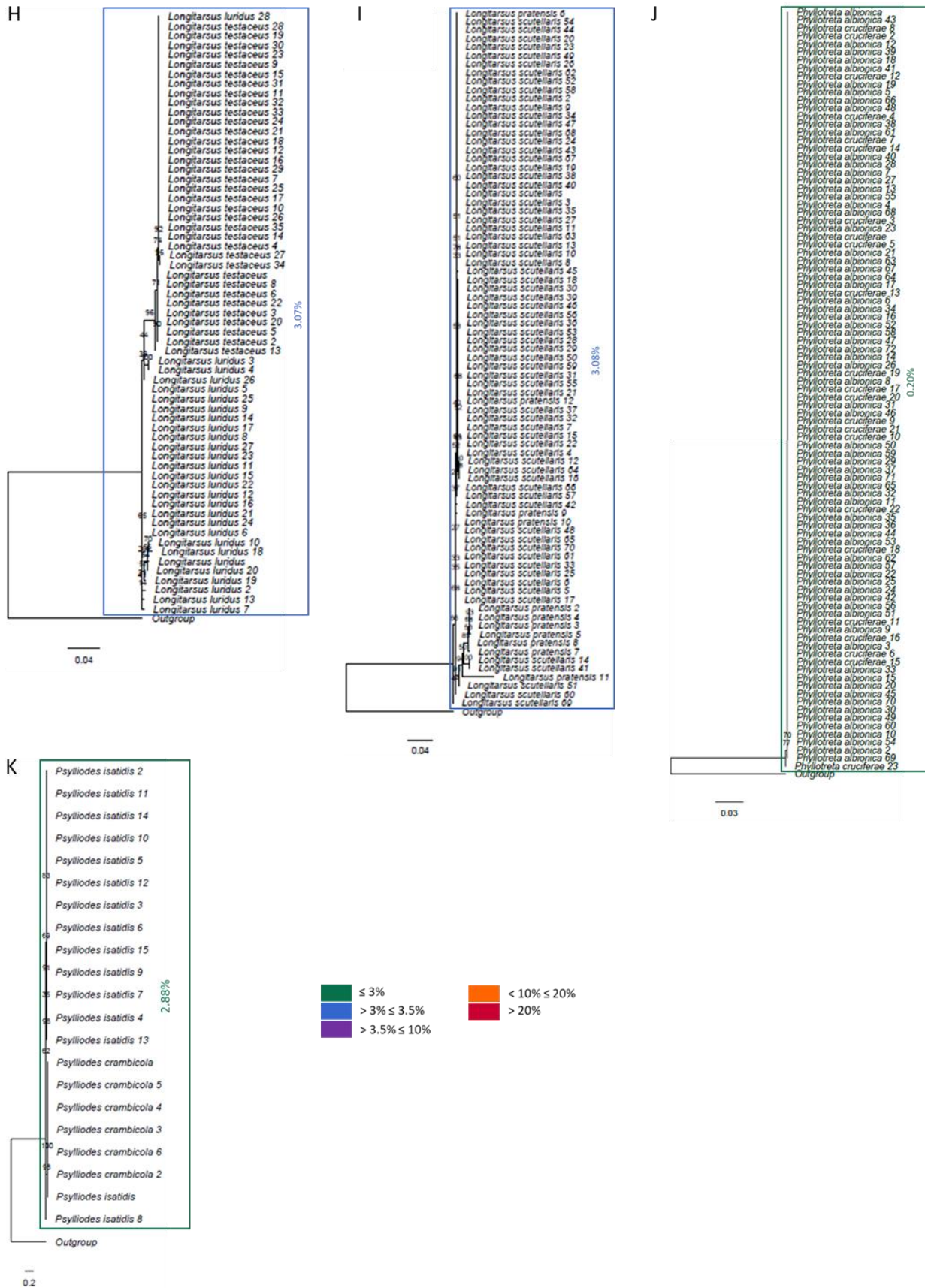


Figure 4.11 Maximum likelihood trees of flea beetle species (Hemiptera: Tingidae) based on 501-bp alignments of standard COI barcoding sequences that showed paraphyly possible due to misidentification.

Maximum intraspecific COI p-distances (%) of species in the tribe Alticini

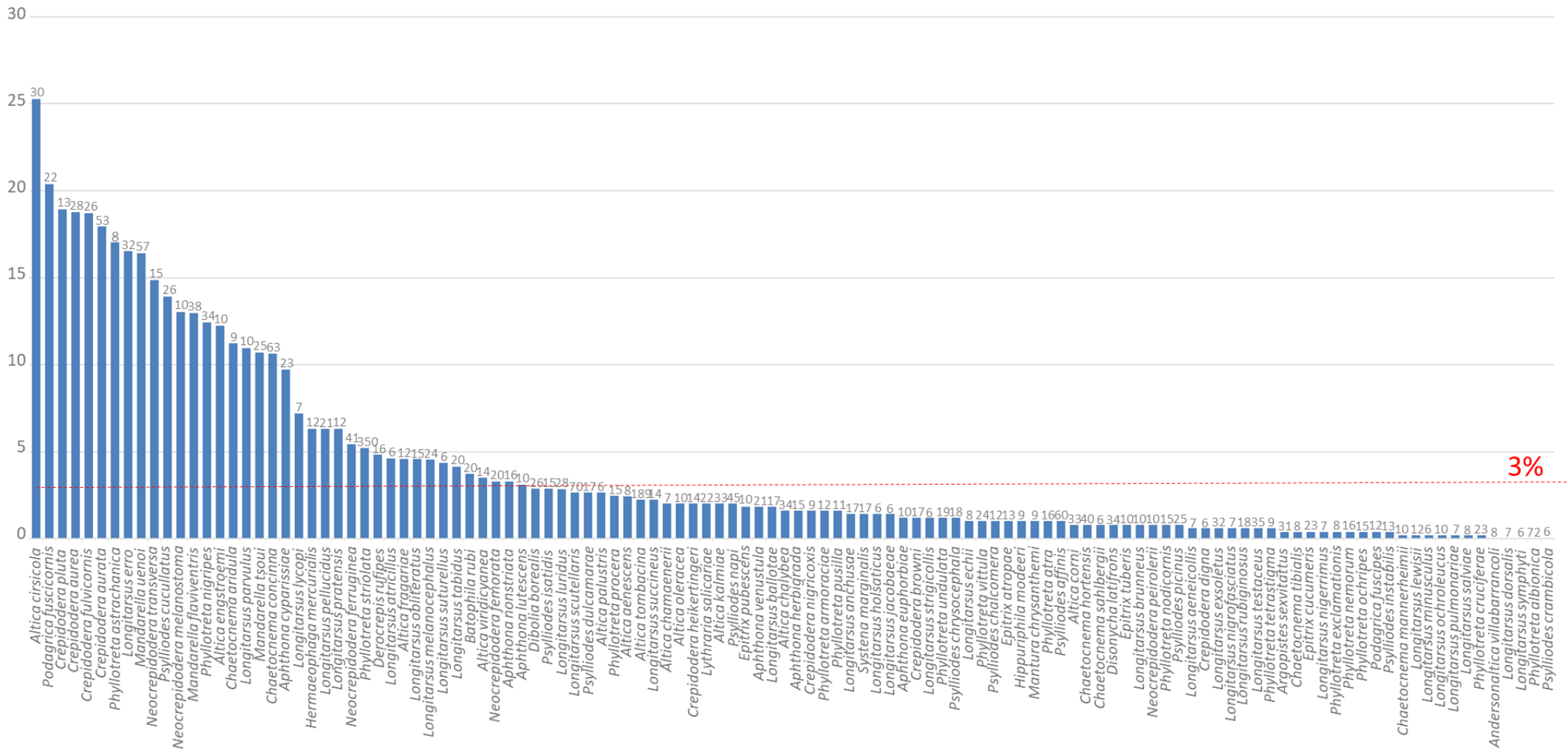


Figure 4.12 Maximum intraspecific p-distances (%; K2P) in 116 species of flea beetles (Chrysomelidae: Alticini) based on a 500 bp sequence alignment of the standard COI barcoding region.

4.4.12 Interspecific divergence in Alticini

Interspecific maximum p-distances between all species pairs ranged from 0.01% to 31.77% and were lowest for the congeneric pair *Phyllotreta albionica* and *Phyllotreta cruciferae*, and highest between the pair *Altica palustris* and *Phyllotreta nodicornis* (Table S13). The interspecific maximum p-distances among congeneric species pairs (*Altica*, *Aphthona*, *Argopistes*, *Chaetocnema*, *Crepidodera*, *Epitrix*, *Longitarsus*, *Mandarella*, *Neocrepidodera*, *Phyllotreta*, *Podagrica*, and *Psylliodes*) ranged from 0.01% to 31.77%, and among all non-congeneric species pairs the range was from 16.40% to 24.99%. The ranges of genetic divergences between congeneric and non-congeneric species overlap, and maximum p-distances are not reliable for inferring congeners in the tribe Alticini. The level of incongruence between genetic clustering and species names, and between maximum intraspecific and average interspecific p-distances indicates a high level of taxonomic inconsistencies and misidentifications for 14.66% of the Alticini species available on GenBank. In many instances, the inconsistency is caused when morphological characters of species do not clearly distinguish one species from another and when morphological characters are interpreted incorrectly. DNA barcoding is a useful tool that should be used to complement taxonomy; therefore, we propose that voucher specimens for Alticini should be revisited after thorough morphological analysis.

4.5 CONCLUSION

The genus *Argopistes* has been poorly studied in South Africa and worldwide. Previously, *Argopistes* species have been reported to feed on wild and cultivated olives in Western Cape province. In this study, we confirmed the identity of two olive flea beetles (*A. capensis* and *A. sexvitattus*) associated with wild and cultivated olive orchards, with *A. sexvitattus* being the dominant species. Three complete mitochondrial genome of *A. capensis* and *A. sexvitattus* morphotypes and COI barcodes for *A. sexvitattus*. The two olive flea beetles formed a close phylogenetic relationship among the tribe Alticini congruent to belonging to the same genus and their utilization of the same host *O. europaea*. The incidence of cases of non-monophyly and genetic divergences incompatible with taxonomic identifications suggests that the COI sequences of Alticini currently available on BOLD systems are likely to include misidentifications and cryptic diversity. Therefore, we recommend appropriate coupling of DNA-based methods and alpha taxonomy for recording and cataloguing Alticini species.

Chapter 5: Conclusion

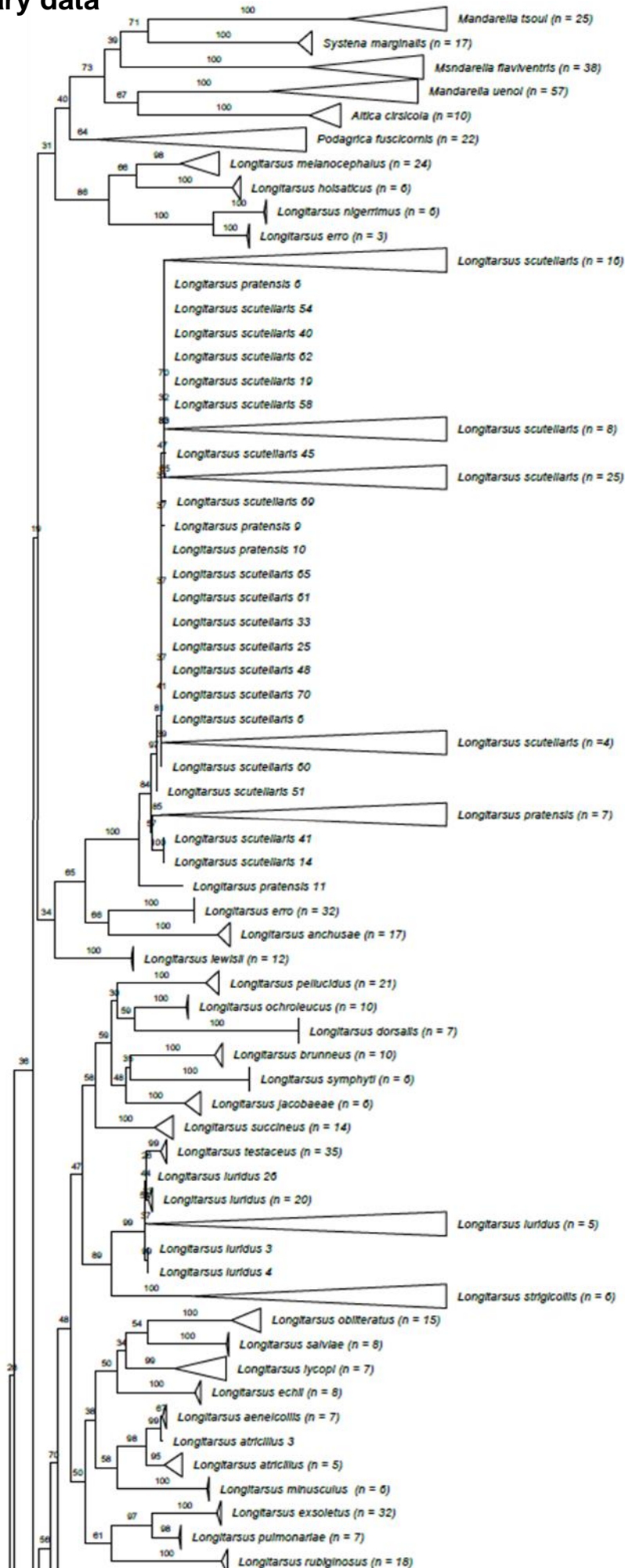
The olive industry in South Africa faces agricultural pests such as olive lace bugs and olive flea beetles and very little is known about these native pests. Apart from the native olive lace bug, *F. olivina* from Australia, olive lace bugs, and olive flea beetle are known not to occur in other olive-producing regions. Currently, growers make use of pesticides to control olive lace bugs and olive flea beetles which can over time cause major problems like adverse effects on humans, environmental contamination, and pesticide resistance in target and non-target organisms.

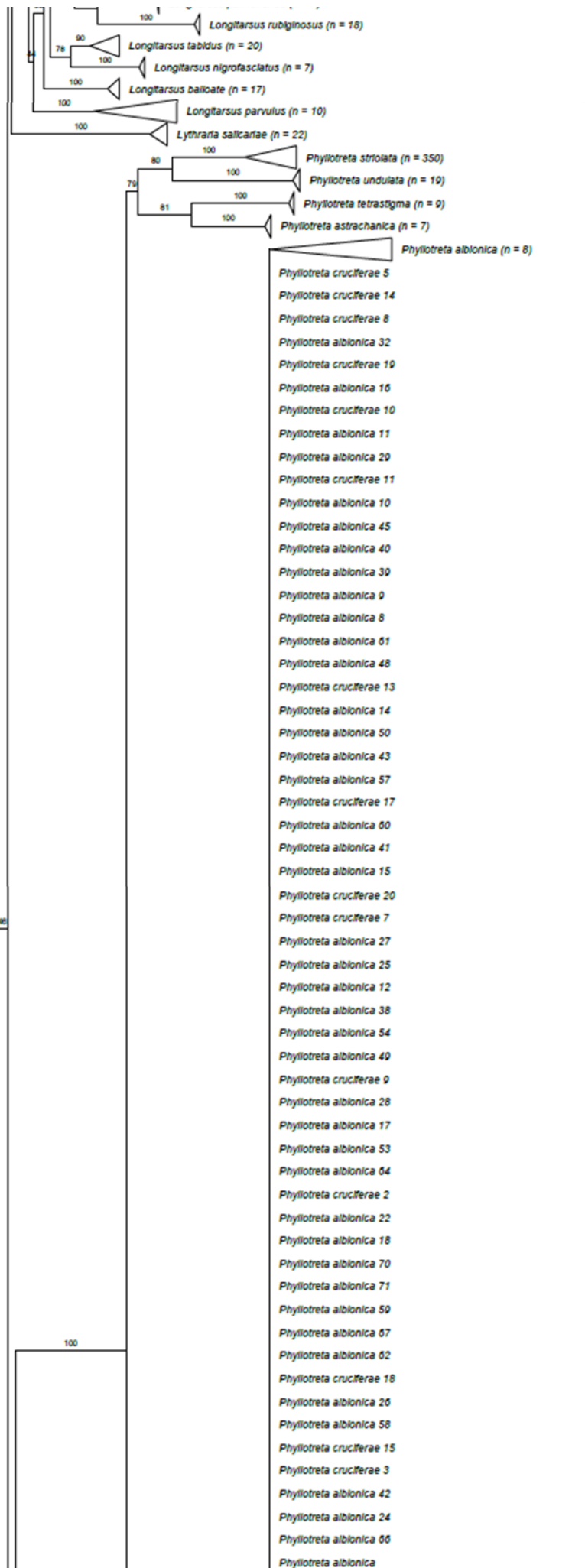
The identification of the pests is very crucial in developing control measures that aid in targeting only the pests and avoiding injury to beneficial organisms. The purpose of this research was to comprehensively catalogue olive lace bug and olive flea beetle species associated with wild and cultivated olives in Western Cape province of South Africa, to determine their phylogenetic position within their respective family/tribe and gain insight into their phylogenetic relationships, and to determine their distribution in olive orchards in the Western Cape province of South Africa.

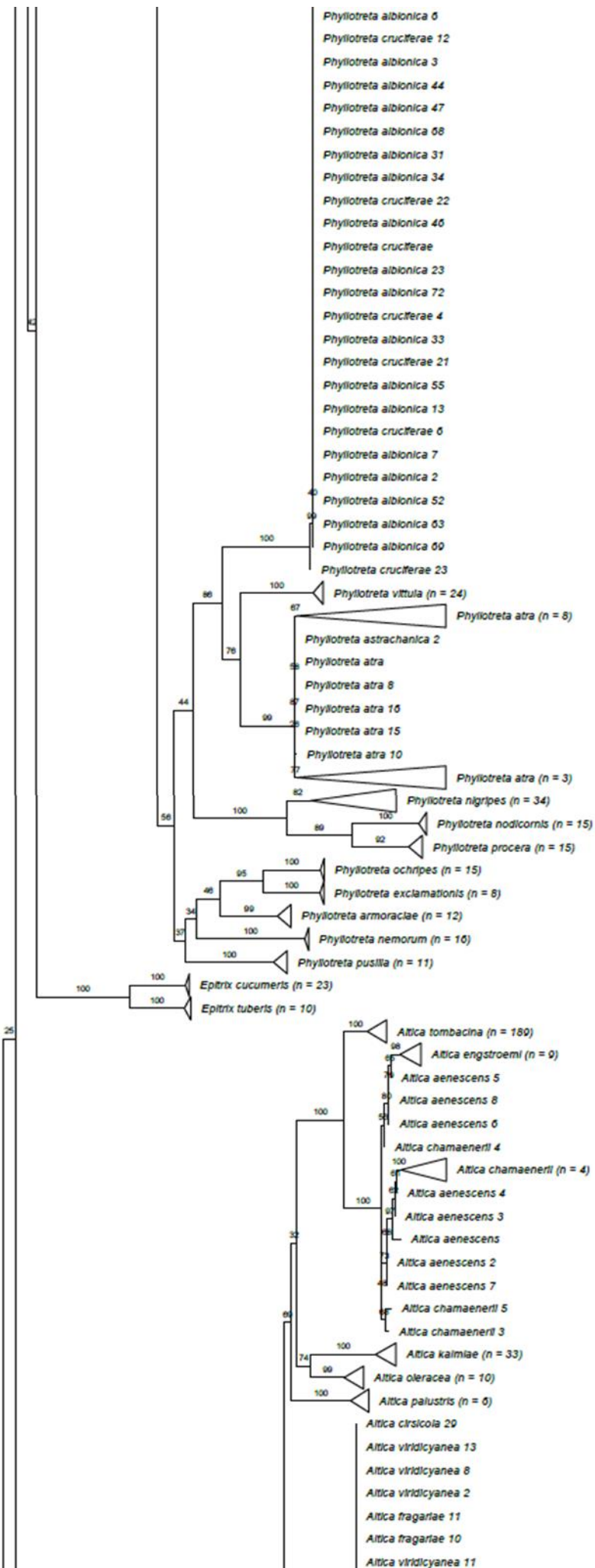
The survey of wild and cultivated olives in the Western Cape province of South Africa confirmed a higher species diversity of olive lace bugs than previously reported and confirmed the presence of two olive flea beetles (*A. capensis* and *A. sexvitattus*) that were previously reported in the region. Before this study, only two olive lace bugs (*P. australis* and *N. paliatseasi*) were previously reported in the Western Cape. The Eastern Cape was surveyed for olive flea beetles; however, no genetic data was generated. This study confirms the accuracy of DNA-based methods as a tool for species identification and has contributed the first DNA barcodes and mitochondrial genomes for several species including olive lace bugs (*C. lineata*, *Neoplerochila* sp., *P. australis*) and olive flea beetles (*A. capensis* and *A. sexvitattus*). In addition, the phylogenetic position of the olive lace bugs and olive flea beetles within their respective family/tribe was determined. The four olive lace bugs and two olive flea beetles clustered together in their respective phylogenetic trees, indicating that the species may have evolutionary adaptation to their host.

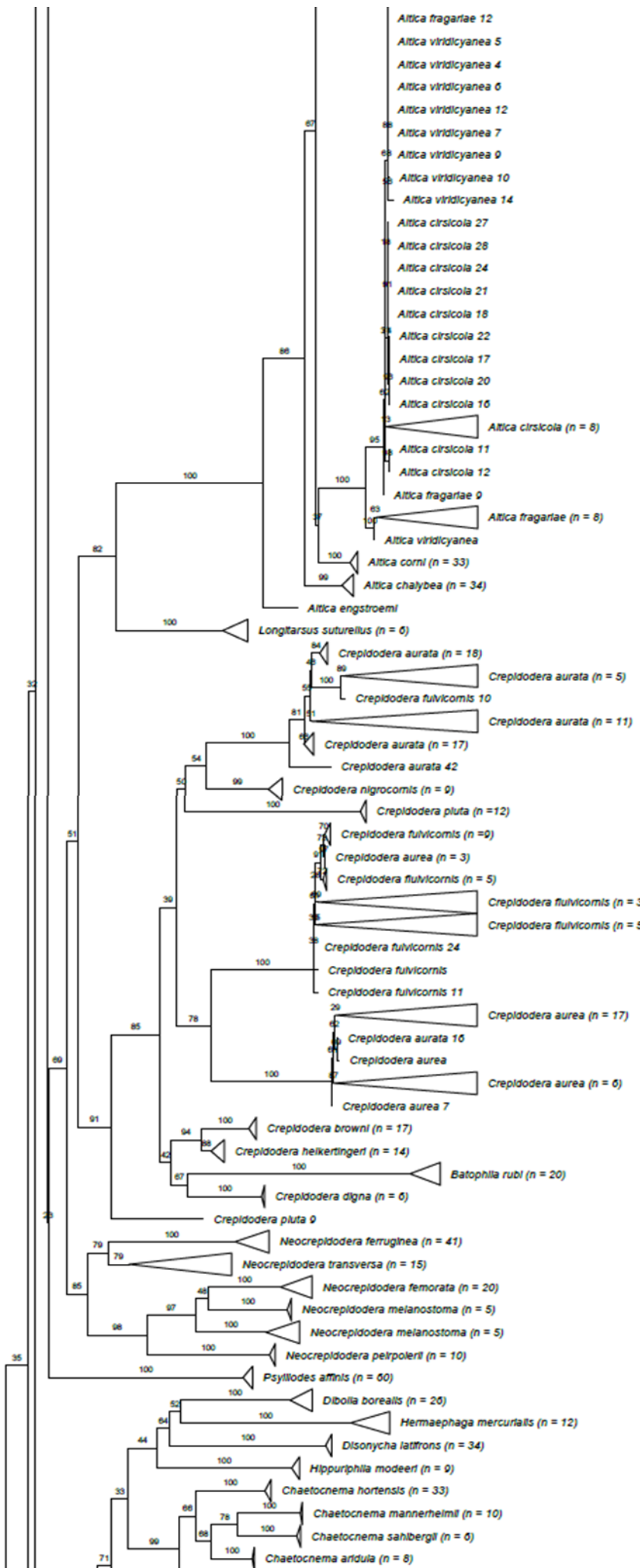
This work presented by this dissertation provides a baseline for future studies. The assessment of species diversity is very important as the correct identification of pests is a necessary step, which will lead to published information such as the insect's history, behaviour, and ecology, all important in the development of suitable control procedures.

Supplementary data









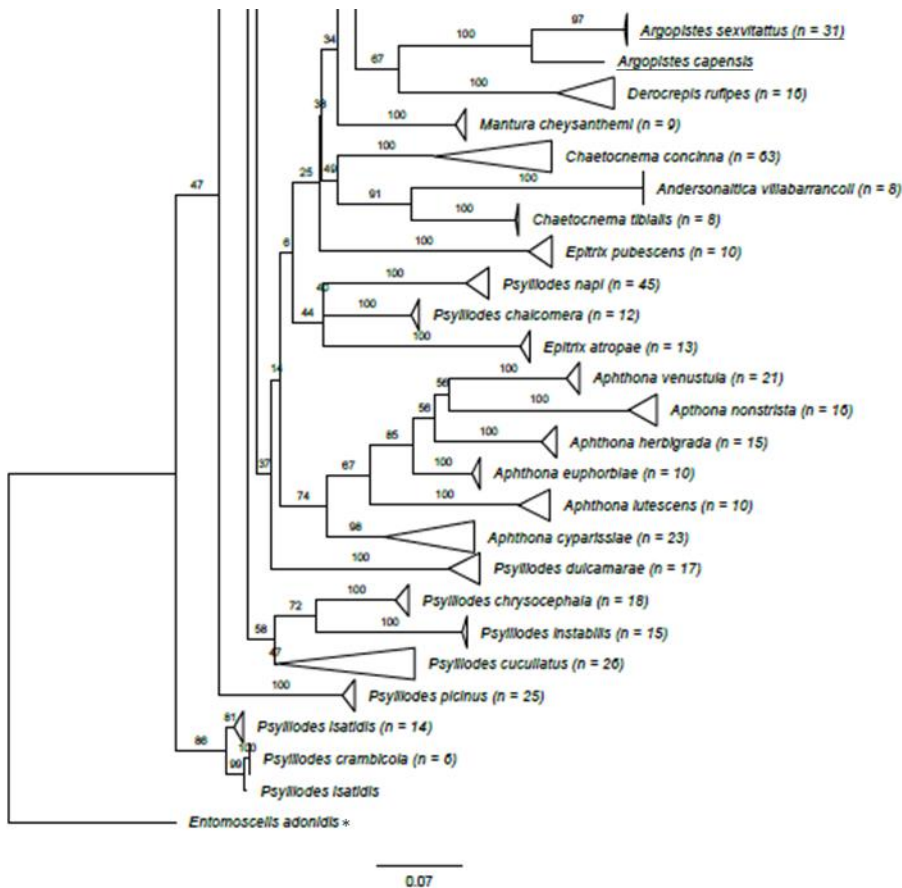
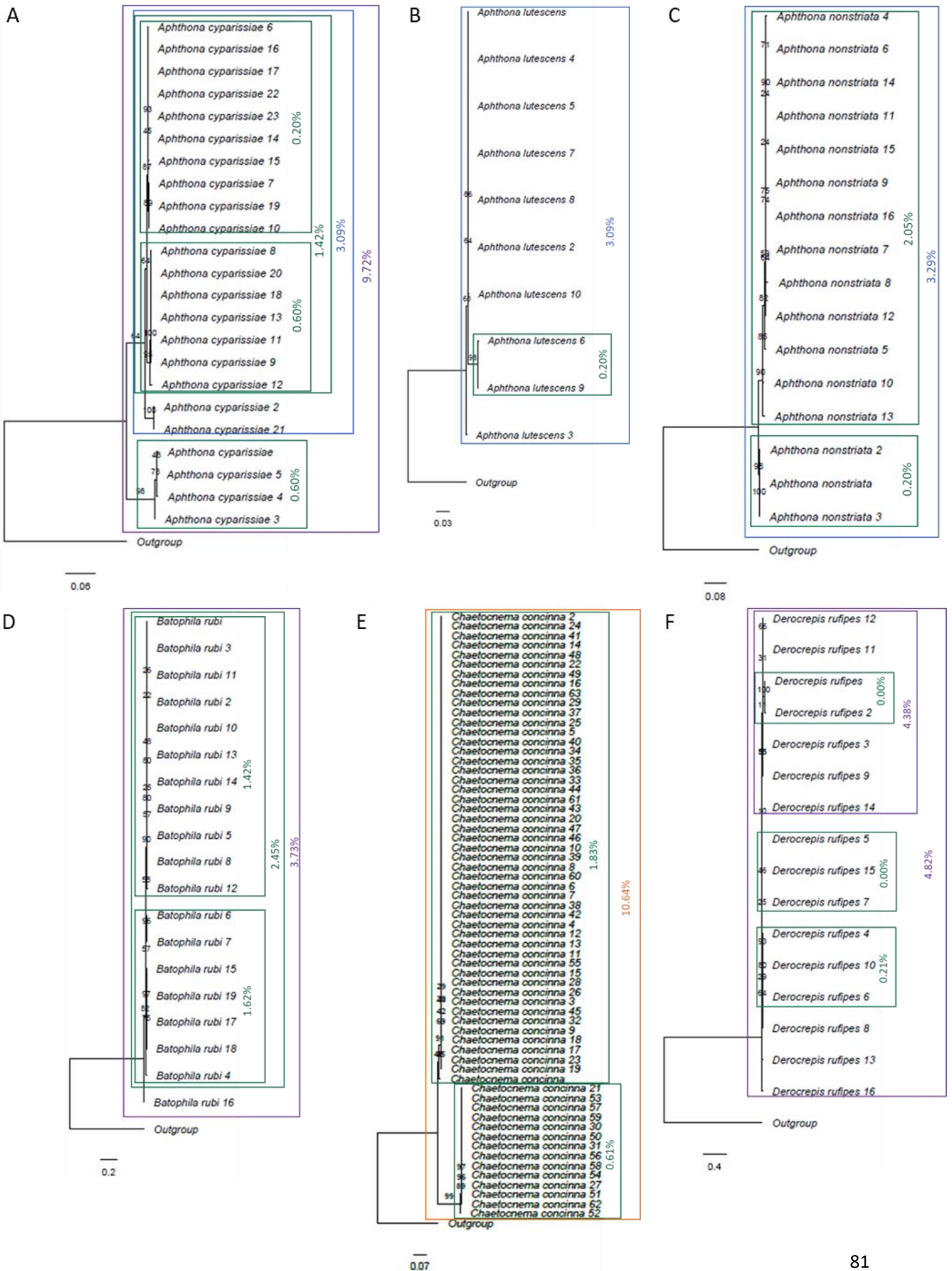
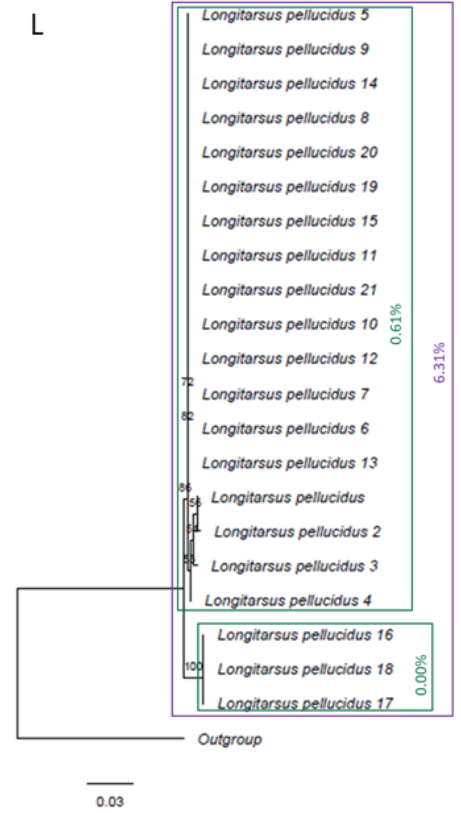
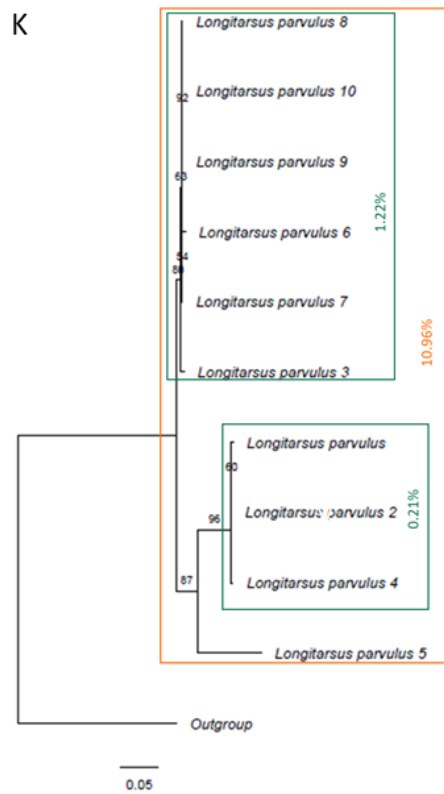
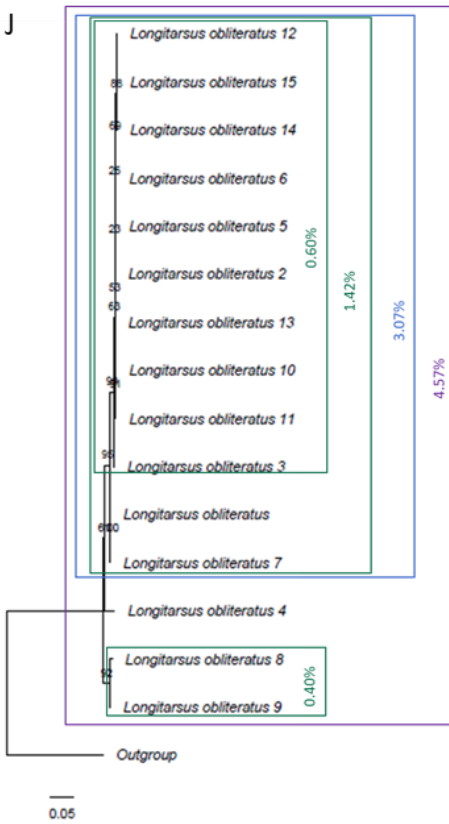
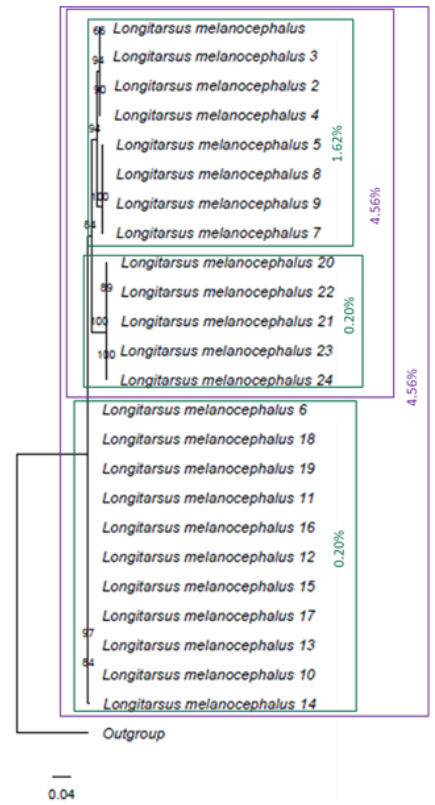
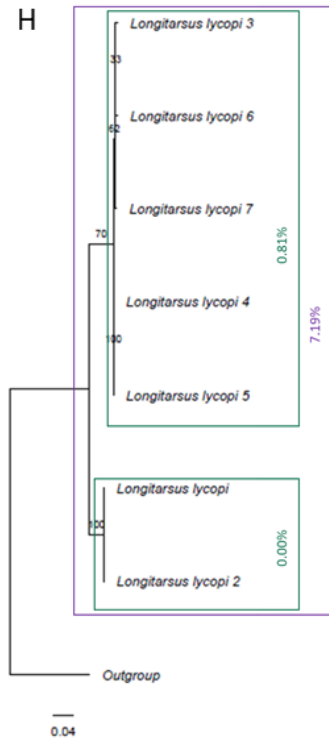
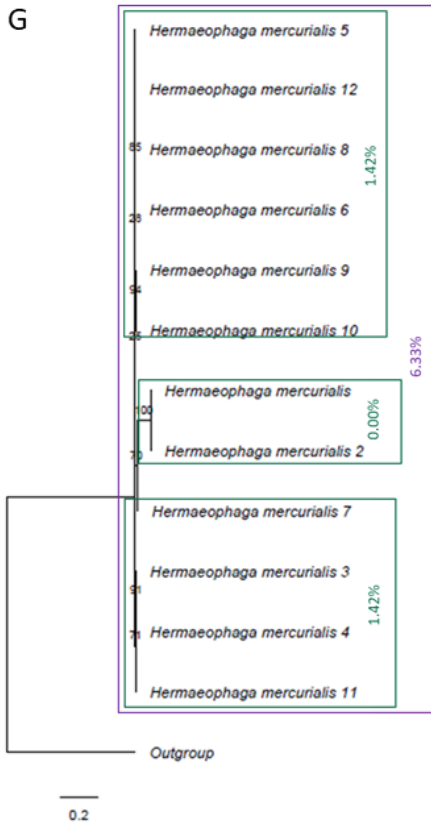


Figure S4. 1 Maximum likelihood tree of flea beetle species (Hemiptera: Tingidae) based on a 501-bp alignment of standard COI barcoding sequences. The analyses included 349 sequences representing 117 species in 22 genera retrieved from GenBank and the new sequences of the olive flea beetles *Argopistes capensis* and *Argopistes sexvittatus* generated in this study (underlined). Triangles represent collapsed groups of sequences belonging to the same species. * Outgroup (Chrysomelidae: Chrysomelini).





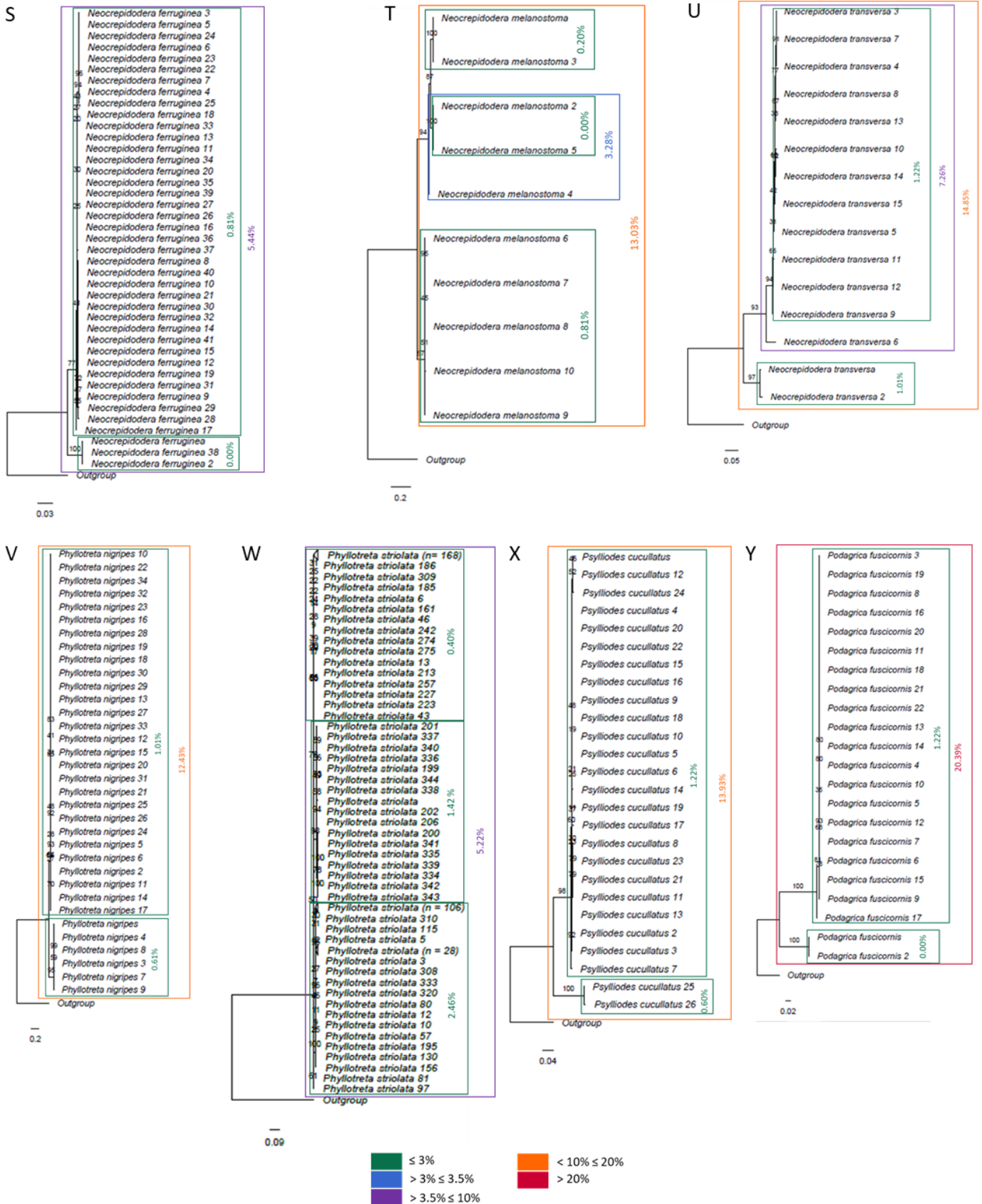


Figure S4. 2 Maximum likelihood trees of flea beetle species (Hemiptera: Tingidae) based on 501-bp alignments of standard COI barcoding sequences that showed multiple lineages possible due to cryptic diversity.

Table S4. 1 List of adult specimens representative of olive flea beetles *Argopistes capensis* and *Argopistes sexvitattus* (Coleoptera: Chrysomelidae) used for imaging, DNA barcoding, and sequencing of complete mitochondrial genomes. Cultivated host: *Olea europaea* subsp. *europaea*; Wild host: *Olea europaea* subsp. *cuspidata*

Collection date	Collection site	Olive Farm / Wild tree on farm vicinity / Ornamental tree	Region	GPS	Host	Use in this study	GenBank accession
10-Nov-15	Foxenburg Estate	Olive Farm	Wellington	33°34'21.0"S 19°03'39.0"E	Cultivated	Complete mitogenome/Image	Upon acceptance
10-Nov-15	Foxenburg Estate	Wild tree on farm vicinity	Wellington	33°34'21.0"S 19°03'39.0"E	Wild	Image	n.a.
06-Feb-20	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	Complete mitogenome	Upon acceptance
06-Feb-20	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	Complete mitogenome	Upon acceptance
30-Dec-15	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	DNA barcode	Upon acceptance
08-Nov-15	Foxenburg Estate	Wild tree on farm vicinity	Wellington	33°34'21.0"S 19°03'39.0"E	Wild	DNA barcode	Upon acceptance
30-Dec-15	Foxenburg Estate	Olive Farm	Wellington	33°34'21.0"S 19°03'39.0"E	Cultivated	DNA barcode	Upon acceptance
30-Dec-15	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
10-Nov-15	Foxenburg Estate	Olive Farm	Wellington	33°34'21.0"S 19°03'39.0"E	Cultivated	DNA barcode	Upon acceptance
10-Nov-15	Foxenburg Estate	Olive Farm	Wellington	33°34'21.0"S 19°03'39.0"E	Cultivated	DNA barcode	Upon acceptance
16-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	DNA barcode	Upon acceptance

10-Nov-15	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	DNA barcode	Upon acceptance
17-Jan-16	Foxenburg Estate	Olive Farm	Wellington	33°34'21.0"S 19°03'39.0"E	Cultivated	DNA barcode	Upon acceptance
17-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	DNA barcode	Upon acceptance
23-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	DNA barcode	Upon acceptance
24-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
24-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
24-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
24-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
24-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
24-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
24-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
28-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
28-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
28-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	DNA barcode	Upon acceptance
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	DNA barcode	Upon acceptance
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	DNA barcode	Upon acceptance
10-Nov-15	Foxenburg Estate	Olive Farm	Wellington	33°34'21.0"S 19°03'39.0"E	Cultivated	DNA barcode	Upon acceptance

04-Nov-15	Foxenburg Estate	Olive Farm	Wellington	33°34'21.0"S 19°03'39.0"E	Cultivated	DNA barcode	Upon acceptance
28-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	DNA barcode	Upon acceptance
10-Feb-20	ARC Nursery	Ornamental tree	Stellenbosch	33°55'27.0"S 18°52'25.0"E	Cultivated	DNA barcode	Upon acceptance
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	Image	n.a.
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	Image	n.a.
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	Image	n.a.
13-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	Image	n.a.
17-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	Image	n.a.
17-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	Image	n.a.
17-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	Image	n.a.
17-Jan-16	IT (SU Campus)	Ornamental tree	Stellenbosch	33°55'34.0"S 18°51'53.0"E	Wild	Image	n.a.
10-Nov-15	Foxenburg Estate	Olive Farm	Wellington	33°34'21.0"S 19°03'39.0"E	Cultivated	Image	n.a.
30-Dec-15	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	Image	n.a.
16-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	Image	n.a.
16-Jan-16	Alexanderhof	Ornamental tree	Stellenbosch	33°56'13.2"S 18°51'13.2"E	Cultivated	Image	n.a.

Table S4. 2 List of the 64 partial and complete mitochondrial sequences used to assess the phylogenetic position of the olive flea beetles *Argopistes capensis* and *Argopistes sexvitattus* within the tribe Alticini. *Chrysomela vigintipunctata* and *Entomoscelis adonidis* (Chrysomelidae: Chrysomelini) were used as outgroups.

Species	Genbank	Size (bp)	Reference	Status
<i>Agasicles hygrophila</i>	NC_028332.1	15,917	Li <i>et al.</i> , 2015	Complete
<i>Altica cirsicola</i>	NC_042876.1	15,864	Nie <i>et al.</i> , 2019	Complete
<i>Altica ericeti</i>	KX943460.1	16,290	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Altica fragariae</i>	NC_042875.1	16,220	Nie <i>et al.</i> , 2019	Complete
<i>Altica viridicyanea</i>	NC_048472.1	16,706	Nie <i>et al.</i> , 2019	Complete
<i>Aphthona albertinae</i>	KX943467.1	16,161	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Aphthona lutescens</i>	KX943361.1	15,606	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Apteropeda orbiculata</i>	KX943507.1	16,822	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Apteropeda ovulum</i>	KX943422.1	16,794	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Argopistes capensis</i>	Upon acceptance	16,543	This study	Complete
<i>Argopistes sexvitattus</i> striped	Upon acceptance	16,566	This study	Complete
<i>Argopistes sexvitattus</i> black	Upon acceptance	16,542	This study	Complete
<i>Argopistes tsekooni</i>	NC_045929.1	16,552	Long <i>et al.</i> , 2020	Complete
<i>Batophila aerata</i>	KX943466.1	15,986	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Chaetocnema arida</i>	KX943445.1	15,830	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Chaetocnema depressa</i>	KX943408.1	16,210	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Chaetocnema hortensis</i>	KX943358.1	15,799	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Chaetocnema obesa</i>	KX943442.1	15,775	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Chaetocnema paganettii</i>	KX943482.1	15,888	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Chaetocnema pelagica</i>	NC_041170.1	16,331	Nie <i>et al.</i> , 2018	Complete
<i>Chaetocnema scheffleri</i>	KX943431.1	15,952	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Chaetocnema tibialis</i>	KX943476.1	15,758	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Crepidodera pluta</i>	KX087265.1	16,339	Hunter <i>et al.</i> , 2016	Partial
<i>Longitarsus aeneus</i>	KX943357.1	15,836	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus atricillus</i>	KX943363.1	14,136	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus candidulus</i>	KX943430.1	15,865	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus cerinthes</i>	KX943478.1	16,169	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus curtus</i>	KX943501.1	15,919	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus exsoletus</i>	KX943418.1	15,735	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus ibericus</i>	KX943455.1	15,984	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus luridus</i>	KX943364.1	13,787	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus melanocephalus</i>	KX943469.1	16,348	Gomez-Rodriguez <i>et al.</i> , 2015	Partial
<i>Longitarsus membranaceus</i>	KX943473.1	15,868	Gomez-Rodriguez <i>et al.</i> , 2015	Partial

<i>Longitarsus niger</i>	KX943504.1	16,212	Gomez-Rodriguez et al., 2015	Partial
<i>Longitarsus nigrocillus</i>	KX943464.1	16,316	Gomez-Rodriguez et al., 2015	Partial
<i>Longitarsus nigrofasciatus</i>	KX943438.1	15,846	Gomez-Rodriguez et al., 2015	Partial
<i>Longitarsus pratensis</i>	KX943360.1	15,642	Gomez-Rodriguez et al., 2015	Partial
<i>Longitarsus rutilus</i>	KX943491.1	15,988	Gomez-Rodriguez et al., 2015	Partial
<i>Longitarsus tabidus</i>	KX943424.1	16,155	Gomez-Rodriguez et al., 2015	Partial
<i>Macrohaltica subplicata</i>	NC_041169.1	15,840	Nie et al., 2019	Complete
<i>Mantura chrysanthemii</i>	KX943486.1	15,986	Gomez-Rodriguez et al., 2015	Partial
<i>Neocrepidodera brevicollis</i>	KX943440.1	16,408	Gomez-Rodriguez et al., 2015	Partial
<i>Orestia punctipennis</i>	KX943441.1	16,191	Gomez-Rodriguez et al., 2015	Partial
<i>Phyllotreta cruciferae</i>	KX943506.1	15,716	Gomez-Rodriguez et al., 2015	Partial
<i>Phyllotreta foudrasi</i>	KX943502.1	15,586	Gomez-Rodriguez et al., 2015	Partial
<i>Phyllotreta hemipoda</i>	KX943496.1	15,640	Gomez-Rodriguez et al., 2015	Partial
<i>Phyllotreta parallela</i>	KX943456.1	16,115	Gomez-Rodriguez et al., 2015	Partial
<i>Phyllotreta striolata</i>	NC_045901.1	15,689	Zu and Yan, 2018	Complete
<i>Phyllotreta tetrastigma</i>	KX943497.1	15,775	Gomez-Rodriguez et al., 2015	Partial
<i>Phyllotreta undulata</i>	KX943475.1	15,698	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes affinis</i>	KX943355.1	16,006	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes chlorophana</i>	NC_053362.1	14,561	Gao et al., 2020	Complete
<i>Psylliodes chrysocephala</i>	KX943483.1	17,128	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes circumdatus</i>	KX943454.1	16,724	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes cupreatus</i>	KX943439.1	15,447	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes cupreus</i>	KX943425.1	16,328	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes fusiformis</i>	KX943421.1	16,033	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes gougeleti</i>	KX943356.1	15,984	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes heydeni</i>	KX943452.1	16,292	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes hispanus</i>	KX943503.1	17,357	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes laevicollis</i>	KX943451.1	16,019	Gomez-Rodriguez et al., 2015	Partial
<i>Psylliodes thlaspi</i>	KX943362.1	15,598	Gomez-Rodriguez et al., 2015	Partial
<i>Chrysomela vigintipunctata*</i>	NC_050933.1	17,474	Yan et al. 2020	Complete
<i>Entomoscelis adonidis*</i>	KX943493.1	16,353	Gomez-Rodriguez et al., 2015	Partial

Table S4. 3 List of COI barcoding sequences of *Argopistes sexvitattus* morphotypes used for the construction of a median-joining network.

Sample	Sample code	Morphotype	Sequence
<i>Argopistes sexvitattus</i> 1	AG001	Striped	Complete mitogenome
<i>Argopistes sexvitattus</i> 2	AG009	Black	DNA barcode
<i>Argopistes sexvitattus</i> 3	AG008	Black	Complete mitogenome
<i>Argopistes sexvitattus</i> 4	AM68	Black	DNA barcode
<i>Argopistes sexvitattus</i> 5	AM69	Black	DNA barcode
<i>Argopistes sexvitattus</i> 6	AM71	Black	DNA barcode
<i>Argopistes sexvitattus</i> 7	AM72	Black	DNA barcode
<i>Argopistes sexvitattus</i> 8	AM74	Black	DNA barcode
<i>Argopistes sexvitattus</i> 9	AM75	Black	DNA barcode
<i>Argopistes sexvitattus</i> 10	AM77	Black	DNA barcode
<i>Argopistes sexvitattus</i> 11	AM78	Black	DNA barcode
<i>Argopistes sexvitattus</i> 12	AS1	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 13	AS78	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 14	AB1	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 15	AB3	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 16	AB5	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 17	AB6	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 18	AE2	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 19	AE5	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 20	AE6	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 21	AE9	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 22	AM1	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 23	AM5	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 24	AM42	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 25	AM80	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 26	AM82	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 27	AM83	Striped	DNA barcode
<i>Argopistes sexvitattus</i> 28	AS3	Black	DNA barcode
<i>Argopistes sexvitattus</i> 29	AS5	Black	DNA barcode
<i>Argopistes sexvitattus</i> 30	AS38	Black	DNA barcode
<i>Argopistes sexvitattus</i> 31	AS120	Black	DNA barcode

Table S4. 4 Intraspecific p-distances (K2P) in 116 species in the tribe Alticini, based on a 500 bp alignment of COI barcoding sequences (n = 2,673). Standard errors were calculated using 1,000 bootstrap replicates.

Species	n	Max	Min	Mean	SE
<i>Altica aenescens</i>	8	2.45	0.00	0.83	0.26
<i>Altica chalybea</i>	34	1.63	0.00	0.51	0.20
<i>Altica chamaenerii</i>	7	2.04	0.00	0.99	0.31
<i>Altica cirsiicola</i>	30	25.28	0.00	11.52	1.15
<i>Altica corni</i>	33	0.81	0.00	0.14	0.07
<i>Altica engstroemi</i>	10	12.24	0.00	2.86	0.38
<i>Altica fragariae</i>	12	4.59	0.00	2.03	0.44
<i>Altica kalmiae</i>	33	2.03	0.00	0.32	0.10
<i>Altica oleracea</i>	10	2.04	0.00	1.20	0.29
<i>Altica palustris</i>	6	2.65	0.00	0.88	0.24
<i>Altica tombacina</i>	189	2.25	0.00	0.28	0.09
<i>Altica viridicyanea</i>	14	3.51	0.00	0.58	0.14
<i>Andersonaltica villabarrancoli</i>	8	0.00	0.00	0.00	0.00
<i>Aphthona cyparissiae</i>	23	9.72	0.00	3.41	0.47
<i>Aphthona euphorbiae</i>	10	1.21	0.00	0.43	0.17
<i>Aphthona herbigrada</i>	15	1.63	0.00	0.78	0.25
<i>Aphthona lutescens</i>	10	3.09	0.00	1.00	0.26
<i>Aphthona nonstriata</i>	16	3.29	0.00	1.20	0.28
<i>Aphthona venustula</i>	21	1.83	0.00	0.75	0.24
<i>Argopistes capensis</i>	1	-	-	-	-
<i>Argopistes sexvitattus</i>	30	0.40	0.00	0.10	0.08
<i>Batophila rubi</i>	20	3.73	0.00	1.21	0.27
<i>Chaetocnema aridula</i>	9	11.22	0.00	2.53	0.35
<i>Chaetocnema concinna</i>	63	10.64	0.00	3.68	0.54
<i>Chaetocnema hortensis</i>	40	0.81	0.00	0.10	0.03
<i>Chaetocnema mannerheimii</i>	10	0.20	0.00	0.04	0.04
<i>Chaetocnema sahlbergii</i>	6	0.81	0.00	0.35	0.18
<i>Chaetocnema tibialis</i>	8	0.40	0.00	0.10	0.07
<i>Crepidodera aurata</i>	53	17.94	0.00	2.49	0.37
<i>Crepidodera aurea</i>	28	18.77	0.00	3.80	0.44
<i>Crepidodera browni</i>	17	1.21	0.00	0.50	0.15
<i>Crepidodera digna</i>	6	0.60	0.00	0.32	0.18
<i>Crepidodera fulvicornis</i>	26	18.71	0.00	2.35	0.31
<i>Crepidodera heikertingeri</i>	14	2.04	0.00	0.38	0.12
<i>Crepidodera nigricoxis</i>	9	1.63	0.00	0.90	0.28
<i>Crepidodera pluta</i>	13	18.92	0.00	3.04	0.33
<i>Derocrepis rufipes</i>	16	4.82	0.00	1.31	0.25
<i>Dibolia borealis</i>	26	2.88	0.00	0.88	0.24
<i>Disonycha latifrons</i>	34	0.81	0.00	0.19	0.06

<i>Epitrix atropae</i>	13	1.01	0.00	0.41	0.17
<i>Epitrix cucumeris</i>	23	0.40	0.00	0.07	0.04
<i>Epitrix pubescens</i>	10	1.84	0.00	0.94	0.31
<i>Epitrix tuberis</i>	10	0.81	0.00	0.16	0.08
<i>Hermaphysa mercurialis</i>	12	6.33	0.00	2.21	0.42
<i>Hippuriphila modeeri</i>	9	1.01	0.00	0.38	0.18
<i>Longitarsus aeneicollis</i>	7	0.61	0.00	0.35	0.20
<i>Longitarsus anchusae</i>	17	1.44	0.00	0.52	0.20
<i>Longitarsus atricillus</i>	6	4.61	0.00	2.24	0.41
<i>Longitarsus ballotae</i>	17	1.83	0.00	0.54	0.17
<i>Longitarsus brunneus</i>	10	0.81	0.00	0.34	0.15
<i>Longitarsus dorsalis</i>	7	0.00	0.00	0.00	0.00
<i>Longitarsus echii</i>	8	1.02	0.00	0.54	0.23
<i>Longitarsus erro</i>	32	16.53	0.00	2.88	0.34
<i>Longitarsus exsoletus</i>	32	0.60	0.00	0.14	0.10
<i>Longitarsus holsaticus</i>	6	1.42	0.00	0.51	0.20
<i>Longitarsus jacobaeae</i>	6	1.42	0.00	0.59	0.22
<i>Longitarsus lewisii</i>	12	0.20	0.00	0.03	0.03
<i>Longitarsus luridus</i>	28	2.86	0.00	0.53	0.14
<i>Longitarsus lycopi</i>	7	7.19	0.00	3.51	0.62
<i>Longitarsus melanocephalus</i>	24	4.56	0.00	2.30	0.43
<i>Longitarsus minusculus</i>	6	0.20	0.00	0.07	0.07
<i>Longitarsus nigerrimus</i>	7	0.40	0.00	0.11	0.08
<i>Longitarsus nigrofasciatus</i>	7	0.60	0.00	0.25	0.16
<i>Longitarsus obliteratus</i>	15	4.57	0.00	1.79	0.34
<i>Longitarsus ochroleucus</i>	10	0.20	0.00	0.04	0.04
<i>Longitarsus parvulus</i>	10	10.96	0.00	4.65	0.66
<i>Longitarsus pellucidus</i>	21	6.31	0.00	0.58	0.18
<i>Longitarsus pratensis</i>	12	6.31	0.00	2.11	0.40
<i>Longitarsus pulmonariae</i>	7	0.20	0.00	0.06	0.06
<i>Longitarsus rubiginosus</i>	18	0.60	0.00	0.14	0.08
<i>Longitarsus salviae</i>	8	0.20	0.00	0.05	0.05
<i>Longitarsus scutellaris</i>	70	2.66	0.00	0.31	0.12
<i>Longitarsus strigicollis</i>	6	1.21	0.00	0.50	0.19
<i>Longitarsus succineus</i>	14	2.25	0.00	0.99	0.27
<i>Longitarsus suturellus</i>	6	4.36	0.00	2.33	0.52
<i>Longitarsus symphyti</i>	6	0.00	0.00	0.00	0.00
<i>Longitarsus tabidus</i>	20	4.14	0.00	0.55	0.13
<i>Longitarsus testaceus</i>	35	0.60	0.00	0.11	0.08
<i>Lythraia salicariae</i>	22	2.04	0.00	0.92	0.30
<i>Mandarella flaviventris</i>	38	12.96	0.00	7.04	0.80
<i>Mandarella tsoui</i>	25	10.70	0.00	6.05	0.80
<i>Mandarella uenoi</i>	57	16.41	0.00	9.97	0.95
<i>Mantura chrysanthemi</i>	9	1.01	0.00	0.38	0.15

<i>Neocrepidodera femorata</i>	20	3.30	0.00	0.78	0.16
<i>Neocrepidodera ferruginea</i>	41	5.44	0.00	0.95	0.20
<i>Neocrepidodera melanostoma</i>	10	13.03	0.00	7.76	0.99
<i>Neocrepidodera peirolerii</i>	10	0.81	0.00	0.27	0.12
<i>Neocrepidodera transversa</i>	15	14.85	0.00	4.61	0.57
<i>Phyllotreta albionica</i>	72	0.00	0.00	0.00	0.00
<i>Phyllotreta armoraciae</i>	12	1.63	0.00	0.54	0.19
<i>Phyllotreta astrachanica</i>	8	17.03	0.00	4.46	0.54
<i>Phyllotreta atra</i>	16	1.01	0.00	0.40	0.17
<i>Phyllotreta cruciferae</i>	23	0.20	0.00	0.02	0.02
<i>Phyllotreta exclamationis</i>	8	0.40	0.00	0.20	0.10
<i>Phyllotreta nemorum</i>	16	0.40	0.00	0.08	0.04
<i>Phyllotreta nigripes</i>	34	12.43	0.00	3.83	0.52
<i>Phyllotreta nodicornis</i>	15	0.81	0.00	0.18	0.09
<i>Phyllotreta ochripes</i>	15	0.40	0.00	0.12	0.08
<i>Phyllotreta procera</i>	15	2.46	0.00	1.19	0.29
<i>Phyllotreta pusilla</i>	11	1.62	0.00	0.85	0.28
<i>Phyllotreta striolata</i>	350	5.22	0.00	1.49	0.35
<i>Phyllotreta tetrastigma</i>	9	0.60	0.00	0.37	0.16
<i>Phyllotreta undulata</i>	19	1.21	0.00	0.54	0.23
<i>Phyllotreta vittula</i>	24	1.02	0.00	0.10	0.04
<i>Podagrica fuscicornis</i>	22	20.39	0.00	3.72	0.42
<i>Podagrica fuscipes</i>	12	0.40	0.00	0.11	0.06
<i>Psylliodes affinis</i>	60	1.01	0.00	0.22	0.11
<i>Psylliodes chalcomera</i>	12	1.02	0.00	0.34	0.14
<i>Psylliodes chrysocephala</i>	18	1.21	0.00	0.46	0.15
<i>Psylliodes crambicola</i>	6	0.00	0.00	0.00	0.00
<i>Psylliodes cucullatus</i>	26	13.93	0.00	2.22	0.30
<i>Psylliodes dulcamarae</i>	17	2.66	0.00	1.18	0.31
<i>Psylliodes instabilis</i>	13	0.40	0.00	0.11	0.08
<i>Psylliodes isatidis</i>	15	2.88	0.00	0.56	0.16
<i>Psylliodes napi</i>	45	2.03	0.00	0.43	0.17
<i>Psylliodes picinus</i>	25	0.81	0.00	0.21	0.11
<i>Systema marginalis</i>	17	1.43	0.00	0.23	0.08

Table S4. 5 Interspecific p-distances (K2P) among 116 species within the tribe Alticini, based on a 500 bp alignment of COI barcoding sequences (n = 2,673). Standard errors were calculated using 1,000 bootstrap replicates.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	<i>Altica aenescens</i>		1.53	0.35	1.60	1.48	0.55	1.54	1.60	1.51	1.65	1.01	1.64	2.31	2.22	2.33	2.16
2	<i>Altica chalybea</i>	10.89		1.55	1.57	1.32	1.38	1.42	1.53	1.34	1.53	1.46	1.45	2.15	2.20	2.39	2.25
3	<i>Altica chamaenerii</i>	1.18	11.18		1.61	1.52	0.68	1.57	1.62	1.53	1.69	1.06	1.63	2.31	2.25	2.32	2.17
4	<i>Altica cirsicola</i>	16.24	15.37	16.31		1.49	1.62	0.97	1.71	1.51	1.64	1.55	0.84	2.01	1.93	1.96	1.98
5	<i>Altica corni</i>	9.74	7.95	10.14	14.71		1.47	1.32	1.61	1.41	1.50	1.49	1.39	2.22	2.21	2.34	2.30
6	<i>Altica engstroemi</i>	3.05	10.40	3.78	16.41	10.48		1.51	1.47	1.44	1.61	1.05	1.61	2.20	2.14	2.27	2.10
7	<i>Altica fragariae</i>	11.31	10.24	11.67	9.88	8.85	11.50		1.54	1.35	1.42	1.45	0.58	2.22	2.17	2.17	2.17
8	<i>Altica kalmiae</i>	11.30	10.59	11.64	16.88	11.09	10.75	11.13		1.31	1.49	1.53	1.66	2.26	2.32	2.23	2.26
9	<i>Altica oleracea</i>	10.79	8.46	11.17	15.11	8.91	10.63	9.74	8.94		1.45	1.40	1.41	2.30	2.14	2.05	2.17
10	<i>Altica palustris</i>	11.49	10.45	12.04	15.78	9.69	11.87	9.73	10.25	9.98		1.64	1.51	2.40	2.32	2.28	2.33
11	<i>Altica tombacina</i>	5.34	9.38	5.82	15.80	9.79	6.52	10.57	10.62	9.30	12.16		1.51	2.26	2.31	2.25	2.21
12	<i>Altica viridicyanea</i>	11.97	9.81	12.05	8.43	9.13	12.13	2.51	11.87	9.73	10.12	11.00		2.22	2.22	2.22	2.21
13	<i>Andersonaltica villabarrancoli</i>	21.14	19.63	21.08	21.72	20.76	20.53	20.84	20.59	20.78	22.86	20.58	20.34		2.16	2.24	2.27
14	<i>Aphthona cyparissiae</i>	21.20	20.92	21.78	21.35	21.65	20.88	21.56	22.21	20.84	23.24	22.30	21.85	20.95		1.62	1.73
15	<i>Aphthona euphorbiae</i>	21.47	22.18	21.31	20.46	21.62	21.50	20.56	20.41	19.58	21.35	21.23	20.94	19.95	13.82		1.50
16	<i>Aphthona herbigrada</i>	19.01	20.01	19.04	20.27	20.12	19.06	19.45	20.63	19.94	21.51	19.29	19.60	20.43	15.09	10.81	
17	<i>Aphthona lutescens</i>	22.40	23.39	22.95	23.04	23.07	22.51	23.27	24.15	22.46	23.54	23.00	23.37	21.34	14.10	14.08	14.93
18	<i>Aphthona nonstriata</i>	26.38	26.23	26.40	25.80	26.23	26.16	26.63	26.44	24.15	27.51	24.47	26.12	27.71	19.15	15.30	17.28
19	<i>Aphthona venustula</i>	21.63	21.21	21.99	21.79	21.95	22.02	22.65	23.95	21.66	23.48	21.44	22.28	20.56	15.49	12.26	13.91
20	<i>Argopistes capensis</i>	21.31	21.51	22.04	23.52	22.88	21.14	22.63	21.59	20.64	23.89	19.94	22.93	20.33	18.98	18.74	18.15
21	<i>Argopistes sexvittatus</i>	21.78	19.80	21.77	20.99	22.47	21.31	21.11	22.92	20.61	23.72	19.65	21.48	17.00	18.09	19.09	19.19
22	<i>Batophila rubi</i>	24.34	25.36	24.72	25.15	25.90	25.11	23.78	24.47	24.00	23.51	22.70	25.09	24.55	23.58	20.22	23.25

23	<i>Chaetocnema aridula</i>	18.41	16.66	18.63	19.30	17.12	18.45	19.26	17.88	18.02	20.03	18.23	19.06	18.18	14.47	16.27	16.66
24	<i>Chaetocnema concinna</i>	22.89	22.15	22.84	22.46	21.19	23.27	23.09	23.20	22.10	22.61	22.96	22.37	22.22	18.01	19.86	19.38
25	<i>Chaetocnema hortensis</i>	17.75	17.89	18.07	20.54	18.94	18.03	20.43	17.82	19.14	20.54	18.59	20.30	19.08	16.44	17.36	18.71
26	<i>Chaetocnema mannerheimii</i>	18.98	19.27	19.33	21.33	19.75	18.79	21.45	18.71	18.97	21.42	19.37	21.15	21.45	16.90	17.53	17.44
27	<i>Chaetocnema sahlbergii</i>	17.69	17.99	17.68	20.64	18.91	17.53	20.55	19.33	18.26	20.65	17.40	20.24	20.79	15.48	16.97	16.13
28	<i>Chaetocnema tibialis</i>	19.48	19.98	19.60	21.85	19.97	19.28	21.91	21.13	19.08	22.71	18.70	21.46	14.82	19.33	17.64	17.68
29	<i>Crepidodera aurata</i>	21.88	23.72	22.46	23.87	22.88	22.13	23.84	22.88	21.69	24.23	21.30	24.20	20.42	17.63	17.49	19.93
30	<i>Crepidodera aurea</i>	23.46	24.32	23.70	24.63	23.65	23.82	24.27	24.34	24.56	23.49	23.18	24.98	23.29	16.98	21.79	19.99
31	<i>Crepidodera browni</i>	21.82	23.74	22.74	24.33	22.90	22.40	23.30	23.56	21.90	23.36	21.03	23.59	23.71	17.59	18.12	18.89
32	<i>Crepidodera digna</i>	22.12	22.09	22.56	25.38	22.34	21.93	24.51	21.71	20.53	21.64	22.52	25.23	24.26	17.26	19.63	21.20
33	<i>Crepidodera fulvicornis</i>	25.15	26.48	25.44	25.00	26.82	25.62	25.70	26.06	25.47	27.43	24.86	26.17	23.33	19.04	20.88	22.28
34	<i>Crepidodera heikertingeri</i>	20.27	22.58	20.47	22.16	21.58	20.73	20.75	22.51	19.69	21.42	19.53	21.35	20.53	18.43	17.84	18.05
35	<i>Crepidodera nigricoxis</i>	21.66	23.78	22.56	24.47	23.16	21.87	23.72	23.90	22.52	22.85	21.71	24.13	23.80	18.68	19.27	20.21
36	<i>Crepidodera pluta</i>	25.24	25.71	25.32	26.62	27.14	26.05	27.74	27.37	25.88	27.69	24.82	27.78	26.30	20.36	20.46	23.32
37	<i>Derocrepis rufipes</i>	20.19	22.79	20.67	22.28	21.45	20.27	20.65	21.86	20.23	20.38	21.26	21.25	21.39	18.84	18.13	18.00
38	<i>Dibolia borealis</i>	22.30	21.98	22.83	22.41	21.23	21.96	22.82	21.84	18.72	23.38	22.18	22.34	20.55	16.78	17.42	20.15
39	<i>Disonycha latifrons</i>	23.25	24.06	23.94	23.51	24.83	23.61	23.29	25.61	22.83	24.87	24.11	23.48	21.18	18.31	18.42	22.22
40	<i>Epitrix atropae</i>	24.86	24.68	24.61	23.42	24.03	25.52	24.43	27.69	24.99	25.42	23.98	24.38	23.38	19.47	18.91	19.74
41	<i>Epitrix cucumeris</i>	21.85	21.35	22.27	23.19	22.13	20.90	22.45	22.03	20.93	24.24	21.29	22.96	24.14	17.12	19.29	19.43
42	<i>Epitrix pubescens</i>	23.19	22.29	23.20	22.75	23.20	23.33	22.30	23.24	21.68	24.52	22.54	22.66	20.77	17.70	17.54	18.60
43	<i>Epitrix tuberis</i>	20.51	20.14	21.19	23.32	21.57	19.84	21.66	21.07	19.58	22.19	20.56	22.12	24.53	18.02	18.70	19.77
44	<i>Hermaeophaga mercurialis</i>	22.06	22.80	21.91	24.96	24.60	22.63	25.34	24.41	21.84	24.42	22.05	24.90	23.34	20.55	21.01	21.27
45	<i>Hippuriphila modeeri</i>	21.77	20.76	21.99	21.57	21.03	21.88	20.64	22.15	19.50	22.51	21.95	21.00	22.65	17.37	18.97	20.35
46	<i>Longitarsus aeneicollis</i>	19.51	20.86	19.31	19.43	19.60	19.20	19.05	20.77	19.46	21.57	19.46	19.07	17.74	16.70	15.08	15.90
47	<i>Longitarsus anchusae</i>	22.23	22.39	22.56	23.11	21.78	22.40	23.55	23.90	19.86	25.15	21.76	23.53	20.90	17.46	18.40	21.14
48	<i>Longitarsus atricillus</i>	19.80	21.76	19.63	20.41	19.86	19.60	19.87	20.99	20.20	20.96	20.06	20.11	17.57	17.60	16.15	15.61
49	<i>Longitarsus ballotae</i>	21.57	21.64	22.10	20.37	21.32	21.49	20.85	23.13	20.70	23.56	21.35	20.59	19.77	13.55	13.50	15.02

50	<i>Longitarsus brunneus</i>	21.82	20.34	22.22	23.23	21.56	21.19	22.34	22.66	19.22	22.79	20.34	22.78	17.31	17.30	18.49	20.17
51	<i>Longitarsus dorsalis</i>	21.14	22.86	21.73	23.82	23.19	21.10	22.93	21.02	19.20	23.79	20.79	23.51	21.18	18.82	21.30	22.39
52	<i>Longitarsus echii</i>	22.33	22.86	22.67	22.88	22.93	22.09	22.64	23.98	22.47	23.97	22.89	23.21	19.64	17.67	13.61	13.97
53	<i>Longitarsus erro</i>	19.84	21.81	19.67	23.47	20.95	20.36	23.24	20.60	19.19	21.23	20.17	23.67	20.65	17.34	14.50	16.91
54	<i>Longitarsus exsoletus</i>	22.36	23.31	23.01	23.08	23.91	22.61	23.42	24.91	23.18	24.04	22.47	23.78	18.95	16.54	15.65	15.33
55	<i>Longitarsus holsaticus</i>	21.15	20.31	21.21	21.05	21.25	21.99	21.38	22.40	18.71	21.84	21.77	20.90	19.83	17.48	17.53	19.55
56	<i>Longitarsus jacobaeae</i>	23.64	21.59	24.19	23.09	22.07	23.52	23.57	24.93	20.71	24.89	22.53	23.35	18.87	15.31	17.99	18.54
57	<i>Longitarsus lewisii</i>	21.55	20.88	21.81	21.24	20.22	21.24	21.06	22.61	19.69	23.10	20.32	21.28	20.61	15.82	15.16	16.96
58	<i>Longitarsus luridus</i>	20.68	22.19	21.39	21.25	19.83	20.32	20.57	23.29	20.35	22.35	20.58	20.94	20.07	16.17	15.16	16.44
59	<i>Longitarsus lycopi</i>	20.20	20.59	20.70	21.54	19.42	20.63	20.93	21.06	18.75	23.50	19.69	20.78	19.14	17.24	16.68	17.77
60	<i>Longitarsus melanocephalus</i>	21.30	19.34	21.60	21.07	21.03	21.30	21.87	21.60	18.63	22.09	21.16	21.68	20.30	16.90	17.20	19.78
61	<i>Longitarsus minusculus</i>	21.88	22.86	22.54	20.27	20.11	22.22	20.18	22.33	20.83	21.98	21.53	20.88	19.07	15.78	15.54	15.94
62	<i>Longitarsus nigerrimus</i>	19.93	22.52	20.01	23.28	21.09	20.48	23.40	22.22	20.79	22.23	20.40	23.03	20.30	21.64	18.67	21.87
63	<i>Longitarsus nigrofasciatus</i>	23.40	20.48	23.70	22.83	21.57	23.04	21.33	21.49	20.90	22.67	20.72	21.84	19.77	16.38	15.34	17.76
64	<i>Longitarsus obliteratedus</i>	23.99	21.43	23.94	22.03	22.28	24.33	22.43	23.87	21.35	24.46	23.35	22.32	23.89	16.22	17.10	17.87
65	<i>Longitarsus ochroleucus</i>	18.47	18.26	19.38	20.94	18.47	17.95	19.83	21.23	18.98	21.20	19.09	20.29	17.32	16.93	18.86	17.55
66	<i>Longitarsus parvulus</i>	23.38	22.11	23.24	21.51	21.69	23.77	21.89	24.77	21.34	24.13	22.47	21.96	20.81	16.78	15.00	15.34
67	<i>Longitarsus pellucidus</i>	18.84	21.75	18.92	22.37	21.71	19.43	22.18	24.87	20.91	23.53	19.36	22.78	20.39	16.60	16.12	16.96
68	<i>Longitarsus pratensis</i>	21.15	22.40	21.21	21.65	20.94	20.99	21.80	21.26	20.50	23.43	21.34	22.00	18.84	17.07	13.65	16.75
69	<i>Longitarsus pulmonariae</i>	21.21	22.12	21.20	22.93	22.34	21.21	22.64	22.82	23.17	22.98	21.98	22.71	17.73	17.71	16.88	16.30
70	<i>Longitarsus rubiginosus</i>	20.03	22.65	20.51	22.24	21.87	20.14	21.71	23.41	21.30	22.63	22.00	22.40	18.35	18.39	17.48	15.54
71	<i>Longitarsus salviae</i>	20.34	20.30	20.01	21.31	20.25	20.35	20.00	20.90	19.03	21.74	19.54	20.06	18.79	15.16	14.91	16.67
72	<i>Longitarsus scutellaris</i>	21.72	22.78	21.80	21.79	21.28	21.48	22.31	21.61	21.48	23.78	22.33	22.36	19.49	17.83	13.94	17.26
73	<i>Longitarsus strigicollis</i>	20.74	23.47	20.87	21.46	20.28	20.86	22.06	22.65	21.48	22.20	21.36	21.63	20.12	14.55	16.22	16.84
74	<i>Longitarsus succineus</i>	20.86	20.23	21.27	21.67	19.91	20.40	21.35	23.13	20.02	21.49	20.46	22.16	17.90	16.45	17.34	16.78
75	<i>Longitarsus suturellus</i>	19.89	20.86	20.20	22.05	19.83	20.37	20.68	20.11	18.91	21.04	21.69	21.81	21.83	18.64	19.23	19.22
76	<i>Longitarsus symphyti</i>	25.39	25.81	25.51	25.57	25.75	24.82	25.26	26.17	23.03	26.79	24.50	26.09	18.78	19.06	18.77	22.62
77	<i>Longitarsus tabidus</i>	20.53	18.36	20.86	19.97	18.49	20.63	19.28	20.41	17.17	20.72	18.67	19.46	17.71	14.93	12.53	14.33

78	<i>Longitarsus testaceus</i>	20.83	22.21	21.54	21.40	20.21	20.45	20.88	23.88	20.28	22.88	20.37	21.25	20.55	16.28	15.42	16.18
79	<i>Lythriaria salicariae</i>	21.62	22.54	21.89	22.00	21.39	22.37	21.61	21.60	19.86	22.43	20.34	21.54	20.42	20.18	19.06	19.86
80	<i>Mandarella flaviventris</i>	26.45	26.66	27.19	25.09	27.01	25.79	26.04	28.57	27.64	27.04	26.77	26.07	26.41	21.50	20.44	21.99
81	<i>Mandarella tsoui</i>	24.45	23.94	24.40	24.37	24.26	24.39	24.03	25.40	24.90	24.83	25.54	24.41	22.33	22.64	22.47	22.43
82	<i>Mandarella uenoi</i>	26.13	25.88	26.72	24.72	27.18	26.39	26.50	28.91	27.64	28.76	25.83	26.49	25.38	23.51	25.07	23.66
83	<i>Mantura chrysanthemi</i>	17.34	18.33	17.39	18.56	18.13	17.74	17.78	19.01	16.74	20.45	17.37	17.91	18.38	15.97	13.83	15.96
84	<i>Neocrepidodera femorata</i>	22.79	22.63	23.64	23.68	23.15	23.01	24.85	27.01	21.66	23.42	24.33	24.95	22.75	17.96	20.37	23.62
85	<i>Neocrepidodera ferruginea</i>	20.87	22.02	21.19	22.97	21.19	21.16	22.87	21.90	21.31	23.15	22.06	23.31	21.89	19.21	19.20	19.72
86	<i>Neocrepidodera melanostoma</i>	23.50	24.27	23.76	24.41	24.42	23.58	24.34	24.44	22.91	23.68	24.07	24.79	22.87	19.24	19.58	21.96
87	<i>Neocrepidodera peirolerii</i>	26.39	25.39	26.60	24.65	25.46	26.10	25.29	25.33	24.77	26.41	26.10	25.84	24.21	18.20	17.15	20.11
88	<i>Neocrepidodera transversa</i>	21.66	21.10	22.22	22.74	22.05	22.11	22.13	22.49	21.05	22.76	22.05	22.86	22.72	17.88	19.19	17.06
89	<i>Phyllotreta albionica</i>	23.06	23.27	23.59	23.85	21.64	22.75	23.20	23.87	21.74	21.71	22.75	22.98	26.04	20.53	19.53	20.13
90	<i>Phyllotreta armoraciae</i>	23.19	22.16	23.77	23.67	21.51	22.42	22.82	22.20	21.01	23.29	22.99	22.90	24.81	22.44	21.03	19.10
91	<i>Phyllotreta astrachanica</i>	26.52	28.22	26.82	26.55	27.10	26.54	27.63	27.91	25.16	27.92	26.23	27.56	26.52	21.21	20.88	24.38
92	<i>Phyllotreta atra</i>	22.86	26.80	23.38	26.02	24.76	24.24	25.90	26.67	25.12	26.72	23.57	26.91	25.57	22.87	21.39	20.97
93	<i>Phyllotreta cruciferae</i>	23.06	23.27	23.59	23.85	21.63	22.75	23.20	23.87	21.74	21.71	22.75	22.98	26.03	20.52	19.52	20.12
94	<i>Phyllotreta exclamationis</i>	25.76	24.27	26.05	26.96	23.47	25.86	27.06	27.13	24.63	25.99	26.10	27.44	28.71	21.31	22.13	20.49
95	<i>Phyllotreta nemorum</i>	22.99	23.29	23.27	23.58	24.35	23.31	23.88	25.76	22.88	22.34	22.79	23.83	26.36	22.82	21.04	20.93
96	<i>Phyllotreta nigripes</i>	27.54	28.30	28.28	27.06	27.05	27.13	28.37	27.84	25.72	27.40	27.00	27.49	25.04	22.43	22.63	23.30
97	<i>Phyllotreta nodicornis</i>	28.37	30.25	28.53	28.48	31.23	28.83	30.24	29.73	27.66	31.77	28.29	30.37	24.99	24.55	23.03	24.25
98	<i>Phyllotreta ochripes</i>	25.47	23.55	25.58	25.98	23.56	26.15	26.94	26.59	23.54	26.69	24.60	26.42	26.16	20.14	21.20	23.07
99	<i>Phyllotreta procera</i>	24.67	27.48	24.77	25.76	26.18	25.38	27.24	26.46	25.41	27.89	25.73	26.84	25.37	23.83	22.11	23.73
100	<i>Phyllotreta pusilla</i>	24.09	22.64	24.18	23.18	23.04	23.79	23.86	25.18	21.90	24.31	24.18	23.73	24.33	24.09	20.64	20.97
101	<i>Phyllotreta striolata</i>	24.47	23.42	24.57	25.63	24.64	24.38	26.20	25.86	23.59	25.66	25.18	25.43	25.43	24.20	22.77	22.57
102	<i>Phyllotreta tetrastigma</i>	27.46	27.62	27.31	26.42	26.27	26.98	25.30	25.54	25.28	26.70	25.82	25.75	26.72	20.80	22.99	22.75
103	<i>Phyllotreta undulata</i>	27.88	27.79	27.66	27.00	26.73	28.43	27.18	24.84	24.02	27.08	28.37	27.10	29.18	22.69	22.67	22.84
104	<i>Phyllotreta vittula</i>	25.38	27.05	25.11	23.11	24.79	25.97	24.41	27.02	24.08	25.73	24.72	23.64	23.01	24.41	22.41	21.07

105	<i>Podagrica fuscicornis</i>	21.41	21.54	21.62	22.56	21.91	21.60	22.48	23.10	22.04	23.41	21.37	23.09	20.21	17.61	19.92	19.19
106	<i>Podagrica fuscipes</i>	21.20	21.25	21.39	21.76	20.88	21.67	21.04	21.18	19.47	23.63	20.60	21.07	19.43	17.34	18.97	19.48
107	<i>Psylliodes affinis</i>	25.93	26.48	25.87	25.45	28.10	25.06	26.48	27.33	25.23	29.02	25.32	26.22	22.44	19.76	18.85	19.61
108	<i>Psylliodes chalcomera</i>	18.14	17.88	17.99	18.15	16.38	17.99	17.83	19.25	15.69	19.78	18.24	17.78	16.56	13.56	13.76	15.35
109	<i>Psylliodes chrysocephala</i>	17.00	17.32	17.70	18.57	17.19	17.91	17.65	17.85	16.43	17.54	17.58	17.41	16.92	15.82	15.79	14.77
110	<i>Psylliodes crambicola</i>	19.42	20.86	19.56	20.67	19.03	19.58	20.96	21.81	19.73	21.92	20.00	20.87	18.28	15.33	14.38	16.16
111	<i>Psylliodes cucullatus</i>	19.15	19.62	19.50	19.80	19.80	20.00	19.40	21.04	18.74	20.50	19.34	19.33	17.14	17.12	16.30	17.33
112	<i>Psylliodes dulcamarae</i>	20.67	22.61	21.16	21.40	20.38	21.52	20.85	22.64	19.85	21.78	20.21	21.46	25.21	19.60	19.59	20.77
113	<i>Psylliodes instabilis</i>	20.39	21.33	20.50	21.53	20.50	20.59	21.32	20.18	18.40	20.46	20.80	21.63	21.07	17.33	16.05	15.23
114	<i>Psylliodes isatidis</i>	18.45	20.52	18.68	20.59	18.77	18.93	20.72	21.08	19.44	21.56	19.40	20.68	19.42	16.15	14.42	15.26
115	<i>Psylliodes napi</i>	22.35	22.03	22.82	22.08	21.27	22.65	21.74	22.41	21.70	22.84	21.54	22.54	22.06	16.59	14.16	16.12
116	<i>Psylliodes picinus</i>	20.53	20.70	21.10	20.06	20.27	20.82	20.59	22.04	18.22	21.72	19.93	20.39	18.80	17.14	17.72	18.49
117	<i>Systema marginalis</i>	24.10	24.45	24.57	23.70	23.91	23.73	24.78	25.33	24.15	26.24	23.96	24.88	21.01	18.50	20.17	21.12

17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
2.34	2.61	2.32	2.27	2.36	2.58	2.05	2.30	2.07	2.20	2.07	2.14	2.31	2.40	2.35	2.32	2.58	2.29	2.34	2.57	2.17
2.52	2.65	2.34	2.38	2.22	2.58	1.95	2.25	2.07	2.27	2.11	2.21	2.46	2.41	2.43	2.31	2.65	2.41	2.51	2.61	2.41
2.37	2.61	2.35	2.33	2.37	2.61	2.06	2.30	2.09	2.23	2.07	2.15	2.35	2.43	2.40	2.34	2.61	2.31	2.40	2.59	2.19
2.14	2.38	2.09	2.14	1.98	2.24	1.83	1.99	1.95	2.06	2.00	2.05	2.18	2.24	2.25	2.34	2.32	2.12	2.29	2.39	2.08
2.43	2.64	2.44	2.43	2.37	2.64	2.00	2.21	2.11	2.28	2.17	2.18	2.47	2.43	2.42	2.37	2.68	2.35	2.49	2.71	2.36
2.29	2.54	2.26	2.21	2.29	2.55	1.99	2.28	2.01	2.10	1.99	2.05	2.26	2.39	2.34	2.26	2.57	2.26	2.31	2.56	2.12
2.37	2.60	2.39	2.39	2.30	2.46	2.06	2.27	2.22	2.36	2.26	2.29	2.49	2.43	2.47	2.50	2.59	2.27	2.51	2.67	2.27
2.51	2.58	2.51	2.38	2.48	2.56	2.07	2.29	2.08	2.18	2.24	2.31	2.46	2.39	2.49	2.34	2.68	2.45	2.54	2.72	2.33
2.29	2.39	2.21	2.23	2.29	2.45	1.95	2.21	2.09	2.16	2.06	2.16	2.34	2.39	2.33	2.23	2.55	2.17	2.43	2.57	2.19
2.48	2.73	2.52	2.52	2.47	2.45	2.20	2.29	2.32	2.43	2.31	2.45	2.57	2.39	2.49	2.37	2.72	2.37	2.47	2.77	2.29
2.47	2.46	2.30	2.20	2.25	2.44	2.04	2.30	2.15	2.25	2.07	2.09	2.28	2.34	2.32	2.32	2.52	2.27	2.33	2.54	2.25
2.38	2.63	2.39	2.43	2.35	2.55	2.07	2.22	2.21	2.38	2.30	2.29	2.53	2.52	2.50	2.57	2.66	2.35	2.59	2.69	2.34

2.26	2.76	2.24	2.24	2.00	2.58	2.01	2.34	2.14	2.37	2.27	1.86	2.23	2.42	2.50	2.48	2.51	2.32	2.48	2.62	2.28
1.65	2.00	1.69	2.05	1.98	2.33	1.66	1.93	1.90	1.91	1.78	2.05	1.90	1.75	1.94	1.88	2.10	1.95	2.02	2.11	2.00
1.78	1.90	1.60	2.02	2.14	2.24	1.89	2.12	2.01	2.03	1.95	2.04	1.98	2.19	2.06	2.14	2.36	2.05	2.14	2.22	2.03
1.78	2.03	1.75	2.06	2.22	2.40	1.85	2.05	2.10	2.01	1.89	2.08	2.15	2.14	2.05	2.21	2.36	2.03	2.16	2.39	1.92
	2.22	1.89	2.07	2.22	2.49	2.00	2.04	1.96	2.11	2.02	1.88	2.07	2.27	2.20	2.30	2.38	2.19	2.26	2.33	2.15
20.1		1.90	2.33	2.53	2.64	2.18	2.43	2.38	2.28	2.26	2.50	2.43	2.52	2.52	2.48	2.62	2.55	2.56	2.45	2.44
15.4	16.1		2.13	2.18	2.36	1.89	2.07	1.93	2.07	1.97	2.09	2.08	2.10	2.14	2.09	2.39	2.08	2.19	2.19	2.04
18.5	21.9	18.8		1.44	2.30	1.96	2.21	1.99	1.99	1.98	2.17	2.09	2.25	2.26	2.31	2.34	2.31	2.20	2.40	2.01
20.4	23.5	19.0	9.68		2.18	1.90	2.16	2.00	2.22	2.05	2.14	2.12	2.38	2.19	2.30	2.26	2.08	2.15	2.40	2.11
24.1	26.7	22.8	21.5	20.1		2.29	2.44	2.43	2.63	2.42	2.48	2.04	2.34	2.24	2.31	2.33	2.12	2.09	2.60	2.49
17.7	20.8	16.6	17.5	16.9	22.0		2.01	1.40	1.38	1.37	2.10	1.96	2.02	2.19	2.05	2.33	2.05	2.27	2.12	2.07
18.6	23.3	18.7	20.5	20.2	23.2	18.0		2.11	2.13	2.16	2.01	2.30	2.29	2.28	2.36	2.43	2.32	2.36	2.45	2.12
16.3	22.5	16.5	16.7	17.3	22.9	10.0	18.8		1.63	1.63	1.98	2.10	2.18	2.13	2.16	2.48	2.11	2.32	2.33	2.07
18.5	21.7	17.6	16.5	19.6	24.3	9.47	19.5	11.7		1.45	2.10	2.34	2.30	2.46	2.12	2.55	2.45	2.41	2.44	1.95
17.9	21.9	18.3	16.6	17.6	22.4	10.0	19.6	12.2	9.48		2.05	2.10	2.12	2.34	2.04	2.43	2.26	2.33	2.34	2.00
15.7	23.6	18.6	17.7	18.3	23.7	19.3	17.9	16.5	17.8	18.5		2.00	2.27	2.20	2.21	2.42	2.26	2.36	2.56	2.10
17.9	23.7	19.4	18.6	18.3	19.0	17.6	21.5	19.4	21.1	18.9	17.8		1.87	1.86	1.92	1.89	1.80	1.68	1.99	2.08
22.6	24.7	20.0	20.6	21.9	23.2	20.2	21.7	21.1	22.7	20.9	20.8	16.8		1.93	1.96	1.85	1.76	2.11	2.14	2.11
19.3	24.5	18.6	20.3	19.5	20.3	20.3	21.9	19.1	23.9	22.0	20.3	15.4	17.3		1.81	1.98	1.08	1.60	2.05	2.07
20.3	24.1	19.1	20.9	20.2	20.7	18.5	22.4	19.2	18.5	17.8	20.1	15.9	18.3	14.2		2.21	1.58	1.73	2.08	2.09
21.6	25.3	21.9	21.7	20.3	21.3	21.7	23.4	23.4	23.9	23.5	22.7	16.5	16.2	16.3	19.1		1.82	2.05	2.15	2.47
19.7	24.9	18.5	21.2	18.2	18.8	18.8	22.2	19.3	23.6	21.2	20.9	14.7	15.4	6.15	11.8	14.8		1.59	1.99	2.09
21.5	24.8	19.3	20.0	18.7	18.6	20.6	22.7	22.0	22.3	21.4	22.6	13.9	19.3	12.3	13.5	18.0	12.1		1.92	2.21
22.2	23.1	20.6	23.4	22.8	26.0	19.2	23.4	21.6	22.7	20.9	25.6	18.0	20.8	19.0	19.7	20.1	18.0	16.1		2.29
19.7	23.4	18.4	16.9	18.9	24.9	18.9	19.1	18.1	16.7	17.9	18.2	18.6	19.6	18.7	18.5	23.4	19.2	20.6	23.5	
18.2	21.9	17.2	18.4	19.4	24.9	14.5	20.1	15.3	16.0	16.2	18.4	20.3	22.9	20.5	18.5	23.5	20.9	21.5	21.1	19.6

18.9	23.5	19.1	19.3	19.0	22.6	16.6	20.7	16.5	20.1	19.2	19.3	20.1	22.2	20.8	21.4	23.8	20.6	22.5	23.1	21.2
18.4	23.7	15.8	22.4	19.8	23.3	21.9	18.5	22.2	22.9	22.2	22.4	21.1	21.7	20.3	19.5	22.3	18.6	20.6	25.1	21.3
18.1	23.6	22.5	17.5	19.8	23.0	20.4	21.0	18.0	19.6	19.7	19.2	21.1	20.6	21.1	21.2	24.9	20.9	23.5	25.2	22.3
14.7	23.3	17.9	20.2	19.9	25.5	21.0	20.7	19.9	21.9	20.6	17.0	20.2	24.0	19.9	21.5	24.7	19.5	21.4	23.1	19.8
20.8	23.4	20.8	20.3	21.1	20.4	19.6	20.0	18.2	21.2	19.7	21.3	20.4	19.7	19.8	19.0	22.9	19.9	20.7	24.3	20.6
21.2	24.6	21.0	19.6	22.5	24.8	18.9	22.3	20.1	20.5	17.9	18.9	20.2	23.6	22.9	23.1	25.1	22.3	23.8	23.5	23.0
19.5	23.3	19.3	17.6	18.8	22.9	16.8	19.7	18.4	20.1	19.4	19.0	18.0	21.3	21.4	22.2	23.2	21.2	21.3	23.3	19.3
15.1	21.6	15.5	19.6	17.5	21.1	18.3	19.2	17.5	19.4	15.5	16.0	17.5	20.9	19.3	18.8	21.5	17.3	18.3	19.6	17.6
19.0	21.9	19.3	21.7	20.6	21.1	17.4	20.6	18.7	19.8	18.6	18.2	18.9	19.2	21.7	20.7	21.3	20.5	23.1	23.7	22.1
14.4	23.3	15.6	18.7	17.1	22.8	19.2	18.5	18.1	19.3	17.9	15.2	18.0	20.3	19.5	19.3	21.8	17.7	19.8	21.2	17.6
15.8	19.3	13.0	18.2	18.3	20.6	14.9	16.1	14.8	16.3	16.2	16.0	19.4	19.9	17.6	20.2	21.0	18.7	18.9	22.4	17.5
19.6	22.3	15.5	16.6	15.8	19.9	16.9	20.6	15.7	17.2	15.1	17.0	18.7	20.8	18.9	18.2	21.0	18.2	18.6	21.5	20.0
20.6	25.1	18.9	16.0	16.7	23.6	19.6	20.0	19.5	21.1	19.8	19.2	18.3	19.8	20.3	22.4	20.2	21.1	21.2	23.9	21.2
17.7	21.1	14.4	19.1	17.9	20.8	19.4	17.5	18.2	19.8	19.8	18.4	18.4	21.3	18.7	19.9	22.3	17.9	18.0	21.1	17.6
15.4	20.5	15.9	20.7	20.8	21.6	18.9	18.7	17.9	18.4	18.5	16.5	18.1	21.9	18.5	16.0	23.5	17.7	19.5	23.1	19.4
18.2	23.3	14.0	17.1	18.0	21.7	19.1	18.3	19.0	20.0	17.4	19.5	19.8	19.8	21.2	22.7	24.2	20.5	19.7	21.3	19.8
18.4	21.4	17.4	19.3	19.6	21.7	19.1	19.9	17.0	20.1	16.9	18.2	19.9	21.2	20.5	20.0	22.3	19.6	22.6	21.6	19.9
18.3	23.0	15.9	18.9	17.9	20.9	19.5	18.5	17.7	18.9	19.0	15.3	18.8	19.9	18.1	19.7	18.3	18.5	19.5	22.0	18.5
15.8	21.4	15.9	19.5	17.3	21.1	14.5	16.2	16.4	17.0	17.4	18.5	17.6	19.9	17.2	17.8	22.0	17.5	17.8	19.7	19.4
16.7	21.6	14.6	19.3	18.1	21.0	19.6	17.4	19.2	18.7	20.7	16.1	19.7	21.4	17.5	18.1	20.1	18.5	19.1	20.9	18.2
19.2	22.0	18.3	19.7	17.9	23.9	17.4	20.5	17.3	18.9	18.2	17.4	18.9	22.4	19.1	19.3	22.1	19.0	21.3	23.4	18.2
17.4	21.3	17.0	18.5	17.2	21.7	18.0	19.4	16.9	19.2	18.8	16.6	18.9	21.0	20.5	21.3	22.4	20.1	21.7	22.3	20.2
16.3	24.0	16.7	21.5	18.9	21.7	16.9	18.2	19.9	20.2	18.6	17.8	16.9	19.8	19.4	20.3	20.8	18.6	20.3	23.6	18.5
20.8	23.0	20.9	21.3	22.5	22.1	19.2	20.7	18.7	21.7	21.3	18.2	20.4	23.3	21.2	22.4	23.7	22.4	22.0	24.2	23.3
16.7	20.9	15.5	18.6	16.6	17.8	17.0	18.0	17.3	16.4	17.1	17.8	17.4	21.9	19.3	20.4	22.4	17.7	18.9	20.2	19.4
16.9	21.3	16.1	20.5	20.2	24.2	19.7	19.0	18.7	18.5	18.1	18.9	19.0	22.5	21.2	20.4	22.6	21.8	21.8	22.6	21.3

19.8	22.7	14.7	18.0	17.3	21.4	18.8	19.7	17.8	19.3	17.6	15.5	18.6	18.3	19.5	21.0	21.4	20.1	20.7	21.9	18.2
16.9	19.7	14.6	20.6	18.8	20.9	18.7	18.8	17.4	18.6	17.7	17.7	19.0	20.2	21.0	19.4	21.3	18.7	20.5	22.1	19.8
15.8	22.1	15.1	17.6	17.1	23.5	17.9	17.9	16.1	18.0	17.7	16.3	18.4	20.9	20.8	21.1	20.1	20.0	21.0	21.2	19.1
14.9	22.2	17.4	19.8	19.0	22.5	16.2	17.2	15.9	18.2	18.0	15.3	18.4	23.2	20.4	20.4	25.0	18.8	20.5	20.6	19.1
17.1	22.2	13.4	18.3	18.3	22.0	20.3	18.9	19.1	20.1	19.8	18.3	20.4	21.3	21.4	21.3	23.7	19.6	20.8	22.8	17.7
16.9	25.3	19.3	20.4	21.1	24.3	21.1	20.2	20.7	21.7	21.7	17.0	19.3	20.7	21.4	24.2	22.8	19.8	21.8	25.4	19.0
16.4	20.3	17.6	18.2	19.5	22.7	18.4	17.0	17.2	19.3	19.3	16.5	17.6	21.2	18.0	20.8	22.7	19.3	19.2	20.5	16.2
15.0	23.7	18.1	20.6	19.6	23.0	16.8	17.3	16.2	18.3	18.6	15.3	19.1	23.9	20.8	21.2	25.0	19.3	20.5	20.4	19.4
16.2	21.0	14.8	19.5	18.3	22.4	19.8	17.8	17.7	20.3	19.2	17.8	17.6	17.8	19.3	21.0	20.1	18.3	19.6	20.4	17.0
16.2	23.4	16.7	18.1	16.0	22.6	18.8	21.2	18.4	19.7	17.6	17.1	19.1	20.4	19.6	21.0	21.3	19.3	19.7	20.9	20.8
18.8	22.1	18.1	21.3	18.6	24.0	18.3	23.1	18.5	18.3	17.3	22.2	22.2	21.0	18.1	19.0	20.9	17.6	20.3	20.5	19.4
20.4	25.1	17.8	20.7	18.1	21.5	22.3	23.5	20.9	22.3	21.4	19.3	19.2	22.0	21.4	19.7	21.1	19.2	21.7	23.9	24.2
15.8	19.8	12.5	18.0	15.7	18.8	15.0	18.1	15.7	16.2	16.0	15.9	16.9	18.5	18.8	19.8	21.6	17.8	19.2	20.9	16.9
17.1	21.1	13.8	19.8	18.3	21.3	19.4	18.0	19.2	18.2	20.3	16.7	20.3	21.0	18.4	18.0	20.3	18.3	19.5	20.7	18.4
22.2	22.3	19.0	20.5	21.3	20.3	18.8	20.1	19.5	20.0	18.0	19.4	18.7	19.5	19.9	20.1	21.4	18.2	21.0	22.6	21.0
20.1	24.9	20.4	20.8	22.0	26.2	20.6	22.7	20.9	22.0	20.7	21.5	23.8	23.3	23.1	23.6	25.7	23.1	23.4	27.3	21.7
23.4	27.3	21.9	21.6	20.4	26.3	22.7	21.2	22.8	21.9	22.1	23.6	23.4	22.4	24.7	24.0	21.8	23.2	23.0	22.3	20.8
23.5	28.1	22.9	24.1	22.6	26.3	22.8	25.3	23.8	24.0	22.0	24.0	24.7	24.0	23.6	24.4	22.3	24.5	24.0	26.6	23.9
14.7	20.7	16.2	17.6	17.9	22.0	15.2	17.3	15.4	16.6	16.0	12.5	15.2	19.5	18.1	18.5	22.0	18.0	20.0	20.4	15.7
20.4	27.1	17.9	22.3	21.0	23.1	19.8	20.3	21.8	20.8	20.7	21.2	19.9	23.6	22.9	22.4	23.3	22.7	23.5	25.9	22.4
21.5	22.0	17.4	22.4	20.8	25.6	20.4	18.1	18.7	21.8	20.8	19.9	20.5	22.4	20.2	23.4	24.5	22.2	23.2	23.7	21.0
21.4	24.7	19.3	21.7	21.3	22.2	20.8	21.0	21.0	21.5	19.8	20.5	19.4	21.7	22.0	20.5	22.1	20.7	21.6	23.2	22.2
19.0	23.3	16.1	21.7	18.5	22.2	21.0	20.5	20.4	21.7	20.2	23.3	18.3	24.3	20.3	21.0	21.5	21.1	21.4	24.2	22.0
21.6	23.8	19.6	21.5	19.5	22.4	18.5	21.7	19.1	19.9	19.7	21.8	19.8	21.2	22.3	20.3	23.4	19.8	20.7	21.0	21.4
21.3	26.9	21.5	24.7	23.8	27.9	19.9	23.8	20.8	21.7	21.0	21.3	21.7	23.1	20.7	23.5	25.4	21.0	22.5	24.7	20.4
22.8	26.3	23.4	21.5	21.9	25.7	16.7	23.4	19.7	18.5	20.1	20.1	19.9	23.8	22.5	22.7	24.7	21.7	23.7	27.4	20.4

21.1	26.2	22.6	25.1	25.4	28.0	20.3	23.3	21.1	22.0	21.6	23.6	21.6	26.7	25.2	24.1	26.4	25.8	25.9	24.1	24.5
22.5	27.7	22.8	24.6	22.9	23.4	19.7	23.7	22.2	23.4	21.7	21.7	20.3	22.3	20.9	22.9	23.6	20.2	22.2	22.1	21.6
21.3	26.8	21.5	24.6	23.8	27.8	19.9	23.7	20.8	21.7	20.9	21.3	21.6	23.0	20.7	23.5	25.3	21.0	22.5	24.7	20.3
23.1	24.5	20.8	24.7	24.7	28.8	20.1	23.2	20.8	22.5	23.1	24.1	21.2	23.5	22.8	21.3	27.3	23.8	25.5	26.3	21.9
22.3	25.2	22.1	25.7	21.8	24.7	21.9	24.0	20.9	22.5	22.0	23.0	22.8	21.0	22.4	23.5	24.1	20.4	21.9	24.4	22.0
21.5	26.6	22.8	22.0	24.2	26.2	20.2	23.8	21.1	22.4	21.8	22.1	22.2	26.8	24.3	23.5	27.2	23.8	25.3	27.8	24.7
23.4	27.0	25.1	24.4	24.9	28.7	20.9	24.8	21.1	22.2	21.6	23.4	23.1	27.9	23.7	23.9	25.6	23.6	24.8	27.2	26.3
23.5	27.1	21.8	22.9	23.3	26.6	19.5	21.3	21.2	20.7	21.1	23.3	21.7	22.5	22.9	22.9	27.0	23.9	23.1	25.3	21.1
22.7	26.9	23.2	25.4	24.7	26.2	21.0	24.1	21.2	21.4	20.9	22.4	22.1	26.2	21.9	22.1	24.1	22.1	25.0	25.9	24.1
22.0	26.0	22.2	23.4	23.0	27.1	18.6	23.9	18.5	20.0	19.6	20.7	20.7	22.6	24.5	23.3	24.6	21.7	24.1	25.0	21.4
25.2	24.9	22.2	28.2	26.0	26.6	20.9	24.2	21.6	22.6	23.3	25.4	23.2	26.1	26.0	23.8	26.7	25.3	25.9	27.9	25.9
24.7	26.6	25.0	28.1	27.7	28.9	22.1	23.7	23.4	24.7	23.9	25.8	23.4	24.7	26.4	25.4	25.5	26.2	25.3	24.1	25.0
25.0	27.7	24.5	26.9	27.8	29.2	21.1	23.7	23.0	22.9	24.0	26.6	24.9	25.8	25.8	23.9	26.8	24.8	27.2	23.5	26.2
22.2	27.9	23.3	23.3	22.4	26.4	21.7	23.8	19.8	25.0	23.1	20.0	22.6	22.9	21.8	24.8	23.4	21.3	25.3	26.1	21.6
18.2	24.8	20.7	18.4	18.7	22.4	19.2	19.8	18.5	18.4	19.7	18.0	19.7	20.6	20.6	21.3	21.2	20.3	22.1	25.4	20.6
15.4	25.8	19.1	19.1	18.0	22.9	18.8	18.2	19.5	21.4	21.1	16.3	17.5	19.9	20.5	20.6	20.4	18.0	22.5	23.5	21.1
21.0	23.3	20.6	23.4	21.5	24.6	20.5	21.4	22.3	21.6	20.7	18.8	17.6	23.6	22.6	23.6	22.2	23.3	19.7	22.3	20.7
16.9	21.8	16.1	18.9	16.5	21.0	13.5	16.7	15.2	16.2	17.2	15.6	16.7	18.6	19.8	17.4	19.7	15.6	18.6	21.9	17.1
15.9	18.9	16.4	17.5	19.0	21.2	13.6	16.3	15.2	15.6	14.7	15.5	17.8	19.4	19.6	18.1	23.6	17.7	19.3	22.2	15.7
16.0	19.6	16.3	19.0	19.3	23.4	16.5	18.6	15.7	16.7	16.5	15.8	18.2	20.8	19.0	19.5	20.3	19.6	18.8	20.9	16.9
18.4	21.0	16.4	18.9	15.4	18.9	16.2	18.9	16.8	16.7	16.8	18.6	18.2	21.1	19.1	17.3	21.6	16.8	16.9	22.5	16.8
20.1	23.1	21.2	21.9	22.2	22.7	19.8	20.2	20.5	20.9	21.2	21.1	21.9	23.9	20.2	19.1	25.3	18.9	19.8	22.5	21.3
17.1	21.0	16.9	20.6	20.9	21.6	16.4	18.7	17.5	15.0	16.5	17.1	18.9	22.8	22.6	19.5	24.3	20.4	21.5	21.4	18.8
16.5	20.4	16.6	19.4	19.2	23.5	16.5	17.7	16.2	17.2	16.5	15.8	18.4	20.9	18.5	20.1	20.6	18.7	19.4	21.1	17.0
16.0	22.9	16.5	20.5	22.4	21.8	17.5	15.4	16.0	18.9	19.5	17.7	19.8	18.5	17.2	19.7	20.9	17.0	21.7	20.7	18.8
19.7	22.1	17.5	18.8	17.6	22.2	16.2	18.4	17.0	17.2	17.9	16.1	19.2	22.0	21.2	20.4	22.9	19.1	20.2	23.1	17.6

20.5	23.6	21.0	20.7	18.6	24.8	18.6	20.7	19.7	20.8	19.9	20.4	20.2	22.7	22.6	21.4	22.2	21.2	21.6	22.7	21.2
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38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
2.36	2.47	2.57	2.32	2.38	2.24	2.33	2.32	2.18	2.34	2.15	2.33	2.36	2.38	2.45	2.11	2.41	2.27	2.51	2.24
2.40	2.55	2.51	2.32	2.34	2.23	2.35	2.34	2.28	2.38	2.31	2.36	2.28	2.52	2.49	2.30	2.49	2.26	2.35	2.28
2.39	2.50	2.59	2.35	2.40	2.28	2.32	2.35	2.18	2.35	2.14	2.36	2.39	2.41	2.46	2.09	2.43	2.30	2.55	2.25
2.07	2.18	2.16	2.16	2.08	2.19	2.24	2.05	1.91	2.14	1.94	1.95	2.21	2.23	2.23	2.09	2.16	1.98	2.18	2.04
2.35	2.54	2.52	2.35	2.40	2.33	2.49	2.28	2.23	2.38	2.22	2.32	2.40	2.50	2.52	2.25	2.50	2.31	2.39	2.21
2.28	2.45	2.55	2.17	2.30	2.14	2.29	2.30	2.10	2.27	2.06	2.26	2.26	2.33	2.33	2.06	2.34	2.28	2.43	2.18
2.35	2.45	2.50	2.36	2.30	2.33	2.48	2.25	2.15	2.41	2.16	2.22	2.41	2.47	2.45	2.30	2.45	2.26	2.47	2.25
2.37	2.67	2.72	2.35	2.43	2.29	2.50	2.45	2.28	2.47	2.23	2.45	2.47	2.36	2.49	2.17	2.61	2.38	2.63	2.38
2.04	2.34	2.46	2.23	2.23	2.15	2.27	2.18	2.12	2.12	2.15	2.14	2.16	2.16	2.40	1.98	2.39	2.09	2.27	2.09
2.43	2.57	2.54	2.55	2.52	2.42	2.50	2.41	2.34	2.56	2.26	2.53	2.49	2.56	2.56	2.29	2.54	2.40	2.61	2.45
2.35	2.53	2.50	2.29	2.35	2.22	2.32	2.37	2.17	2.33	2.16	2.31	2.25	2.33	2.49	2.19	2.39	2.35	2.42	2.17
2.35	2.47	2.52	2.41	2.31	2.38	2.48	2.29	2.17	2.44	2.20	2.23	2.46	2.55	2.51	2.34	2.51	2.24	2.48	2.28
2.26	2.32	2.48	2.50	2.22	2.43	2.43	2.45	2.00	2.28	1.94	2.22	2.02	2.40	2.21	2.21	2.19	2.22	2.18	2.22
1.88	1.90	2.06	1.92	1.95	1.96	2.13	1.96	1.87	1.93	1.80	1.58	1.99	2.14	1.92	1.83	1.85	1.94	1.76	1.81
1.98	2.08	2.15	2.17	1.99	2.11	2.21	2.14	1.83	2.03	1.85	1.70	2.11	2.31	1.75	1.74	1.91	2.05	2.10	1.88
2.13	2.23	2.24	2.15	2.04	2.19	2.18	2.16	1.95	2.26	1.88	1.82	2.21	2.47	1.76	1.88	1.92	2.16	2.09	2.00
2.03	2.05	2.13	2.07	1.85	2.26	2.22	2.16	1.80	2.16	1.72	1.85	2.19	2.28	2.07	1.83	2.03	2.12	2.13	1.92
2.32	2.44	2.51	2.40	2.45	2.44	2.44	2.41	2.31	2.33	2.35	2.14	2.35	2.51	2.29	2.18	2.41	2.27	2.40	2.33
1.96	2.08	1.87	2.40	2.00	2.23	2.24	2.15	1.86	2.06	1.79	1.65	1.82	2.10	1.85	1.82	1.75	1.94	1.89	1.91
2.16	2.21	2.44	2.10	2.20	2.29	2.17	2.16	2.17	2.33	2.04	2.07	2.01	1.93	2.20	2.20	2.01	2.18	2.16	2.17
2.18	2.10	2.24	2.31	2.19	2.37	2.37	2.21	2.05	2.23	1.96	2.11	1.99	1.96	2.19	2.30	2.07	2.23	2.09	2.05
2.54	2.37	2.31	2.44	2.59	2.18	2.47	2.45	2.28	2.28	2.35	2.26	2.20	2.47	2.36	2.25	2.34	2.28	2.26	2.32
1.76	1.92	2.29	2.21	2.23	2.11	2.03	1.93	2.08	1.94	2.03	1.80	2.00	2.18	2.20	2.00	2.08	2.08	2.17	1.83

2.15	2.19	2.08	2.22	2.21	2.16	2.30	2.16	2.11	2.17	1.99	1.81	2.26	2.15	1.98	2.03	1.98	2.13	2.05	1.81
1.92	1.94	2.37	2.13	2.23	2.11	2.19	2.09	2.07	2.10	2.06	1.82	1.98	2.20	2.17	1.96	2.15	1.93	2.07	1.93
1.94	2.29	2.42	2.24	2.32	2.32	2.25	2.29	2.17	2.22	2.10	1.98	2.06	2.38	2.28	2.02	2.24	2.23	2.14	2.06
1.94	2.13	2.37	2.26	2.21	2.22	2.03	2.16	1.93	2.09	2.00	1.91	1.89	2.25	2.23	1.99	1.97	1.95	2.17	2.06
2.14	2.17	2.38	2.16	1.96	2.32	2.14	2.20	1.91	2.08	1.82	1.95	1.98	2.23	2.18	1.90	2.10	2.04	1.90	2.07
2.10	2.17	2.25	2.24	2.19	2.14	2.16	2.10	1.95	2.09	1.96	2.09	2.13	2.05	2.13	1.99	2.15	2.17	2.17	2.05
2.33	2.19	2.26	2.12	2.39	2.06	2.42	2.29	2.15	2.05	2.05	2.13	2.24	2.22	2.25	2.19	2.15	2.27	2.14	2.10
2.16	2.30	2.23	2.29	2.20	2.17	2.43	2.40	2.18	2.32	2.13	2.04	2.18	2.31	2.15	1.96	2.30	2.31	2.13	2.02
2.16	2.36	2.16	2.38	2.38	2.17	2.46	2.42	2.11	2.25	2.07	2.28	2.05	2.36	2.26	1.85	2.34	2.20	2.17	2.09
2.40	2.50	2.38	2.54	2.55	2.34	2.55	2.55	2.33	2.36	2.31	2.35	2.33	2.27	2.41	2.50	2.53	2.41	2.11	2.37
2.16	2.25	2.11	2.27	2.17	2.16	2.36	2.30	1.99	2.20	1.98	2.10	2.10	2.35	2.06	1.99	2.27	2.25	2.10	2.03
2.26	2.39	2.21	2.47	2.27	2.23	2.49	2.36	2.05	2.36	2.04	2.15	2.14	2.32	2.10	2.05	2.19	2.37	2.17	2.08
2.19	2.37	2.55	2.51	2.39	2.48	2.38	2.36	2.15	2.37	2.19	2.38	2.30	2.51	2.33	2.33	2.30	2.37	2.37	2.19
2.21	2.28	2.31	2.46	2.20	2.24	2.40	2.30	1.96	2.31	1.89	2.05	2.19	2.33	2.08	2.05	2.17	2.20	2.12	2.12
	2.00	2.44	2.21	2.30	2.14	1.94	1.93	2.03	2.19	2.00	1.86	2.10	2.13	2.13	2.13	2.22	2.16	1.98	2.00
16.9		2.34	2.30	2.34	2.35	2.16	1.94	1.95	2.05	1.93	1.88	2.25	2.09	2.22	2.09	2.04	2.04	1.92	2.00
23.2	23.2		2.22	2.13	2.16	2.59	2.38	1.95	2.21	1.93	2.05	2.21	2.34	2.07	2.10	1.92	2.05	2.09	1.99
19.6	20.9	19.9		2.13	1.49	2.35	2.31	2.12	2.29	2.07	2.10	2.30	2.48	2.15	2.14	2.14	2.10	2.20	2.17
22.2	21.8	19.1	18.4		2.35	2.41	2.30	1.93	2.06	1.88	1.98	2.22	2.33	2.04	1.96	1.99	2.10	2.10	2.17
19.5	22.3	18.5	9.72	20.8		2.42	2.26	2.04	2.23	2.00	2.05	2.27	2.40	2.10	2.11	2.16	2.10	2.16	1.88
17.6	19.0	25.8	21.9	23.7	23.3		2.05	2.18	2.27	2.22	2.12	2.30	2.35	2.18	2.09	2.22	2.31	2.31	2.33
16.4	17.1	21.9	20.5	21.2	20.3	18.5		2.10	2.17	2.12	2.09	2.37	2.24	2.26	2.02	2.34	2.15	2.14	2.12
18.0	17.2	16.7	18.4	16.2	18.4	20.7	18.7		1.94	0.76	1.63	1.97	1.98	1.65	1.73	1.71	1.90	1.85	1.83
20.3	17.5	20.9	20.8	18.2	20.3	21.4	18.9	16.9		1.93	1.65	2.12	2.12	2.05	1.75	1.98	2.09	1.98	1.93
18.6	17.6	16.3	18.5	16.7	18.5	21.8	19.3	3.95	17.3		1.62	1.93	1.85	1.62	1.69	1.59	1.88	1.76	1.75
15.6	16.3	17.5	18.5	17.5	17.9	20.2	17.8	12.0	13.2	13.0		2.02	2.03	1.71	1.67	1.77	1.97	1.69	1.52

18.1	19.5	20.3	20.2	19.5	20.5	21.5	20.9	15.4	18.6	15.9	16.7		2.03	2.04	2.08	1.87	1.95	1.66	1.94
18.4	18.7	21.9	22.7	21.6	22.5	21.7	19.4	17.0	18.4	15.4	16.4	16.2		2.19	2.19	1.88	2.08	1.92	2.17
18.7	20.1	17.1	18.7	17.5	18.4	19.9	19.8	12.1	19.1	12.6	13.1	16.9	19.4		1.86	1.66	1.96	1.87	1.88
20.3	19.7	19.0	19.0	17.1	18.6	20.4	17.9	15.1	14.5	15.3	14.9	18.8	20.3	15.4		1.87	1.84	1.96	1.70
20.8	18.1	16.0	18.9	18.2	20.0	21.2	21.6	13.3	17.6	12.7	13.8	13.6	14.5	12.2	16.5		1.90	1.88	1.86
19.9	18.4	17.3	18.6	18.8	18.6	22.2	18.9	16.1	18.3	16.6	17.6	17.0	17.9	16.3	16.2	15.9		1.97	1.95
16.5	16.7	17.3	19.5	18.3	19.2	21.4	18.2	13.8	16.1	13.8	13.0	12.1	14.7	15.0	16.9	14.6	16.1		1.78
18.1	17.7	16.6	18.1	19.0	14.8	22.8	18.0	14.6	16.0	14.5	11.5	16.2	19.5	15.0	13.9	15.4	16.2	14.1	
18.2	18.6	15.4	19.6	16.9	18.0	24.6	19.8	10.3	17.1	10.1	10.5	14.3	16.5	12.7	15.7	14.7	17.9	12.1	11.0
16.5	20.2	20.0	17.4	18.4	18.2	21.3	19.3	12.5	19.4	13.5	14.5	16.6	18.8	12.3	16.7	17.5	17.3	15.2	14.8
18.4	17.3	17.9	18.4	18.4	17.3	22.6	17.3	16.6	17.3	16.7	16.9	17.7	20.2	15.2	15.7	16.7	11.4	15.6	13.7
19.3	19.6	18.2	19.7	16.7	17.4	23.8	20.2	10.5	17.2	11.1	12.6	17.9	17.9	13.5	15.2	14.9	16.7	14.0	12.2
20.1	20.4	21.5	21.4	19.7	20.4	21.1	20.9	18.0	17.6	18.7	18.4	20.4	20.8	16.2	15.9	18.9	15.0	18.1	16.7
18.1	18.3	17.8	17.5	18.1	17.2	21.1	18.3	12.7	18.1	13.2	14.1	12.1	18.5	11.6	16.7	12.2	17.2	13.1	14.5
18.2	19.9	18.5	20.3	18.7	19.9	22.6	19.6	12.3	20.0	12.8	14.6	18.6	19.1	13.7	17.1	17.7	17.2	14.0	14.9
18.3	18.3	19.4	17.8	20.3	16.8	21.2	16.8	12.1	18.9	12.2	13.9	12.8	14.0	13.7	17.6	13.9	15.5	10.6	15.5
17.5	19.1	16.7	17.8	18.8	18.0	20.2	19.0	13.0	16.8	13.2	12.5	16.4	18.6	13.3	15.5	15.8	14.9	13.1	14.3
16.2	16.8	18.7	20.2	17.7	21.2	19.1	17.3	12.4	18.1	11.2	12.8	14.9	16.1	14.1	17.7	13.6	18.4	11.3	15.3
18.2	18.9	18.6	17.1	16.8	18.4	23.4	18.5	14.4	13.9	13.7	13.3	16.8	19.7	15.0	12.5	16.5	17.8	15.8	11.3
21.3	19.9	15.5	19.2	17.2	19.6	23.1	20.7	11.8	18.8	10.7	14.1	14.3	15.0	11.6	15.6	7.80	15.4	13.9	16.4
21.7	21.8	22.0	21.1	17.8	20.6	19.9	20.3	14.6	17.8	13.6	15.4	17.8	18.6	13.9	18.3	15.3	19.2	16.5	16.8
19.0	19.0	19.4	17.8	15.9	16.7	18.6	17.6	12.3	16.9	12.3	13.6	16.4	19.3	12.5	15.3	15.9	16.7	13.9	13.0
19.4	19.3	18.8	17.1	16.2	19.4	24.6	18.9	14.5	15.0	13.7	13.9	17.2	20.8	15.2	13.3	16.5	18.4	16.1	12.0
19.4	18.3	15.4	19.4	19.1	18.0	22.2	18.4	11.5	17.0	11.0	14.3	15.5	15.9	14.3	14.8	12.7	14.7	11.8	12.6
18.0	19.2	19.2	18.0	17.8	17.8	21.2	17.4	11.8	17.6	11.7	14.4	13.6	16.9	13.8	17.5	15.5	17.3	13.1	15.8
19.5	21.3	19.8	22.3	19.4	22.0	23.2	22.5	14.8	18.0	15.5	16.5	17.2	16.7	16.5	18.7	16.7	18.2	16.5	17.0

18.3	20.3	18.9	20.2	19.8	20.0	23.5	19.6	14.9	18.0	14.1	17.5	14.1	17.8	16.7	17.8	16.3	19.5	13.0	16.3
16.2	17.2	16.2	16.3	16.1	14.8	20.9	18.2	11.4	12.7	11.7	10.8	12.3	16.7	11.1	13.1	11.8	12.3	11.5	11.7
18.2	19.5	14.6	19.3	17.0	18.1	24.6	20.8	10.6	17.3	10.9	11.3	13.9	18.0	12.3	16.1	14.9	17.9	12.2	10.9
19.0	18.5	22.5	18.6	20.1	18.2	20.5	19.3	16.4	17.0	17.1	16.2	16.7	17.5	18.3	17.6	17.4	15.2	17.8	17.1
22.9	22.6	21.5	21.6	22.2	22.5	24.4	22.7	20.8	22.6	19.9	19.7	22.9	21.9	20.6	22.0	19.2	21.9	21.4	21.3
22.2	24.5	23.8	24.3	23.5	23.6	23.2	23.3	19.9	22.7	19.6	21.0	21.2	22.5	19.6	24.5	21.2	25.1	19.7	20.4
23.5	21.9	24.0	24.5	24.7	24.0	26.0	24.4	21.3	22.7	21.7	23.6	20.8	24.2	23.9	25.2	21.6	22.3	22.5	24.0
15.5	15.5	18.3	18.4	15.2	19.7	18.0	15.1	12.7	17.1	13.4	14.4	16.8	16.4	15.6	15.4	16.1	16.2	15.7	14.4
20.0	19.0	20.9	23.1	21.3	21.9	23.8	19.9	19.2	16.0	20.5	17.8	18.8	20.5	21.4	18.2	19.0	18.5	16.1	19.6
19.8	20.0	22.7	20.4	22.8	20.8	22.1	21.4	19.0	20.0	18.6	16.0	19.5	19.6	16.8	18.4	17.3	19.2	19.1	17.3
20.6	18.9	23.7	20.9	21.3	21.2	22.3	20.7	17.1	19.7	17.7	18.6	17.9	19.4	21.4	18.3	18.7	19.4	15.9	19.2
21.1	18.8	22.3	21.8	20.6	21.8	24.7	18.5	18.8	21.6	19.7	21.0	17.8	18.8	20.9	19.3	18.3	19.5	18.8	19.0
21.7	19.9	21.6	20.9	19.8	20.6	23.2	22.2	17.2	21.0	18.1	17.4	20.0	22.7	18.8	19.8	18.8	18.6	17.5	17.7
22.3	21.9	21.1	23.8	21.5	22.2	24.9	20.8	18.5	21.6	18.1	19.0	24.3	23.0	19.8	19.5	20.5	22.1	19.9	19.7
23.7	22.7	25.5	22.4	23.5	22.2	23.7	19.9	21.1	23.1	21.0	20.5	23.4	23.0	21.7	20.3	24.6	20.9	22.5	20.4
21.0	23.1	23.4	22.0	22.6	24.0	24.7	20.9	22.5	23.5	21.9	19.9	24.6	24.5	22.7	20.8	23.3	20.1	23.1	19.6
22.7	21.8	22.7	24.0	21.0	23.2	25.6	21.1	19.0	20.7	18.4	20.0	21.4	23.3	18.8	20.7	19.0	19.8	18.7	19.6
22.3	21.9	21.1	23.8	21.5	22.2	24.8	20.7	18.5	21.6	18.1	19.0	24.3	23.0	19.8	19.5	20.5	22.1	19.9	19.7
23.4	24.4	23.8	24.2	24.5	25.5	26.1	21.3	22.4	25.3	22.2	20.3	24.5	25.8	22.0	20.5	25.7	21.2	22.1	20.5
23.2	21.9	22.7	23.1	23.4	23.9	25.7	19.5	20.9	24.2	21.2	22.3	24.6	26.1	22.9	20.4	24.3	21.5	22.9	21.6
20.7	23.2	24.5	23.5	25.6	25.5	23.9	21.1	22.4	23.3	22.2	22.6	22.1	23.7	22.2	20.3	24.2	22.2	22.0	23.6
22.2	21.1	27.1	24.9	26.0	26.6	24.0	21.6	25.2	24.5	24.8	23.9	25.2	25.8	24.1	21.2	24.8	23.5	24.2	22.4
21.8	21.2	23.0	24.2	23.2	23.6	24.5	19.0	21.0	22.8	20.9	19.9	22.3	22.3	23.6	20.6	22.2	20.1	21.4	19.7
21.2	22.3	24.2	26.1	24.9	26.0	23.1	20.3	22.5	21.1	23.0	21.1	26.6	24.8	21.6	18.0	25.7	22.3	23.3	21.5
20.7	22.2	23.0	23.2	21.8	21.6	22.1	18.8	20.1	22.4	19.8	20.9	22.8	23.4	20.6	20.3	23.6	20.6	21.2	19.9
25.3	27.1	23.2	23.8	25.4	23.5	27.4	22.0	23.2	23.0	23.2	23.3	21.2	23.9	23.2	20.8	22.8	20.5	21.7	21.5

23.8	27.2	26.5	24.0	24.8	22.9	25.0	23.1	21.1	25.9	20.3	21.8	25.7	23.9	20.6	22.8	24.3	24.3	23.5	20.3
23.3	27.9	25.7	25.9	27.4	25.3	25.6	21.7	24.6	26.2	24.4	23.4	25.5	23.5	24.6	21.3	24.5	23.6	23.1	21.7
24.2	22.5	21.0	23.5	22.7	23.2	25.2	20.8	18.5	19.1	18.1	19.5	23.3	24.2	20.3	20.8	20.8	18.9	20.8	21.3
19.4	19.4	21.2	19.2	20.9	20.0	22.0	18.4	18.8	19.8	18.3	16.8	16.8	18.5	19.2	20.2	19.0	18.3	15.4	14.9
19.2	16.3	17.4	20.2	18.3	21.1	21.3	19.0	16.2	16.8	15.7	17.9	19.4	16.0	19.0	18.6	17.5	16.0	13.8	15.5
22.3	22.6	21.0	21.9	20.3	21.1	22.4	21.8	18.3	22.2	18.6	19.8	20.9	21.6	17.6	20.4	18.1	21.2	17.0	19.2
15.9	18.9	15.0	17.3	17.2	18.3	20.5	17.2	14.1	15.9	13.3	13.3	17.3	18.7	16.4	14.6	16.3	16.3	16.2	14.4
18.0	21.0	18.5	17.6	17.8	16.5	18.7	18.3	14.1	19.1	14.6	15.5	18.4	21.2	14.5	15.9	17.8	15.4	17.4	14.6
16.4	19.8	17.7	17.5	17.2	17.8	19.2	18.8	12.6	18.4	13.8	14.1	17.2	21.7	14.3	18.3	16.4	18.2	15.9	14.0
18.8	19.9	15.8	19.1	17.8	19.0	22.6	22.0	15.2	19.5	15.4	15.0	16.0	19.9	16.0	17.6	16.1	16.9	17.5	16.5
21.6	23.7	18.7	21.2	21.4	21.4	22.3	20.2	18.9	22.9	19.0	19.6	20.4	22.7	20.2	19.5	21.2	19.8	20.8	18.0
18.0	22.4	19.6	19.0	19.0	21.2	20.9	19.3	14.4	19.7	15.0	17.2	19.0	22.2	14.0	16.0	17.4	17.6	18.2	16.9
17.3	19.8	17.7	17.5	17.7	17.8	19.6	19.5	12.9	18.2	13.4	14.1	17.3	21.5	14.8	17.7	16.2	18.4	16.5	13.2
19.2	20.7	18.8	18.9	20.2	18.6	21.3	19.6	15.6	21.4	15.5	16.5	20.7	22.6	14.3	17.1	18.3	20.2	18.1	15.9
14.7	18.9	20.5	18.4	17.5	18.3	20.8	19.8	16.0	18.2	15.9	14.4	17.3	21.7	15.4	17.4	17.0	17.5	15.9	16.0
20.0	18.8	23.2	21.4	20.2	22.0	21.9	22.0	18.6	20.8	19.7	19.2	21.0	22.4	19.0	20.5	22.3	22.3	20.9	20.8

58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77
2.23	2.07	2.21	2.35	2.19	2.46	2.43	2.10	2.36	2.14	2.18	2.32	2.24	2.21	2.27	2.24	2.21	2.20	2.61	2.23
2.35	2.09	2.12	2.43	2.39	2.28	2.31	2.09	2.21	2.36	2.25	2.37	2.40	2.26	2.31	2.49	2.14	2.30	2.67	2.15
2.27	2.08	2.23	2.37	2.19	2.49	2.41	2.17	2.35	2.15	2.18	2.34	2.28	2.19	2.27	2.25	2.24	2.20	2.62	2.25
1.99	1.92	1.99	1.96	2.12	2.15	2.06	2.00	1.96	2.09	1.96	2.16	2.07	2.02	2.03	2.02	2.05	2.08	2.40	1.90
2.22	2.06	2.26	2.22	2.30	2.36	2.35	2.15	2.24	2.35	2.23	2.44	2.38	2.28	2.31	2.27	2.21	2.22	2.68	2.16
2.12	2.03	2.18	2.30	2.15	2.35	2.39	2.01	2.33	2.09	2.12	2.22	2.18	2.12	2.19	2.19	2.11	2.15	2.50	2.17
2.20	2.14	2.28	2.19	2.39	2.30	2.33	2.18	2.21	2.35	2.19	2.41	2.27	2.19	2.29	2.34	2.26	2.25	2.65	2.14
2.44	2.12	2.31	2.37	2.40	2.32	2.44	2.33	2.46	2.52	2.20	2.41	2.46	2.24	2.28	2.39	2.43	2.23	2.69	2.23

2.15	1.95	2.03	2.23	2.26	2.26	2.20	2.14	2.09	2.23	2.05	2.38	2.27	2.15	2.19	2.29	2.19	2.05	2.40	1.95
2.39	2.35	2.32	2.39	2.38	2.45	2.45	2.31	2.42	2.49	2.40	2.50	2.43	2.34	2.47	2.43	2.31	2.37	2.75	2.31
2.24	2.05	2.23	2.31	2.24	2.30	2.42	2.17	2.31	2.17	2.21	2.35	2.41	2.17	2.31	2.30	2.23	2.32	2.56	2.14
2.25	2.12	2.29	2.27	2.38	2.33	2.34	2.26	2.22	2.40	2.21	2.44	2.34	2.22	2.29	2.33	2.34	2.34	2.73	2.17
2.18	2.02	2.19	2.08	2.31	2.21	2.38	2.05	2.15	2.29	1.97	2.08	2.17	2.19	2.08	2.26	2.00	2.24	2.15	2.07
1.82	1.83	1.84	1.82	2.23	1.88	1.81	1.91	1.75	1.86	1.80	1.94	2.05	1.81	1.91	1.70	1.87	1.93	2.12	1.73
1.86	1.88	1.98	1.93	2.18	1.87	1.90	2.10	1.73	1.92	1.67	2.02	2.03	1.82	1.76	1.96	2.03	2.06	2.18	1.67
1.98	1.99	2.14	1.93	2.38	2.08	2.02	2.07	1.75	1.99	1.82	2.02	1.90	1.95	1.93	2.01	2.01	2.05	2.45	1.81
1.95	2.10	1.98	1.97	2.37	1.99	1.97	2.19	1.92	1.87	1.76	1.99	2.01	1.96	1.81	1.94	1.95	2.04	2.25	1.94
2.33	2.28	2.31	2.47	2.49	2.29	2.25	2.34	2.13	2.32	2.37	2.36	2.56	2.27	2.53	2.25	2.44	2.29	2.61	2.24
1.81	1.99	1.92	1.92	2.27	1.87	1.86	1.79	1.69	1.79	1.97	1.74	2.16	2.02	2.09	1.79	1.92	2.01	1.98	1.62
2.13	2.09	2.05	2.28	2.33	2.11	2.25	2.11	2.15	2.10	2.08	2.04	2.18	2.09	2.16	2.26	2.04	2.25	2.29	2.07
2.03	2.04	1.97	2.09	2.45	1.98	2.19	2.11	2.10	2.10	2.11	2.10	2.32	2.21	2.16	2.13	1.99	2.03	2.15	1.97
2.32	2.40	2.31	2.35	2.44	2.08	2.53	2.39	2.19	2.56	2.35	2.33	2.46	2.41	2.45	2.32	2.43	2.48	2.34	2.10
2.21	1.91	1.97	1.97	2.09	1.95	2.19	2.11	1.93	1.99	1.83	2.15	2.16	2.06	1.94	2.15	2.07	2.06	2.32	1.78
1.95	2.05	2.01	1.95	2.23	2.05	2.05	2.15	1.97	1.95	1.88	2.04	2.14	1.95	1.92	2.01	2.25	2.33	2.44	2.03
2.18	1.88	1.92	2.13	2.12	2.06	2.10	2.06	1.91	1.98	1.81	2.10	2.19	2.04	1.91	2.03	2.09	2.08	2.26	1.86
2.11	1.99	2.18	2.22	2.39	2.03	2.16	2.19	2.02	2.07	2.01	2.22	2.29	2.12	2.08	2.24	2.21	2.15	2.41	1.94
2.31	1.99	2.05	2.05	2.34	2.03	2.08	2.06	1.89	2.04	1.96	2.16	2.30	2.18	2.08	2.11	2.06	1.97	2.32	1.98
1.98	1.91	1.92	1.97	2.12	2.11	2.10	1.97	1.93	1.97	1.77	2.02	1.98	2.00	1.81	2.08	1.96	2.34	2.18	1.96
2.18	1.99	2.10	1.90	2.27	2.03	2.07	2.16	2.03	2.17	1.99	2.14	2.12	2.03	2.08	2.03	2.12	2.26	2.12	1.98
2.26	2.20	2.18	2.10	2.39	2.27	2.29	2.00	2.05	2.23	2.23	2.25	2.23	2.24	2.35	1.98	2.17	2.08	2.37	2.00
2.01	2.04	2.17	2.18	2.27	2.14	2.29	2.20	2.15	2.27	2.17	2.27	2.43	2.10	2.25	2.14	2.21	2.01	2.37	2.10
2.12	2.04	2.26	2.29	2.43	2.26	2.25	2.28	2.08	2.30	2.15	2.24	2.57	2.32	2.26	2.22	2.35	2.04	2.24	2.17
2.23	2.23	2.36	2.26	2.50	2.37	2.30	2.31	2.21	2.27	2.53	2.51	2.47	2.44	2.56	2.21	2.32	2.18	2.28	2.32
2.12	1.99	2.15	2.11	2.39	2.04	2.30	2.18	1.97	2.26	2.08	2.17	2.30	2.18	2.16	2.07	2.18	1.93	2.24	2.04

2.15	2.19	2.28	2.20	2.34	2.10	2.28	2.25	2.15	2.25	2.18	2.18	2.32	2.17	2.23	2.15	2.18	2.17	2.35	2.15
2.26	2.35	2.29	2.47	2.49	2.17	2.38	2.36	2.24	2.26	2.20	2.38	2.65	2.27	2.22	2.26	2.25	2.18	2.48	2.29
2.02	1.95	2.09	2.03	2.38	2.19	2.25	2.08	2.08	2.13	2.00	2.03	2.11	1.94	2.09	1.99	2.25	2.03	2.50	1.92
2.08	1.89	2.00	2.08	2.21	2.07	2.03	2.10	1.84	1.90	1.97	2.25	2.32	2.11	2.12	2.12	2.02	2.10	2.05	1.89
2.08	2.20	1.93	2.06	2.29	2.13	2.15	2.02	1.93	1.95	2.03	2.11	2.36	2.19	2.12	1.99	2.07	2.18	2.24	1.96
1.87	2.14	1.96	2.11	2.38	2.03	2.03	2.21	1.88	2.12	2.04	1.93	2.39	2.23	2.08	1.93	2.14	2.17	2.15	1.95
2.24	1.91	2.07	2.27	2.42	2.02	2.21	2.08	1.99	2.29	1.94	2.13	2.29	2.05	2.00	2.20	2.09	2.43	2.29	2.04
1.93	2.01	2.06	1.97	2.30	2.10	2.08	2.23	1.98	2.07	1.86	1.97	2.07	1.94	1.86	2.14	2.01	2.09	2.17	1.95
2.07	1.97	1.95	2.04	2.43	1.99	2.17	1.98	2.00	2.30	2.10	2.17	2.27	2.05	2.22	2.07	2.07	2.35	2.23	1.84
2.50	2.16	2.27	2.31	2.31	2.23	2.38	2.31	2.04	2.14	2.25	2.31	2.09	2.09	2.39	2.34	2.25	2.34	2.41	2.23
2.23	2.08	1.98	2.15	2.34	2.17	2.16	2.01	2.03	2.06	2.02	2.22	2.19	2.14	2.08	2.18	2.06	2.38	2.24	2.07
1.53	1.59	1.87	1.47	2.07	1.70	1.61	1.70	1.59	1.63	1.72	1.61	1.85	1.67	1.79	1.61	1.57	1.76	1.89	1.64
1.93	2.05	1.99	2.01	2.04	2.06	2.12	2.16	1.85	2.07	1.67	2.06	2.03	1.97	1.82	2.01	2.00	1.92	2.09	1.59
1.43	1.60	1.85	1.46	2.07	1.64	1.58	1.67	1.55	1.50	1.61	1.47	1.73	1.61	1.67	1.50	1.50	1.79	1.75	1.56
1.50	1.73	1.89	1.65	2.09	1.80	1.69	1.83	1.55	1.68	1.62	1.80	1.90	1.72	1.74	1.73	1.80	1.87	2.09	1.50
1.87	1.91	2.00	2.12	2.26	1.67	2.17	1.74	1.84	1.91	1.90	1.86	2.11	2.04	1.98	1.97	1.83	2.05	1.88	1.72
1.97	2.06	2.13	2.07	2.30	2.14	2.17	1.82	2.06	1.99	2.14	1.91	2.17	2.25	2.26	2.01	2.06	1.98	2.15	1.99
1.71	1.60	1.89	1.79	1.98	1.59	1.68	1.83	1.60	1.86	1.79	1.64	1.76	1.64	1.85	1.83	1.82	1.93	2.04	1.61
1.80	1.82	1.73	1.79	1.89	1.94	1.87	2.01	1.70	2.04	1.51	1.80	2.02	1.80	1.64	1.72	2.01	1.99	2.06	1.65
1.88	2.03	1.90	1.83	2.14	1.61	2.05	1.87	1.78	1.75	1.85	1.29	1.91	2.00	1.93	1.70	1.91	1.91	1.98	1.62
2.05	1.92	1.47	2.01	1.92	1.96	1.97	1.94	1.69	2.11	1.96	1.85	2.19	1.97	2.06	1.84	2.02	2.05	2.16	1.60
1.62	1.84	1.86	1.78	2.12	1.72	1.79	1.55	1.61	1.53	1.86	1.85	2.04	1.81	1.96	1.65	1.77	1.97	1.82	1.64
1.55	1.76	1.69	1.67	2.03	1.86	1.78	1.95	1.72	1.87	1.56	1.94	2.00	1.74	1.68	1.62	1.94	2.01	2.04	1.63
	1.69	1.89	1.77	2.13	1.68	1.69	1.54	1.67	1.72	1.69	1.74	1.88	1.75	1.76	1.43	1.54	1.84	1.89	1.54
13.90		1.77	1.62	1.80	1.80	1.70	1.73	1.65	1.76	1.73	1.78	1.87	1.65	1.79	1.71	1.82	1.99	1.92	1.70
16.31	16.32		1.90	1.78	2.01	1.76	1.90	1.73	1.89	1.72	1.86	2.10	1.85	1.77	1.79	1.84	2.10	2.14	1.60

13.25	12.24	16.01		2.06	1.86	1.75	1.89	1.73	1.80	1.71	1.83	1.96	1.83	1.78	1.69	1.78	2.06	2.01	1.59
18.32	16.02	14.51	17.95		2.21	2.12	2.22	2.00	2.05	1.98	2.17	2.18	1.96	2.05	1.86	2.01	2.24	2.19	1.86
12.32	15.02	16.93	15.15	18.97		1.88	1.75	1.69	1.88	1.76	1.65	2.01	1.73	1.79	1.72	1.70	1.97	1.85	1.32
13.31	14.34	15.15	14.89	19.40	15.19		1.89	1.67	1.81	1.91	1.90	2.15	1.72	1.97	1.79	1.79	2.17	1.99	1.74

11.0	14.5	16.2	14.6	19.5	12.6	15.4		1.60	1.74	1.96	1.78	1.76	1.78	2.04	1.64	1.51	1.93	1.96	1.71
14.0	15.5	15.9	14.7	18.2	14.2	15.2	13.1		1.72	1.65	1.73	1.77	1.75	1.73	1.59	1.80	1.90	1.81	1.50
13.4	15.0	16.1	14.8	17.8	14.6	14.8	12.7	14.2		2.00	1.97	1.92	1.76	2.07	1.75	1.70	2.04	1.90	1.77
13.8	15.4	14.5	14.0	16.7	14.5	17.2	16.7	14.5	17.4		1.75	1.81	1.67	0.46	1.79	1.83	1.97	1.95	1.51
12.9	15.2	15.7	14.4	19.0	12.4	16.4	12.9	14.4	15.6	15.6		1.70	1.80	1.84	1.78	1.84	1.89	1.89	1.61
15.0	16.7	18.4	16.2	19.6	17.3	19.1	13.4	15.8	15.5	16.5	12.2		1.68	1.90	2.09	1.76	2.07	2.06	1.84
13.2	14.2	15.7	15.1	16.4	13.3	13.3	13.1	15.2	13.6	13.4	13.3	12.0		1.73	1.87	1.79	1.96	2.04	1.75
13.8	15.8	14.3	14.2	16.6	14.3	17.0	17.2	14.9	17.7	2.07	15.5	16.9	13.8		1.90	1.94	2.06	2.06	1.65
9.47	14.1	15.6	13.0	15.8	13.5	14.9	11.7	13.6	13.5	15.4	13.2	17.4	14.3	15.9		1.82	2.04	1.77	1.47
11.2	15.7	15.7	13.3	17.3	12.8	15.1	11.2	15.5	13.5	16.2	14.5	13.5	13.9	16.7	13.9		1.97	1.88	1.66
15.6	18.6	19.6	18.0	21.3	16.7	19.8	16.1	17.9	18.1	18.4	15.9	18.4	17.8	18.9	18.1	17.1		2.15	2.01
14.1	16.7	18.3	16.4	19.3	14.6	17.1	15.6	14.9	14.8	16.3	15.1	17.2	17.1	17.1	13.2	15.0	19.0		1.75
10.4	12.5	12.5	11.2	14.8	8.59	13.5	11.9	12.0	13.3	11.8	11.2	14.5	13.0	12.6	10.1	11.9	16.9	12.9	
1.88	14.5	17.0	13.8	17.7	11.4	12.8	11.6	13.7	14.0	14.6	13.5	15.0	13.2	14.6	9.78	11.3	15.5	13.1	9.81
19.6	18.3	17.3	17.2	17.3	18.6	20.8	17.8	17.1	18.9	17.0	18.9	21.9	19.5	17.3	18.4	19.1	18.8	19.2	14.7
20.7	22.4	21.5	21.3	21.5	20.4	20.2	19.8	19.9	19.2	21.7	20.7	21.9	21.2	21.3	22.1	20.2	21.5	22.7	20.0
19.6	23.5	21.6	22.1	23.4	20.2	23.0	18.8	20.9	18.5	20.9	19.1	20.0	19.7	21.0	21.7	20.0	20.5	20.9	19.8
22.6	24.5	21.9	22.1	23.8	23.5	23.2	23.3	22.3	22.9	24.1	23.6	23.9	23.8	24.1	23.6	22.0	24.0	23.9	22.3
13.2	13.5	15.2	15.1	16.0	14.5	15.5	14.5	16.1	13.5	15.4	16.7	15.9	12.7	15.8	14.3	15.8	18.8	17.1	12.4
17.5	21.0	20.1	20.1	20.8	18.1	19.1	17.5	18.6	18.8	19.3	20.1	21.3	19.2	19.6	18.4	18.2	20.2	20.6	16.5
18.5	20.4	20.4	21.1	21.1	18.6	18.1	16.3	17.6	17.6	19.9	17.7	19.3	17.1	20.6	18.1	20.1	17.9	21.8	17.8

18.0	21.0	21.5	19.6	21.7	17.6	18.0	17.4	18.0	19.5	19.4	19.5	20.1	19.2	19.6	17.7	18.5	21.2	18.8	17.1
20.0	20.6	21.9	18.7	23.4	17.5	20.7	19.3	20.4	19.7	20.3	17.8	22.4	21.7	20.8	18.1	19.5	19.8	18.8	17.5
18.4	20.6	19.6	17.3	20.7	17.9	19.7	17.2	16.9	18.4	17.9	18.1	18.5	18.1	17.9	20.3	17.2	17.9	23.1	16.0
17.6	21.0	19.3	18.6	22.2	22.5	21.7	20.6	19.1	20.4	18.9	20.8	23.0	20.8	19.0	17.1	21.0	21.3	22.8	19.3
21.1	19.3	21.4	19.1	21.8	21.9	23.6	20.1	20.1	21.9	18.4	22.7	20.9	20.4	18.1	22.5	21.5	23.8	20.2	18.8
19.5	22.5	20.2	20.9	20.9	22.8	21.6	23.2	19.1	21.0	20.3	24.1	24.5	22.2	20.4	21.8	21.5	23.1	22.8	21.3
18.6	20.7	20.3	16.2	20.1	20.8	21.9	19.8	19.2	19.2	19.8	20.9	22.2	21.9	19.3	20.1	20.4	22.0	20.9	17.6
17.6	21.0	19.3	18.6	22.2	22.5	21.7	20.6	19.1	20.4	18.9	20.8	23.0	20.8	19.0	17.1	21.0	21.3	22.8	19.3
21.6	23.1	22.1	21.1	22.5	23.0	20.8	22.2	17.5	22.4	21.1	23.2	24.8	22.8	21.2	20.8	21.8	23.6	22.8	21.1
20.7	21.6	19.1	22.0	22.8	21.1	21.5	20.4	19.5	20.3	21.2	23.0	23.2	21.7	20.8	19.9	21.5	23.4	23.1	21.1
22.0	20.7	22.1	22.1	21.9	22.8	23.6	22.6	22.3	22.6	19.9	23.7	25.5	22.5	20.4	21.9	23.5	25.0	22.0	20.2
24.9	23.2	22.8	24.9	23.5	23.2	23.7	25.8	23.2	24.0	20.8	26.1	26.6	24.3	21.1	25.5	24.9	25.6	24.4	22.9
20.2	21.9	20.2	19.5	22.6	21.2	21.3	20.2	19.9	19.6	20.7	22.4	25.4	21.1	21.0	18.6	22.4	24.4	21.0	19.3
22.2	21.3	19.9	21.9	22.0	23.8	20.8	24.3	21.1	23.6	19.8	24.1	25.4	22.6	19.8	23.1	24.6	23.6	22.8	20.9
20.9	19.5	19.8	20.8	22.4	21.8	22.0	20.8	19.5	20.6	19.0	21.8	20.8	20.6	18.4	21.5	20.6	23.7	19.6	19.3
20.7	20.3	21.1	23.1	20.3	24.0	23.5	20.4	20.2	22.1	20.5	21.8	21.9	23.1	20.3	20.3	22.0	24.4	20.2	21.3
20.2	23.5	27.3	22.2	24.9	21.7	23.1	22.5	22.2	23.6	22.3	22.6	24.5	19.4	23.0	22.1	22.1	22.8	22.6	23.0
21.9	23.3	23.9	21.4	25.4	24.2	22.9	24.4	21.4	24.9	20.6	23.5	25.9	22.6	21.2	22.9	25.0	24.0	23.2	22.7
18.8	21.1	21.9	20.4	20.6	23.6	21.5	21.9	19.9	20.2	20.7	21.1	22.5	21.3	20.7	17.7	22.8	22.6	21.1	18.0
18.3	19.4	14.9	17.6	19.7	16.9	18.5	17.8	19.3	16.1	18.3	19.1	17.7	17.8	18.5	20.6	16.6	19.3	18.6	16.8
17.7	18.2	14.0	16.8	17.5	16.2	16.8	18.7	18.5	15.4	16.4	18.3	17.1	18.4	16.3	17.2	18.4	18.8	17.1	15.1
19.5	21.6	19.1	20.8	22.8	20.3	21.7	19.7	18.2	19.1	18.9	20.3	22.5	20.5	19.0	18.8	20.7	22.0	21.4	18.8
13.9	14.9	15.5	14.9	16.8	16.6	16.6	17.1	14.7	15.7	12.6	16.1	17.6	14.8	13.1	14.4	16.9	17.4	17.4	11.7
18.3	14.4	15.1	14.5	17.5	16.5	18.1	15.4	17.3	17.8	15.2	16.0	16.7	14.7	15.6	17.1	16.9	19.8	21.3	13.7
13.4	15.6	16.6	15.6	15.0	17.0	16.7	16.5	16.8	15.0	15.3	15.2	16.1	13.0	15.5	15.4	14.3	18.9	18.8	13.9
15.5	16.7	15.9	16.1	20.3	16.2	18.6	17.6	16.0	17.8	16.1	17.1	20.3	18.2	16.3	15.7	16.9	17.6	19.0	13.8

19.2	20.0	21.2	20.4	21.6	19.9	20.4	20.9	20.3	20.5	17.9	20.9	19.8	17.5	18.0	21.0	19.5	21.5	24.6	19.5
15.2	18.0	17.1	17.5	19.5	15.3	17.2	17.7	16.1	17.8	16.6	17.9	20.6	18.2	16.6	16.1	18.6	18.4	19.0	14.4
13.1	15.8	16.6	15.4	16.2	16.8	17.4	16.6	16.9	15.5	15.4	15.1	15.7	13.6	15.6	15.3	14.3	18.1	18.6	13.6
17.3	16.8	17.9	16.5	20.2	15.3	17.5	17.3	16.8	18.2	15.4	18.4	18.1	15.6	15.5	17.6	19.0	22.3	21.1	15.3
17.0	15.0	17.8	14.6	18.8	16.3	17.3	17.8	16.5	16.6	15.3	17.3	19.0	15.5	15.7	18.4	17.9	19.6	19.1	13.1
18.9	19.2	19.9	19.8	23.4	20.3	19.6	20.2	21.1	18.4	21.3	21.5	21.7	19.5	21.6	21.3	18.9	22.8	21.8	20.1

78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97
2.27	2.31	2.47	2.37	2.43	1.99	2.41	2.21	2.25	2.61	2.18	2.45	2.45	2.48	2.42	2.45	2.60	2.43	2.51	2.68
2.36	2.44	2.50	2.35	2.40	2.08	2.39	2.28	2.37	2.58	2.15	2.41	2.30	2.58	2.75	2.41	2.49	2.43	2.61	2.77
2.31	2.35	2.52	2.38	2.45	1.99	2.46	2.23	2.28	2.66	2.21	2.47	2.46	2.50	2.45	2.47	2.62	2.45	2.56	2.71
2.01	2.13	2.14	2.12	2.11	1.80	2.15	2.09	2.17	2.31	2.01	2.22	2.11	2.24	2.36	2.22	2.41	2.19	2.22	2.47
2.24	2.32	2.58	2.40	2.50	2.11	2.45	2.23	2.38	2.60	2.23	2.31	2.29	2.56	2.60	2.31	2.51	2.54	2.51	2.85
2.16	2.32	2.39	2.30	2.39	1.94	2.37	2.16	2.23	2.52	2.15	2.34	2.33	2.42	2.46	2.34	2.56	2.38	2.40	2.64
2.23	2.32	2.40	2.28	2.42	2.01	2.46	2.31	2.34	2.58	2.18	2.45	2.32	2.51	2.63	2.45	2.66	2.50	2.51	2.79
2.50	2.31	2.65	2.45	2.67	2.12	2.74	2.32	2.37	2.55	2.23	2.50	2.32	2.59	2.75	2.50	2.74	2.61	2.49	2.75
2.15	2.20	2.55	2.37	2.49	1.89	2.26	2.21	2.21	2.46	2.14	2.23	2.20	2.35	2.57	2.23	2.45	2.32	2.38	2.56
2.45	2.39	2.54	2.36	2.64	2.26	2.46	2.42	2.32	2.67	2.32	2.30	2.43	2.64	2.72	2.30	2.61	2.36	2.53	2.91
2.23	2.26	2.52	2.45	2.40	2.00	2.54	2.29	2.32	2.63	2.27	2.44	2.44	2.49	2.48	2.44	2.67	2.48	2.54	2.72
2.27	2.35	2.42	2.35	2.43	2.05	2.52	2.38	2.42	2.67	2.25	2.44	2.32	2.51	2.71	2.44	2.67	2.50	2.48	2.82
2.22	2.20	2.43	2.24	2.28	2.17	2.40	2.34	2.28	2.44	2.28	2.59	2.46	2.49	2.57	2.59	2.74	2.64	2.43	2.45
1.83	2.10	2.12	2.25	2.21	1.87	2.03	2.04	1.88	2.00	1.89	2.05	2.18	2.04	2.18	2.05	2.21	2.31	2.12	2.38
1.86	2.12	2.09	2.27	2.39	1.82	2.26	2.17	1.95	2.08	2.07	2.20	2.24	2.12	2.28	2.20	2.35	2.36	2.29	2.43
1.94	2.19	2.16	2.35	2.21	2.00	2.46	2.14	2.13	2.20	1.88	2.19	2.10	2.36	2.17	2.18	2.23	2.32	2.27	2.41
1.99	2.37	2.07	2.36	2.27	1.84	2.26	2.31	2.12	2.18	2.17	2.30	2.39	2.13	2.33	2.30	2.50	2.36	2.17	2.44
2.26	2.36	2.40	2.70	2.62	2.24	2.69	2.31	2.26	2.43	2.38	2.67	2.66	2.51	2.69	2.67	2.53	2.62	2.54	2.65

1.75	2.10	2.08	2.29	2.20	1.93	2.03	2.02	1.93	1.89	2.08	2.29	2.48	2.24	2.29	2.29	2.29	2.46	2.34	2.59
2.19	2.20	2.12	2.17	2.31	2.05	2.33	2.37	2.07	2.35	2.24	2.53	2.27	2.36	2.46	2.52	2.61	2.66	2.23	2.46
2.06	2.33	2.21	2.09	2.09	2.09	2.32	2.26	2.17	2.14	2.11	2.50	2.26	2.46	2.39	2.50	2.58	2.47	2.47	2.59
2.32	2.23	2.61	2.62	2.43	2.36	2.44	2.61	2.14	2.32	2.24	2.67	2.52	2.74	2.48	2.67	2.80	2.62	2.52	2.80
2.19	2.07	2.03	2.26	2.15	1.89	2.16	2.26	2.09	2.25	1.99	2.12	1.85	2.12	2.16	2.12	2.19	2.34	2.06	2.19
2.02	2.19	2.22	2.11	2.38	1.98	2.24	1.96	2.10	2.15	2.20	2.37	2.34	2.27	2.37	2.37	2.35	2.40	2.28	2.44
2.20	2.14	2.11	2.30	2.22	1.93	2.38	2.18	2.12	2.25	2.08	2.21	2.16	2.17	2.41	2.21	2.30	2.34	2.21	2.29
2.10	2.26	2.20	2.26	2.33	2.01	2.32	2.38	2.20	2.37	2.11	2.33	2.11	2.34	2.50	2.32	2.46	2.45	2.31	2.32
2.31	2.10	2.08	2.27	2.11	1.99	2.31	2.26	2.06	2.22	2.10	2.20	2.17	2.25	2.30	2.20	2.34	2.34	2.18	2.19
2.02	2.27	2.10	2.34	2.21	1.73	2.29	2.21	2.03	2.39	2.24	2.29	2.11	2.31	2.32	2.29	2.47	2.37	2.24	2.47
2.26	2.09	2.31	2.36	2.25	1.86	2.22	2.19	1.96	1.96	2.05	2.26	2.12	2.24	2.16	2.25	2.29	2.40	2.15	2.34
2.26	2.06	2.22	2.24	2.18	2.15	2.38	2.30	2.10	2.36	2.13	2.30	2.35	2.46	2.22	2.30	2.36	2.18	2.45	2.64
2.10	2.21	2.27	2.48	2.18	2.11	2.35	2.23	2.12	2.28	2.28	2.23	2.30	2.39	2.21	2.23	2.35	2.38	2.37	2.50
2.11	2.20	2.39	2.45	2.31	2.14	2.44	2.45	2.10	2.35	2.15	2.39	2.41	2.51	2.40	2.39	2.33	2.45	2.29	2.49
2.21	2.29	2.41	2.26	2.11	2.36	2.43	2.51	2.15	2.30	2.35	2.56	2.43	2.47	2.35	2.56	2.62	2.44	2.50	2.60
2.15	2.04	2.27	2.39	2.29	2.07	2.32	2.38	2.07	2.27	2.07	2.23	2.22	2.51	2.18	2.23	2.37	2.24	2.28	2.50
2.19	2.28	2.30	2.39	2.27	2.25	2.47	2.35	2.11	2.26	2.18	2.36	2.43	2.57	2.32	2.36	2.58	2.34	2.42	2.55
2.23	2.34	2.58	2.25	2.38	2.25	2.62	2.42	2.25	2.48	2.17	2.48	2.70	2.29	2.23	2.48	2.53	2.56	2.59	2.70
2.07	2.25	2.15	2.08	2.25	1.88	2.40	2.31	2.17	2.35	2.17	2.17	2.18	2.32	2.24	2.17	2.34	2.44	2.40	2.56
2.07	2.09	2.20	2.25	2.21	1.88	2.24	2.22	2.09	2.21	2.23	2.21	2.36	2.16	2.32	2.21	2.35	2.40	2.12	2.31
2.16	2.13	2.18	2.34	2.10	1.90	2.15	2.24	1.89	2.13	2.07	2.26	2.33	2.24	2.25	2.26	2.44	2.39	2.35	2.26
1.83	2.40	2.21	2.45	2.26	2.16	2.20	2.40	2.24	2.33	2.16	2.30	2.59	2.33	2.38	2.30	2.54	2.35	2.44	2.71
2.21	2.12	2.20	2.46	2.32	2.15	2.45	2.26	2.15	2.42	2.26	2.47	2.33	2.25	2.56	2.47	2.50	2.35	2.37	2.52
1.96	2.22	2.22	2.33	2.26	1.91	2.27	2.42	2.06	2.23	2.06	2.29	2.41	2.25	2.25	2.28	2.54	2.47	2.43	2.64
2.12	2.08	2.30	2.48	2.28	2.22	2.33	2.27	2.14	2.37	2.19	2.38	2.42	2.44	2.47	2.38	2.65	2.48	2.51	2.73
2.51	2.21	2.34	2.26	2.37	2.06	2.43	2.35	2.17	2.42	2.28	2.41	2.37	2.33	2.45	2.41	2.55	2.52	2.29	2.40

2.31	2.17	2.22	2.36	2.33	1.81	2.19	2.31	2.09	2.07	2.28	2.18	2.18	2.12	2.16	2.18	2.26	2.20	2.16	2.35
1.58	1.95	2.10	2.07	2.06	1.71	2.13	2.06	1.81	2.14	1.93	2.11	2.23	2.22	2.09	2.10	2.43	2.26	2.27	2.64
1.95	1.97	2.25	2.25	2.14	2.02	1.93	2.18	2.02	2.31	2.09	2.30	2.32	2.25	2.18	2.30	2.48	2.51	2.27	2.43
1.51	1.92	1.99	1.97	2.05	1.72	2.16	2.00	1.80	2.15	1.90	2.03	2.17	2.10	1.97	2.02	2.36	2.24	2.20	2.53
1.57	2.00	2.06	2.16	2.29	1.80	2.05	1.86	1.91	2.29	1.88	2.16	2.20	2.01	2.12	2.16	2.21	2.36	2.23	2.46
1.87	1.96	2.30	2.18	2.03	2.08	2.10	2.16	1.88	2.06	2.16	2.50	2.44	2.46	2.34	2.50	2.58	2.60	2.29	2.55
2.12	2.07	2.17	2.26	2.30	1.99	2.28	2.20	2.06	2.10	2.32	2.42	2.44	2.47	2.42	2.42	2.68	2.63	2.40	2.57
1.67	2.12	2.16	2.04	2.31	1.99	2.33	1.95	2.12	2.27	2.08	2.23	2.33	2.32	2.12	2.23	2.35	2.44	2.31	2.53
1.85	1.96	2.18	2.44	2.31	1.79	2.03	1.97	1.83	2.14	1.99	2.09	2.14	2.13	2.23	2.08	2.20	2.18	2.02	2.25
1.94	1.97	2.04	2.18	2.14	1.95	2.13	1.99	2.02	2.11	2.00	2.23	2.52	2.37	2.11	2.22	2.60	2.55	2.43	2.48
2.07	1.82	2.19	2.47	2.16	1.94	2.09	2.09	1.96	2.17	1.98	2.33	2.29	2.09	2.18	2.33	2.24	2.30	2.26	2.42
1.66	2.08	2.16	2.11	2.16	1.95	1.96	2.11	1.71	2.15	1.94	2.16	2.36	2.24	2.13	2.16	2.34	2.42	2.23	2.45
1.56	2.06	2.16	2.13	2.26	1.80	2.15	1.98	1.99	2.19	1.96	2.23	2.29	2.10	2.22	2.23	2.36	2.36	2.36	2.40
0.59	2.20	2.17	2.04	2.13	1.70	1.96	2.07	1.85	2.20	1.93	2.11	2.28	2.09	2.10	2.11	2.40	2.29	2.27	2.56
1.76	1.99	2.16	2.24	2.20	1.67	2.18	2.16	2.04	2.20	2.15	2.21	2.01	2.17	2.23	2.21	2.35	2.16	2.06	2.38
1.96	1.97	2.11	2.20	2.12	1.77	2.11	2.18	2.09	2.26	2.04	2.08	2.27	2.02	2.21	2.08	2.28	2.15	2.24	2.42
1.86	2.07	2.16	2.22	2.12	1.85	2.18	2.20	1.96	2.10	1.93	2.12	2.14	2.18	1.95	2.12	2.37	2.34	2.22	2.53
2.06	2.10	2.17	2.33	2.24	1.96	2.32	2.27	2.18	2.53	2.16	2.37	2.41	2.25	2.27	2.37	2.42	2.48	2.22	2.52
1.61	2.17	2.13	2.11	2.26	1.89	2.05	2.10	1.86	2.04	1.94	2.40	2.32	2.35	2.30	2.39	2.48	2.35	2.31	2.44
1.66	2.25	2.10	2.27	2.19	1.92	2.14	2.01	1.87	2.24	2.02	2.32	2.39	2.14	2.34	2.32	2.22	2.27	2.37	2.48
1.59	2.08	2.08	2.03	2.18	1.84	2.05	1.94	1.84	2.11	1.95	2.25	2.19	2.29	2.18	2.25	2.40	2.27	2.30	2.63
1.64	1.89	2.07	2.13	2.07	1.80	2.04	1.89	1.80	2.12	1.75	2.02	2.06	1.93	2.01	2.01	1.88	2.03	2.19	2.31
1.77	2.11	1.99	1.97	2.19	1.80	2.19	2.04	2.04	2.25	2.01	2.26	2.36	2.16	2.15	2.26	2.48	2.34	2.30	2.57
1.77	2.00	2.10	2.06	2.23	1.86	2.06	2.17	1.94	2.17	1.90	2.11	2.00	2.08	2.16	2.10	2.30	2.22	2.02	2.24
1.81	2.14	2.12	2.00	2.26	1.99	2.17	1.97	2.00	2.01	1.91	2.28	2.35	2.36	2.25	2.27	2.45	2.47	2.29	2.53
1.89	2.32	2.15	2.02	2.25	1.90	2.32	2.15	2.05	2.32	1.94	2.45	2.15	2.37	2.34	2.45	2.48	2.40	2.32	2.54

1.76	2.18	2.16	2.06	2.25	1.74	2.20	1.95	1.98	2.32	1.96	2.25	2.16	2.21	2.29	2.25	2.41	2.33	2.26	2.52
1.84	2.08	2.11	2.10	2.25	1.94	2.10	2.27	2.00	2.26	1.94	2.21	2.04	2.14	2.18	2.21	2.39	2.25	2.08	2.30
1.46	2.05	2.26	2.25	2.20	1.88	2.05	2.07	1.80	2.07	2.09	1.98	2.30	2.18	2.14	1.98	2.34	2.18	2.22	2.50
1.53	2.18	2.04	2.06	2.08	1.92	2.10	2.17	1.93	2.18	1.92	2.32	2.29	2.16	2.26	2.32	2.34	2.32	2.32	2.49
1.86	2.09	2.20	2.16	2.19	2.10	2.12	1.99	2.06	2.16	1.87	2.23	2.41	2.29	2.24	2.23	2.39	2.42	2.35	2.49
1.80	2.16	2.34	2.19	2.29	2.14	2.24	2.32	1.99	2.09	2.33	2.40	2.18	2.38	2.31	2.40	2.38	2.48	2.28	2.55
1.48	1.80	2.08	2.10	2.15	1.69	1.93	1.99	1.81	2.04	1.80	2.20	2.12	2.20	2.08	2.20	2.31	2.38	2.14	2.43
	2.30	2.18	2.04	2.20	1.72	1.99	2.10	1.85	2.27	1.94	2.17	2.35	2.10	2.15	2.17	2.43	2.34	2.31	2.57
20.6		2.28	2.26	2.21	2.06	2.19	2.22	1.89	2.13	2.21	2.44	2.37	2.23	2.26	2.43	2.62	2.54	2.48	2.62
21.0	23.0		2.22	2.02	1.91	2.47	2.36	2.19	2.38	2.33	2.46	2.31	2.32	2.31	2.46	2.51	2.33	2.36	2.49
19.6	22.1	23.3		2.24	2.27	2.31	2.32	2.18	2.41	2.18	2.52	2.36	2.48	2.33	2.52	2.62	2.43	2.63	2.67
23.1	23.9	22.6	24.9		2.15	2.24	2.49	2.14	2.29	2.34	2.46	2.46	2.33	2.22	2.46	2.38	2.41	2.59	2.53
13.2	17.5	18.8	23.0	22.3		2.08	2.16	1.80	2.14	2.18	2.21	2.30	2.05	2.08	2.21	2.51	2.37	2.11	2.44
17.9	19.9	24.3	24.1	24.4	17.8		2.18	1.51	1.96	1.95	2.54	2.53	2.36	2.54	2.54	2.55	2.55	2.39	2.69
18.5	20.3	23.2	24.1	27.5	18.7	19.3		2.10	2.05	1.84	2.39	2.55	2.34	2.49	2.39	2.43	2.55	2.50	2.41
18.0	18.7	23.9	23.8	24.4	16.8	12.8	20.5		1.79	2.01	2.37	2.35	2.19	2.29	2.37	2.38	2.38	2.31	2.50
21.1	18.3	24.0	25.2	24.9	18.8	15.8	17.7	17.1		2.07	2.51	2.46	2.40	2.52	2.51	2.49	2.51	2.53	2.67
18.3	21.0	25.1	23.1	26.2	20.0	18.0	17.3	20.0	19.0		2.27	2.24	2.23	2.25	2.27	2.24	2.38	2.34	2.47
18.2	23.9	25.1	25.2	26.9	19.6	24.8	23.9	25.8	25.0	23.4		1.76	1.85	1.66	0.01	1.88	1.87	1.95	2.20
21.4	21.9	24.0	23.8	27.1	20.7	25.2	26.0	25.3	24.4	23.2	13.1		1.98	1.88	1.76	1.65	1.79	1.84	2.14
19.6	22.1	24.0	26.7	26.0	19.2	23.6	23.9	24.0	23.8	23.7	16.8	16.8		1.78	1.85	1.88	1.96	1.94	2.13
18.6	21.1	23.8	23.6	23.7	18.3	24.3	25.5	24.0	25.2	22.6	11.5	14.1	14.7		1.66	1.93	2.14	1.95	2.22
18.2	23.9	25.1	25.1	26.9	19.5	24.8	23.9	25.8	25.0	23.4	0.01	13.1	16.8	11.5		1.88	1.87	1.95	2.20
21.5	26.4	26.3	26.2	26.8	24.4	24.9	23.8	25.3	24.3	22.4	14.5	11.5	15.7	14.9	14.5		1.90	2.02	2.11
21.3	24.0	23.5	24.1	26.5	21.4	24.4	25.3	24.7	23.4	23.9	14.8	13.7	17.5	17.3	14.7	15.1		2.19	2.34
22.2	25.5	25.6	29.5	29.7	19.9	23.8	26.5	25.7	26.2	25.6	17.9	17.5	18.8	17.6	17.9	19.3	21.6		1.77

24.9	25.6	26.6	28.6	28.3	22.3	26.7	23.8	26.2	25.9	25.1	20.1	19.6	20.1	20.2	20.1	19.7	21.6	15.0	
20.8	23.1	26.4	24.3	26.1	21.2	23.3	25.4	23.1	24.1	24.0	14.4	12.3	15.2	14.3	14.4	10.0	14.6	18.6	18.9
22.2	24.1	26.1	26.1	27.1	19.0	23.4	25.3	23.6	25.3	24.4	16.1	17.6	16.7	17.1	16.1	17.4	18.5	15.4	10.6
21.2	22.1	26.0	23.8	24.0	19.0	23.4	23.4	23.8	23.3	22.8	15.2	11.0	16.0	14.3	15.2	15.2	15.8	17.5	18.8
21.3	21.6	26.4	28.4	25.3	21.7	24.9	24.4	24.5	23.6	25.1	18.0	14.3	17.6	17.1	18.0	16.4	17.2	20.6	21.6
20.5	22.3	27.6	25.5	29.3	22.0	27.1	23.5	27.2	24.3	24.3	19.0	17.3	14.1	17.5	19.0	17.0	20.0	20.8	22.5
22.2	24.4	29.5	27.3	29.1	22.4	25.2	28.0	24.0	27.5	25.5	17.8	16.5	17.6	17.9	17.8	17.1	21.5	19.7	22.5
18.7	21.9	24.4	23.8	25.6	18.8	23.5	24.0	24.3	26.7	26.6	13.4	14.7	17.9	10.4	13.3	15.8	16.7	16.5	19.8
18.8	19.1	21.9	20.0	22.8	17.1	23.0	20.2	20.9	20.5	21.1	25.6	23.1	24.9	22.9	25.6	26.2	25.5	24.6	24.9
18.5	17.3	21.1	22.8	23.7	15.6	18.3	20.9	19.0	17.3	21.5	23.2	21.8	22.6	21.4	23.2	25.9	21.8	25.2	25.6
20.0	19.3	25.3	22.5	26.9	19.4	24.3	20.7	21.5	20.7	22.1	20.1	22.3	19.6	20.3	20.1	23.4	23.0	26.5	25.9
13.9	17.6	20.3	21.2	24.3	12.4	17.7	18.7	18.5	20.2	17.2	18.7	18.0	18.4	19.4	18.7	20.7	20.2	20.0	23.1
18.5	17.2	21.3	19.2	23.6	15.3	20.2	18.4	20.5	22.1	17.9	22.7	19.9	24.7	23.5	22.7	22.4	21.6	24.0	26.4
13.2	18.9	19.2	18.3	21.4	14.2	21.9	18.3	21.7	19.6	19.2	20.6	21.7	21.0	21.1	20.5	24.2	22.0	25.2	26.0
15.0	19.2	23.5	20.7	23.0	16.2	18.7	19.4	20.5	19.6	16.3	20.9	22.3	21.8	21.9	20.9	22.8	22.9	23.9	24.4
19.1	21.5	23.5	23.8	23.7	18.9	22.7	23.8	22.9	22.1	20.1	23.4	22.7	22.7	23.3	23.4	24.8	23.5	27.4	27.3
15.2	19.4	20.8	21.5	23.2	15.9	19.9	21.8	21.7	22.5	20.2	21.7	22.1	23.4	20.2	21.6	21.6	22.4	24.2	24.4
12.8	19.2	19.3	18.3	21.5	14.8	22.0	18.0	21.6	21.0	19.0	21.0	21.3	21.3	20.1	21.0	23.6	22.0	24.7	26.3
17.4	20.8	20.3	19.3	23.3	17.1	22.5	23.1	22.3	21.9	20.5	21.4	21.2	22.3	22.2	21.4	21.0	21.8	23.1	23.5
16.2	18.9	21.0	22.7	23.4	14.9	19.8	20.0	19.5	22.9	18.9	22.7	21.3	22.0	20.2	22.7	23.2	23.5	22.0	25.3
19.9	22.1	22.3	21.7	22.0	19.3	22.5	19.8	23.8	21.4	21.9	25.7	24.2	22.9	22.1	25.7	24.3	23.7	26.8	26.1

98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117
2.50	2.47	2.49	2.38	2.68	2.72	2.57	2.19	2.31	2.66	2.12	1.98	2.16	2.18	2.28	2.34	2.09	2.40	2.24	2.5
2.41	2.57	2.32	2.28	2.71	2.72	2.73	2.25	2.32	2.70	2.09	2.06	2.33	2.11	2.38	2.43	2.28	2.42	2.38	2.5
2.53	2.50	2.50	2.39	2.68	2.71	2.58	2.19	2.32	2.67	2.11	2.02	2.17	2.19	2.32	2.35	2.11	2.43	2.27	2.5

2.29	2.26	2.13	2.25	2.38	2.40	2.12	2.03	2.07	2.41	1.75	1.79	1.98	1.85	1.95	2.09	1.94	2.06	1.86	2.1
2.38	2.53	2.42	2.40	2.62	2.62	2.55	2.26	2.27	2.82	2.02	2.04	2.17	2.13	2.24	2.47	2.14	2.36	2.31	2.4
2.50	2.44	2.39	2.31	2.55	2.67	2.55	2.15	2.30	2.53	2.02	2.03	2.10	2.17	2.31	2.27	2.05	2.36	2.22	2.4
2.57	2.59	2.42	2.46	2.51	2.61	2.47	2.26	2.28	2.67	1.98	1.99	2.25	2.11	2.15	2.33	2.18	2.26	2.18	2.4
2.58	2.56	2.58	2.44	2.52	2.48	2.72	2.37	2.32	2.81	2.13	2.07	2.40	2.26	2.40	2.27	2.30	2.40	2.34	2.6
2.33	2.40	2.31	2.30	2.48	2.41	2.44	2.23	2.16	2.58	1.86	1.97	2.17	2.03	2.15	2.14	2.11	2.25	2.07	2.4
2.59	2.65	2.55	2.48	2.65	2.70	2.64	2.41	2.49	2.90	2.17	2.06	2.42	2.23	2.38	2.38	2.39	2.44	2.41	2.6
2.52	2.57	2.50	2.47	2.55	2.81	2.53	2.21	2.29	2.61	2.11	2.02	2.22	2.14	2.23	2.41	2.15	2.37	2.20	2.5
2.56	2.59	2.42	2.44	2.56	2.63	2.44	2.32	2.31	2.73	2.02	1.99	2.25	2.12	2.21	2.37	2.18	2.34	2.20	2.4
2.56	2.56	2.42	2.52	2.51	2.83	2.41	2.12	2.20	2.39	2.03	2.08	2.17	1.96	2.59	2.34	2.24	2.44	2.13	2.2
2.04	2.27	2.30	2.34	2.11	2.31	2.31	1.89	1.90	2.07	1.63	1.79	1.78	1.82	2.09	2.01	1.80	1.89	1.87	1.9
2.28	2.32	2.27	2.36	2.44	2.35	2.33	2.19	2.14	2.09	1.74	1.88	1.85	1.84	2.16	1.87	1.82	1.83	2.01	2.2
2.40	2.38	2.22	2.29	2.39	2.41	2.19	2.09	2.20	2.12	1.95	1.85	2.00	1.94	2.23	1.87	1.93	1.92	2.12	2.2
2.41	2.37	2.29	2.55	2.52	2.49	2.31	2.06	1.93	2.27	1.88	1.96	1.95	2.05	2.21	2.04	1.96	1.98	2.09	2.2
2.69	2.72	2.65	2.57	2.59	2.70	2.69	2.47	2.61	2.54	2.30	2.10	2.18	2.29	2.37	2.27	2.27	2.44	2.34	2.4
2.32	2.37	2.35	2.32	2.50	2.50	2.39	2.16	2.12	2.24	2.01	1.99	2.00	1.92	2.29	1.95	2.01	2.03	2.00	2.2
2.39	2.50	2.51	2.68	2.74	2.68	2.42	2.01	2.18	2.48	2.12	2.03	2.10	2.07	2.33	2.28	2.11	2.29	2.09	2.2
2.47	2.51	2.44	2.57	2.76	2.77	2.44	2.00	2.02	2.35	1.95	2.21	2.18	1.84	2.34	2.33	2.16	2.38	2.05	2.1
2.61	2.52	2.62	2.55	2.91	2.81	2.58	2.36	2.40	2.59	2.24	2.29	2.44	2.09	2.33	2.38	2.44	2.39	2.28	2.5
2.05	2.20	2.05	2.19	2.34	2.23	2.22	1.99	2.04	2.25	1.66	1.65	1.97	1.89	2.12	1.92	1.94	2.05	1.85	2.1
2.22	2.41	2.41	2.35	2.39	2.37	2.41	2.02	2.08	2.36	1.93	1.90	2.12	2.07	2.17	2.09	2.01	1.84	1.98	2.2
2.27	2.26	2.14	2.27	2.51	2.42	2.22	2.01	2.17	2.44	1.83	1.84	1.97	1.93	2.23	2.06	1.96	1.99	1.97	2.2
2.22	2.27	2.24	2.37	2.58	2.45	2.60	2.05	2.39	2.44	1.96	1.88	1.98	1.94	2.28	1.95	1.98	2.20	1.97	2.3
2.20	2.18	2.12	2.40	2.53	2.48	2.42	2.09	2.21	2.29	2.00	1.81	1.99	1.92	2.26	2.03	1.98	2.19	2.03	2.1
2.42	2.40	2.21	2.49	2.60	2.64	2.15	1.99	1.95	2.14	1.92	1.97	1.92	2.01	2.27	2.09	1.93	2.08	1.94	2.2
2.27	2.23	2.17	2.28	2.35	2.51	2.27	2.11	2.01	2.02	1.94	2.03	2.03	2.05	2.36	2.15	2.03	2.23	2.10	2.1

2.26	2.50	2.23	2.49	2.37	2.53	2.38	2.09	2.13	2.40	2.04	2.04	2.24	2.22	2.41	2.34	2.23	1.99	2.31	2.3
2.36	2.32	2.44	2.55	2.60	2.52	2.33	2.19	2.27	2.34	2.08	2.18	2.15	2.12	2.21	2.40	2.05	2.01	2.29	2.3
2.40	2.31	2.45	2.38	2.62	2.45	2.54	2.28	2.28	2.54	2.04	2.02	2.23	2.06	2.17	2.21	2.21	2.25	2.37	2.4
2.57	2.40	2.43	2.52	2.50	2.61	2.38	2.28	2.23	2.37	2.18	2.51	2.21	2.31	2.56	2.56	2.24	2.28	2.36	2.3
2.39	2.34	2.28	2.52	2.56	2.49	2.28	2.17	2.09	2.40	1.89	2.02	2.18	1.95	2.16	2.19	2.09	1.96	2.18	2.3
2.39	2.50	2.42	2.51	2.56	2.67	2.52	2.32	2.36	2.19	2.14	2.15	2.10	1.94	2.16	2.32	2.12	2.34	2.28	2.3
2.45	2.54	2.47	2.70	2.47	2.43	2.50	2.52	2.46	2.31	2.32	2.39	2.30	2.42	2.39	2.30	2.30	2.24	2.39	2.3
2.22	2.44	2.31	2.58	2.54	2.63	2.30	2.13	2.28	2.22	1.97	1.92	1.94	1.92	2.30	2.13	1.94	2.15	1.97	2.3
2.18	2.18	2.16	2.47	2.42	2.33	2.42	2.06	2.12	2.37	1.88	2.06	1.96	2.10	2.26	2.11	1.98	2.17	1.80	2.1
2.16	2.28	2.41	2.65	2.66	2.69	2.31	2.03	1.90	2.35	2.03	2.31	2.22	2.15	2.39	2.45	2.22	2.24	2.09	2.1
2.45	2.46	2.40	2.36	2.70	2.65	2.33	2.23	2.01	2.38	1.86	2.15	2.10	1.86	2.07	2.19	2.08	2.17	2.23	2.4
2.59	2.62	2.32	2.42	2.53	2.68	2.46	2.23	2.38	2.39	2.04	2.07	2.03	2.20	2.28	2.22	2.03	2.19	2.09	2.2
2.40	2.47	2.30	2.52	2.51	2.70	2.31	2.21	2.14	2.27	1.94	2.06	2.02	1.96	2.25	2.13	2.01	2.25	1.99	2.1
2.53	2.64	2.26	2.44	2.41	2.61	2.49	2.20	2.33	2.30	2.09	1.99	2.09	2.12	2.35	2.36	2.09	2.19	2.06	2.3
2.40	2.32	2.23	2.58	2.44	2.52	2.45	2.19	2.22	2.36	2.18	2.06	2.12	2.36	2.30	2.31	2.12	2.27	2.21	2.2
2.08	2.18	2.09	2.25	2.35	2.25	2.25	2.04	2.11	2.34	2.08	2.12	2.15	2.38	2.21	2.25	2.18	2.19	2.18	2.4
2.24	2.32	2.16	2.36	2.26	2.47	2.09	2.00	1.92	2.04	1.77	1.79	1.67	1.86	2.09	1.91	1.69	1.91	1.97	2.0
2.28	2.15	2.26	2.33	2.52	2.53	2.05	2.12	2.03	2.40	1.85	2.13	2.09	2.06	2.33	2.19	2.03	2.30	1.98	2.1
2.16	2.32	2.07	2.32	2.13	2.38	1.99	1.93	1.87	2.04	1.65	1.79	1.73	1.84	2.08	1.86	1.70	1.88	1.89	2.0
2.12	2.20	2.24	2.34	2.30	2.35	2.05	1.87	1.99	2.26	1.65	1.89	1.81	1.79	2.13	2.05	1.78	2.02	1.75	2.1
2.37	2.61	2.42	2.27	2.73	2.63	2.45	1.95	2.21	2.32	2.11	2.15	2.09	1.92	2.24	2.23	2.10	2.34	2.09	2.3
2.37	2.50	2.44	2.44	2.44	2.54	2.58	2.05	1.94	2.38	2.19	2.35	2.41	2.17	2.40	2.39	2.36	2.44	2.38	2.4
2.52	2.25	2.23	2.36	2.30	2.55	2.25	2.11	2.25	2.08	2.08	1.83	1.88	1.96	2.30	1.82	1.90	1.83	1.97	2.1
2.20	1.95	2.16	2.12	2.34	2.25	2.19	2.11	2.05	2.19	1.75	1.87	2.03	1.94	2.13	1.92	1.97	2.00	1.96	2.2
2.32	2.51	2.40	2.38	2.49	2.56	2.23	2.08	2.06	2.17	1.94	2.04	1.96	1.88	2.25	1.99	1.96	2.08	1.97	2.3
2.20	2.30	2.22	2.17	2.49	2.49	2.17	2.09	1.96	2.24	1.89	1.89	2.11	1.97	2.16	2.06	2.07	2.29	2.00	2.2

2.26	2.32	2.24	2.27	2.43	2.43	2.23	1.77	1.73	2.06	1.99	2.10	2.01	2.02	2.26	2.19	2.06	2.12	1.96	2.2
2.19	2.25	2.27	2.29	2.27	2.36	2.27	1.80	1.87	2.18	1.81	1.85	1.79	1.88	2.06	2.05	1.69	1.96	1.93	2.3
2.23	2.26	2.23	2.23	2.27	2.31	2.14	2.02	2.08	2.24	1.82	2.14	1.78	1.84	2.16	1.96	1.74	2.03	2.02	2.0
2.25	2.16	2.02	2.02	2.43	2.33	2.18	2.01	2.04	2.20	1.76	1.75	1.83	1.87	2.09	2.05	1.81	1.88	1.85	2.0
2.17	2.12	2.17	2.19	2.67	2.46	2.26	1.76	1.76	2.18	1.84	1.80	1.96	1.80	2.22	2.02	1.95	2.07	2.00	2.2
2.12	2.28	2.23	2.35	2.41	2.32	2.17	1.98	1.95	2.21	1.87	1.80	1.88	1.90	2.27	2.05	1.82	1.99	1.86	2.1
2.41	2.33	2.41	2.24	2.55	2.63	2.20	2.15	2.15	2.47	1.97	2.12	1.94	2.18	2.33	2.28	2.02	2.35	2.19	2.5
2.24	2.44	2.31	2.48	2.39	2.58	2.45	1.94	1.95	2.32	2.04	1.95	2.04	1.92	2.18	1.93	1.99	1.89	1.93	2.2
2.29	2.14	2.27	2.35	2.43	2.42	2.32	2.01	2.00	2.33	1.95	2.02	1.95	1.96	2.20	1.99	1.98	2.00	2.03	2.0
2.21	2.45	2.26	2.12	2.34	2.56	2.40	1.99	2.17	2.22	2.11	1.95	2.00	2.02	2.32	2.09	2.02	2.02	2.16	2.1
2.01	2.11	1.96	2.03	2.28	2.18	2.02	2.05	2.02	1.96	1.70	1.91	1.90	1.77	2.06	1.86	1.90	1.87	1.86	2.1
2.11	2.45	2.24	2.37	2.51	2.56	2.25	1.83	1.90	2.15	1.95	2.06	1.82	2.03	2.18	2.13	1.83	2.10	1.96	2.0
2.21	2.16	2.06	2.16	2.36	2.18	2.12	1.99	1.86	2.04	1.59	1.76	1.81	1.80	2.00	1.88	1.78	1.85	1.81	2.2
2.35	2.36	2.28	2.28	2.36	2.46	2.31	2.10	2.15	2.30	1.98	1.94	1.92	1.95	2.32	2.07	1.91	2.13	2.07	2.2
2.54	2.48	2.19	2.20	2.49	2.65	2.24	1.95	2.04	2.39	2.07	1.96	1.96	2.18	2.26	2.26	1.93	2.06	2.14	2.3
2.30	2.30	2.24	2.36	2.19	2.44	2.32	1.95	2.15	2.25	1.89	1.84	1.74	2.05	2.09	2.16	1.77	1.99	1.97	2.1
2.30	2.20	2.08	2.20	2.47	2.29	2.21	2.05	1.91	2.10	1.70	1.84	1.90	1.87	2.06	1.94	1.87	1.89	1.87	2.3
2.10	2.27	2.31	2.16	2.36	2.35	2.01	2.20	2.06	2.12	1.85	2.06	1.94	1.83	2.30	2.00	1.92	2.08	2.08	2.2
2.41	2.38	2.19	2.28	2.38	2.60	2.37	1.89	2.08	2.26	2.01	2.02	1.86	1.92	2.20	2.17	1.84	2.14	2.12	2.0
2.41	2.31	2.36	2.44	2.34	2.45	2.32	2.11	2.13	2.37	2.00	2.17	2.07	1.94	2.19	2.11	2.01	2.33	2.18	2.3
2.26	2.38	2.12	2.25	2.43	2.47	2.27	2.10	2.03	2.28	2.06	2.27	2.18	2.11	2.56	2.21	2.17	2.29	2.19	2.3
2.13	2.23	2.15	2.27	2.44	2.39	2.05	1.96	1.88	2.11	1.62	1.78	1.88	1.70	2.16	1.81	1.81	1.96	1.66	2.1
2.30	2.26	2.30	2.29	2.31	2.36	2.14	2.07	2.13	2.28	1.85	2.16	1.77	1.83	2.16	1.98	1.73	2.06	1.95	2.1
2.40	2.48	2.38	2.20	2.27	2.47	2.31	2.13	2.10	2.19	2.03	2.03	2.20	2.18	2.31	2.16	2.22	2.32	2.17	2.3
2.52	2.48	2.49	2.46	2.56	2.77	2.41	2.16	2.18	2.42	2.08	2.09	1.97	2.24	2.25	2.08	1.98	2.05	2.05	2.2
2.46	2.50	2.36	2.66	2.48	2.71	2.40	1.99	2.29	2.27	2.20	2.01	2.01	2.10	2.34	2.26	2.00	2.04	2.19	2.1

2.40	2.37	2.20	2.26	2.57	2.67	2.30	2.14	2.26	2.49	2.29	2.22	2.12	2.14	2.23	2.25	2.14	2.16	2.27	2.0
2.25	2.13	2.13	2.31	2.35	2.31	2.10	1.88	1.89	2.22	1.68	1.90	1.87	1.93	2.16	2.00	1.87	2.09	1.85	2.1
2.42	2.40	2.35	2.45	2.73	2.58	2.41	2.27	2.07	2.57	2.03	2.27	2.42	2.06	2.38	2.25	2.38	2.40	2.24	2.4
2.54	2.55	2.41	2.37	2.34	2.74	2.42	2.11	2.29	2.29	2.12	2.16	2.17	2.12	2.42	2.40	2.09	2.50	2.24	2.2
2.18	2.20	2.28	2.24	2.51	2.28	2.30	2.00	1.92	2.16	1.94	2.07	2.17	2.09	2.26	2.21	2.17	2.21	2.05	2.3
2.46	2.53	2.45	2.43	2.52	2.71	2.66	2.14	2.01	2.28	2.20	2.36	2.18	2.17	2.30	2.42	2.25	2.38	2.38	2.3
2.33	2.32	2.23	2.37	2.38	2.48	2.42	2.10	2.18	2.30	1.92	2.02	2.05	1.82	2.07	2.17	2.03	2.21	2.07	2.2
1.78	1.86	1.86	2.10	2.15	2.14	1.80	2.50	2.39	2.24	2.05	2.43	2.23	2.19	2.44	2.38	2.24	2.33	2.40	2.6
1.71	1.99	1.55	1.81	2.11	2.05	1.88	2.34	2.33	2.38	2.03	2.19	2.32	2.28	2.33	2.29	2.27	2.24	2.28	2.5
1.77	1.86	1.83	2.01	1.70	1.98	1.97	2.38	2.27	2.15	1.99	2.38	2.18	2.22	2.28	2.44	2.16	2.27	2.18	2.3
1.85	1.99	1.77	2.06	2.05	2.15	1.54	2.29	2.32	2.28	2.13	2.44	2.25	2.33	2.38	2.20	2.17	2.39	2.19	2.4
1.78	1.86	1.86	2.10	2.15	2.14	1.80	2.50	2.39	2.24	2.04	2.43	2.23	2.19	2.43	2.38	2.24	2.33	2.40	2.6
1.56	1.95	1.89	1.98	2.14	2.11	1.95	2.61	2.59	2.48	2.30	2.39	2.55	2.42	2.47	2.37	2.48	2.27	2.48	2.5
1.90	2.09	1.97	2.05	2.26	2.40	2.01	2.55	2.46	2.44	2.27	2.38	2.32	2.41	2.39	2.40	2.32	2.35	2.44	2.4
1.97	1.80	1.87	2.13	2.19	2.05	1.85	2.34	2.45	2.53	2.02	2.38	2.46	2.34	2.58	2.49	2.39	2.34	2.21	2.5
2.09	1.56	2.14	2.34	2.40	2.40	2.17	2.45	2.56	2.60	2.41	2.58	2.64	2.51	2.69	2.49	2.62	2.47	2.52	2.6
	1.94	1.90	1.87	2.05	2.03	1.84	2.27	2.31	2.38	2.14	2.33	2.27	2.26	2.51	2.43	2.27	2.32	2.25	2.49
16.7		1.92	2.17	2.36	2.11	1.96	2.33	2.27	2.64	2.30	2.51	2.41	2.40	2.54	2.40	2.38	2.31	2.44	2.46
15.0	17.0		1.85	2.03	2.16	1.75	2.28	2.43	2.38	2.12	2.20	2.35	2.13	2.28	2.30	2.32	2.25	2.36	2.50
15.5	20.3	15.1		2.10	2.06	2.15	2.44	2.47	2.40	2.25	2.36	2.37	2.22	2.41	2.42	2.41	2.54	2.58	2.58
17.2	22.2	17.5	17.5		2.15	2.23	2.52	2.58	2.30	2.29	2.44	2.48	2.67	2.58	2.45	2.50	2.53	2.48	2.36
15.5	19.4	19.1	17.0	18.1		2.13	2.67	2.57	2.62	2.31	2.43	2.59	2.49	2.73	2.44	2.53	2.38	2.44	2.77
14.1	17.5	13.9	19.4	19.3	17.2		2.37	2.39	2.33	2.08	2.39	2.36	2.29	2.47	2.50	2.28	2.51	2.28	2.63
22.4	23.6	22.8	24.8	25.2	26.8	23.8		1.49	2.30	2.04	1.98	1.99	2.04	2.33	2.34	2.01	2.21	2.11	2.20
22.9	22.2	22.5	24.7	26.2	25.0	22.5	12.0		2.23	1.92	2.11	2.05	2.08	2.21	2.26	2.01	2.10	2.17	2.14
21.4	25.5	22.1	22.0	21.3	24.8	21.9	22.0	20.2		2.04	2.24	2.16	2.21	2.43	2.37	2.16	2.24	2.18	2.52

19.4	21.9	19.3	20.9	20.9	20.9	18.8	18.5	16.3	15.9		1.80	1.77	1.68	1.90	1.94	1.72	1.79	1.66	2.15
21.9	25.4	19.8	23.2	22.9	23.3	22.9	18.0	17.7	19.0	13.8		1.64	1.59	1.85	1.72	1.62	1.70	1.75	2.11
21.4	24.4	21.1	22.5	23.0	25.4	22.7	18.2	17.6	18.4	13.6	11.8		1.86	1.90	1.97	0.69	1.85	1.73	2.11
21.3	23.2	20.3	21.7	25.1	24.5	22.5	19.6	19.3	18.3	12.7	12.2	15.3		1.93	1.86	1.83	2.00	1.83	2.14
25.1	25.8	22.2	24.1	24.7	27.1	25.2	22.5	21.2	22.5	15.7	15.4	15.7	16.8		2.21	1.88	1.98	2.05	2.21
22.5	22.7	21.3	23.2	23.1	23.1	24.0	21.5	19.8	20.5	14.8	12.5	15.2	15.2	19.4		1.88	1.91	2.01	2.33
21.5	24.7	21.0	23.2	23.7	25.1	21.9	18.3	17.4	18.5	13.2	11.3	2.57	15.0	16.0	14.6		1.80	1.73	2.11
21.3	21.8	20.9	25.5	23.8	22.5	24.2	20.7	18.5	20.0	14.0	12.3	13.8	16.9	17.2	14.9	13.4		2.05	2.24
20.7	24.4	21.9	24.7	23.8	23.3	21.8	19.7	19.9	19.4	12.3	13.8	12.6	14.9	18.1	16.5	12.7	17.3		2.15
24.0	24.2	23.3	25.4	23.2	27.7	26.4	20.5	19.3	23.3	19.3	18.5	18.5	19.6	21.0	20.6	18.8	20.2	18.7	

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