Molecular systematics and colour variation of *Carpophilus* species (Coleoptera: Nitidulidae) of the South Pacific

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by

Samuel David James Brown

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And every creature which is in heaven, and on the earth, and under the earth, and under the sea, and all that are in them, heard I saying "Blessing, and honour and glory, and power, be unto him that sitteth upon the throne, and unto the Lamb for ever and ever."
Revelation 5:13

Addendum

Subsequent to the submission of this thesis, *Carpophilus* sp. 1 was identified by A. G. Kirejtshuk as *C. robustus* Murray. This species was originally described by Murray (1864) as a variety of *C. vittiger*, but has been reinstated as a full species and will be described fully in an upcoming revision by Kirejtshuk.

Abstract of a thesis submitted in partial fulfilment of the requirements of the Degree of Master of Science.

Molecular systematics of *Carpophilus* species (Coleoptera: Nitidulidae) of the South Pacific

by

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The sap beetle genus *Carpophilus* Stephens (Coleoptera: Nitidulidae) is a large genus consisting of over 200 species and are found worldwide. Several species are important pests of crops and stored products, and are frequently intercepted as part of biosecurity operations. The genus is poorly known taxonomically, and there are several species groups that are challenging to identify by morphological methods. In particular, two species found across the Pacific, *C. maculatus* Murray and *C. oculatus* Murray are frequently confused with each other. These two species are similar in size and colour, but differ primarily by the shape of the colour pattern on their elytra. However, this colour pattern is highly variable within both species, leading to ambiguity in the indentification of these species. Within *C. oculatus*, three subspecies have been described based on differences in the male genitalia and pronotal punctation: *C. o. oculatus* and *C. o. gilloglyi* Dobson are distributed widely across the Pacific, while *C. o. cheesmani* Dobson is known only from Vanuatu.

A search of literature records and specimen collections revealed 32 species of *Carpophilus* recorded from the Pacific region. In addition there remain several unidentified specimens representing at least four species, two of which will be described subsequent to this research. A number of species recorded in the literature may have been misidentified, and these require further field

collections and inspection of museum specimens to confirm their presence in the Pacific.

To test the validity of the subspecies of *C. oculatus*, and its distinctiveness from *C. maculatus*, a phylogeny of available specimens of *Carpophilus* was inferred from one mitochondrial gene (cytochrome *c* oxidase subunit I (COI)), and two nuclear genes (28S ribsomal RNA (28S) and the internal transcribed spacer 2 (ITS2)). These data show large genetic distances between the three subspecies of *C. oculatus* of 7–12%. Given these distances are similar to those between other species in the genus, this indicates these subspecies may be elevated to full species. The data also consistently support a monophyletic relationship between *C. o. oculatus* and *C. o. gilloglyi*. Nuclear genes also support *C. o. cheesmani* as part of a clade with the other subspecies, but these relationships are unresolved in COI. *Carpophilus maculatus* was not supported as being the sister taxon of the *C. o. oculatus* and *C. o. gilloglyi* clade. Other relationships within *Carpophilus* were unresolved, possibly due to a combination of incomplete taxon sampling, and saturation of substitutions within the COI gene.

Phylogeographic analysis of specimens collected from several localities within the range of *C. oculatus* showed that, with only one exception, there were no shared haplotypes between archipelagoes. This result suggests it may be possible to determine the provenence of intercepted specimens, providing further information regarding potential invasion pathways. A degree of geographic structuring was also present within *C. o. gilloglyi*, being separated into a western clade found in Fiji and Rotuma and an eastern clade distributed from the Kermadec Islands and Tonga to French Polynesia. This separation was most profound in COI data, with a mean pairwise distance between the clades of 7%. ITS2 data also demonstrates a degree of differentiation between the two clades, based on differences in the insertions and deletions between the clades.

The variability in the shape and colour of the elytral pattern of *C. oculatus* was also investigated. Colour was quantified using a method based on Red-Green-Blue (RGB) colour values derived from digital photographs, while an outline analysis of the elytral pattern was conducted using elliptic Fourier analysis (EFA). Principal Components Analysis of the RGB values and EFA coefficients showed no clear separation between subspecies, nor were any trends correlated with host fruit or collection localities.

Variation at all levels and all measures studied in this thesis show that this geographic region and this genus of beetles offer intruiging insights into speciation, biogeography and biological invasions. There is much scope for further research on the causes and consequences of this variation and the lives of these interesting insects.

Keywords: *Carpophilus oculatus*, taxonomy, systematics, COI, 28S, ITS2, phylogeography, elliptic Fourier analysis, colour quantification.

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2.1 & 4.1) were modified from the publicly available Oceania regional map published by the CIA world factbook.

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Finally, I would like to acknowledge Jesus Christ, my Saviour and Lord. However you made this world and its amazing diversity, it is a privelige indeed to be able to discover more about these creatures that reflect your beauty, love and generosity.

Contents

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Abbreviations

28S: 28S ribosomal RNA, also known as the large subunit of ribosomal RNA.

ANOVA: Analysis of Variance.

BHPBM: Bishop Museum, Honolulu, Hawaii.

BPP: Bayesian posterior probabilities.

COI: Cytochrome *c* oxidase subunit 1.

EFA: Elliptic Fourier analysis.

GTR: General Time Reversible, a model used in the optimisation of phylogenetic trees using maximum likelihood defined as having 8 rate parameters.

HM: Hunterian Museum, Glasgow, Scotland.

ITS2: Internal Transcribed Spacer 2.

L: Length.

LUNZ: Entomology Research Museum, Lincoln University, Canterbury, New Zealand.

NZAC: New Zealand Arthropod Collection, Landcare Research, Tamaki, Auckland.

MAF: New Zealand Ministry of Agriculture and Forestry; responsible for New Zealand's biosecurity operations. When used with regard to specimen data, refers to the MAF Biosecurity Collection, Auckland, New Zealand.

MANOVA: Multivariate Analysis of Variance.

ML: Maximum Likelihood.

OUMNH: Oxford University Museum of Natural History, Oxford, United Kingdom

PCA: Principal Components Analysis.

PCR: Polymerase Chain Reaction.

PNG: Papua New Guinea.

QM: Queensland Museum, Brisbane, Australia.

RGB: Red-Green-Blue colour notation system.

s. str.: *sensu stricto*—in the strict sense.

W: Width.

Chapter 1

Introduction

1.1 *Carpophilus* overview and importance

The sap beetle genus *Carpophilus* (Coleoptera: Nitidulidae) contains over 200 species found throughout the world. Many species are scavengers of rotting fruit, with both adults and larvae feeding on this substrate. Some species are also known to attack fruit on the tree, decreasing the commercial value of the crop, and as such are considered to be important pests in orchards and agricultural situations. *Carpophilus davidsoni* Dobson, *C. hemipterus* (Linnaeus) and *C. mutilatus* Erichson have emerged as serious pests of stone fruit in Australia (Hossain & Williams, 2003; James *et al.*, 1997), while *C. lugubris* Murray is an important pest of corn in the United States (Dowd, 2000) and *C. sayi* Parsons has been implicated in the transmission of oak wilt disease (Cease & Juzwik, 2001). In Australian orchards, *Carpophilus* are believed to have become more of a problem in recent years due to the decreasing use of broad-spectrum insecticides for the control of other pests (James & Vogele, 2000). Damage is caused by mechanical methods, such as chewing, and by vectoring fungal diseases such as *Monilina*

brown rot (Kable, 1969). As well as being found on fresh fruit, several *Carpophilus* species are associated with stored products like dried fruits, grains and processed goods (Dobson, 1955), with some acheiving pest status. The species most commonly found in these situations are *C. hemipterus*, *C. dimidiatus* (Fabricius), *C. ligneus* Murray and *C. obsoletus* Erichson (Dobson, 1955). A number of species are also associated with flowers and are important pollinators, particularly of the Annonaceae (Nagel *et al.*, 1989).

Six species of *Carpophilus* are known from New Zealand (Leschen & Marris, 2005), all of which are considered to be adventive. The majority of these species (*C. hemipterus*, *C. marginellus*, *C. dimidiatus* and *C. ligneus*) are cosmopolitan species and are economically important as pests of stored products (Hinton, 1945; Dobson, 1955). The other two species present (*C. davidsoni* and *C. gaveni*) are confined to Australasia and can be pests of fruit in southern Australia (James *et al.*, 1995).

From their habits of feeding on fruit and stored products, these beetles are easily dispersed by trade and it is likely that this is the method by which the New Zealand *Carpophilus* species were introduced and a number of cosmopolitan species achieved their current distribution. Although the Pacific region is New Zealand's smallest trading region in terms of volume (Statistics NZ, 2006), the amount of stored products and fresh produce imported is not insignificant. In 2004, fruit, vegetables and stored products made up 17.7% of imports from South Pacific nations, with a total value of $18 million NZD (Statistics NZ, 2006). The majority of these imports consist of roots and tubers, which can harbour a number of different *Carpophilus* species. These imports have the potential to assist in the accidental importation of further *Carpophilus* species into New Zealand. Both adults and larvae of a number of *Carpophilus* species have been intercepted at the New

Zealand border in imported fruits and foodstuffs (Archibald & Chalmers, 1983), showing that this pathway is a likely route by which *Carpophilus* species could become established. The need to monitor and control the importation of this genus, means that accurate and reliable identification methods are required. The conservative morphology of both adults and larvae makes *Carpophilus* species difficult to distinguish using traditional methods. Alternative methods of identification are therefore highly desirable.

There has been much published on the ecology and control of the group, as it is considered to be the most economically important genus within the Nitidulidae (Connell, 1981). Unfortunately, the literature involved is fragmented and difficult to access. Williams *et al.* (1983) collated references to all known literature (both ecological and taxonomic) on *Carpophilus* to that date, but much research has been done on the group since then.

1.2 Taxonomy and classification

Within the Nitidulidae, *Carpophilus* is placed in the subfamily Carpophilinae (Kirejtschuk, 2008). While no formal systematic studies have yet been published on the relationships of higher taxa within the Nitidulidae, it is believed that the Carpophilinae form a single lineage with the Epuraeinae and Amphicrossinae (Kirejtschuk, 2008), an arrangement that is consistent with preliminary molecular systematic results of the family (A. Cline pers. comm.).

The Carpophilinae includes six other genera (Table 1.1, *Procarpophilus* de Jong (known only from a fossil specimen), *Nitops* Murray, *Ctilodes* Murray, *Loriarulus* Kirejtshuk, *Vulpixenus* Kirejtshuk and *Urophorus* Murray. *Urophorus* and *Nitops* were orignally described as subgenera, but have since been elevated to generic status (Gillogly, 1962; Kirejtschuk *et al.*, 2007).

Several authorities do not recognise the generic status of *Urophorus*, and so species within this genus continue to be referred to as species of *Carpophilus*, particularly within the applied literature (Connell, 1981). All these genera have similar habits to *Carpophilus*. *Nitops* is associated with flowers as both larvae and adults (Kirejtschuk, 1997). *Ctilodes*, *Loriarulus* and *Vulpixenus* are restricted to the Malayan archipelago and New Guinea and are very poorly known with nothing known of their biology.

Carpophilus is further classified into nine subgenera: *Carpophilus* Stephens *sensu stricto*, *Megacarpolus* Reitter, *Semocarpolus* Kirejtshuk, *Gaplocarpolus* Kirejtshuk, *Askocarpolus* Kirejtshuk, *Plapennipolus* Kirejtshuk, *Ecnomorphus* Motschulsky, *Caplothorax* Kirejtshuk and *Myothorax* Murray (Table 1.1. The majority of these subgenera are fairly small, consisting of only a few species, most of which are confined to South-East Asia. The exceptions are *Carpophilus s. str.*, *Ecnomorphus* and *Myothorax*, which are large and cosmopolitan.

Taxonomically the genus is poorly known with the last global revision being done in the 19th Century (Murray, 1864). Since then, several new species have been described, most notably by Dobson and Kirejtshuk (e.g. Dobson, 1952, 1993a; Kirejtschuk, 2001). Not withstanding this, the genus remains in need of a thorough taxonomic revision. The large size of the genus, the difficulty of identifying the different species and the lack of good characters that show clear differences between the species have been major factors in the lack of revisions of *Carpophilus*. The genus also has a history of having very confusing nomenclatural issues and long-lasting misidentifications which will make the completion of such a revision a great challenge. However, a thorough review would provide the benefits of a solid taxonomic platform for future research on the genus and enable more accurate and

Table 1.1: Classification of *Carpophilus* and subgenera within the Nitidulidae. Classification follows that of Kirejtschuk (2008).

Nitidulidae

- Amphicrossinae
- Calonecrinae
- **Carpophilinae**
  - ***Carpophilus* Stephens**
    - *Askocarpolus* Kirejtshuk
    - *Caplothorax* Kirejtshuk
    - *Carpophilus* Stephens
    - *Ecnomorphus* Motschulsky
    - *Gaplocarpolus* Kirejtshuk
    - *Megacarpolus* Reitter
    - *Myothorax* Murray
    - *Plapennipolus* Kirejtshuk
    - *Semocarpolus* Kirejtshuk
  - *Ctilodes* Murray
  - *Loriarulus* Kirejtshuk
  - *Nitops* Murray
  - *Procarpophilus* de Jong
  - *Urophorus* Murray
  - *Vulpixenus* Kirejtshuk
- Cillaeinae
- Cryptarchinae
- Cybocephalinae
- Epuraeinae
- Maynipeplinae
- Meligethinae
- Nitidulinae

reliable identification of *Carpophilus* species.

Carpophilus is easily identified by having two exposed abdominal tergites and a button-like male 8th sternite (Leschen & Marris, 2005). It is morphologically conservative between species within the genus and there are a number of groups containing several species with very similar appearance and habits. Murray's (1864) descriptions made great use of colour, punctation and shapes of the elytra and pronotum. Unfortunately, interspecific differences in punctation and the shapes of the elytra and pronotum are extremely subtle, and colour is both subtle and can be highly variable within species. Accurate identification requires the comparison of unknown specimens against a good reference collection of accurately identified specimens and the dissection of male genitalia (Leschen & Marris, 2005). Male primary and secondary sexual characters are becoming better known and are proving very useful for identification and taxonomic purposes, but leave females unidentifiable. Female genitalia have also been proposed as being of taxonomic interest (Dobson, 1954), however their characterisation lags far behind that of males.

Within the South Pacific, *Carpophilus maculatus* and *C. oculatus* Murray are very similar species. They are considered by Leschen & Marris (2005) to belong to the subgenus *Myothorax*, along with the cosmopolitan species *C. dimidiatus* and *C. mutilatus*. This subgenus is considered to be a problematic group with a lot of intraspecific variability and only subtle differences that separate the species (Dobson, 1952). Ewing & Cline (2005) do not discuss *Carpophilus* subgenera, but consider *C. maculatus* to be part of the *C. dimidiatus* species group along with *C. nepos*, and *C. mutilatus*. Interestingly, they do not include *C. oculatus* as part of this assemblage. Both *C. maculatus* and *C. oculatus* are widely distributed through the South Pacific,

with *C. maculatus* also being found in Southwest Asia, South America and the West Indies. Neither species is found on the New Zealand mainland, though *C. oculatus* is found in the Kermadec Islands. The key character that differentiates between *C. oculatus* and *C. maculatus* is the extent of red colouration on the elytra. However, this patterning is very variable in both species, and there is significant overlap between them. Other characters used to define the species are differences in the punctation of the head and pronotum, and the presence of spurs on the metatibia of *C. oculatus* (Gillogly, 1962). This last feature has not been backed up by subsequent work, and is here considered to be tenuous. Dobson (1993b) reviewed several specimens of *C. oculatus* and described three subspecies based on differences in punctation and male genitalia. The nominate subspecies, *C. o. oculatus* is found in Tahiti through the central Pacific to Fiji. There is significant overlap in geographic range between this subspecies and *C. o. gilloglyi* which is found in Fiji, Cook Is. and the Kermadecs. The last subspecies, *C. o. cheesmani* was considered by Dobson to be confined to Vanuatu. The genitalia of *C. o. gilloglyi* is very similar to that of *C. maculatus*, leading to questions regarding the status of the two species.

1.3 Philosophy of species and subspecies

Species

Despite the express purpose of taxonomy and systematics as being the description and relationships between species, the existence, definition, and criteria for delimitation of species remains a subject of intense debate and has resulted in the creation of a number of species concepts (Wheeler & Meier, 2000; Hey, 2001; Coyne & Orr, 2004). Some clarity is offered by the

realisation that species are explanatory hypotheses, the purpose of which is to act as a framework for the inference of tokogenetic and phylogenetic theories about organisms (Fitzhugh, 2005). That species are natural entities existing outside of human theory is relatively self-evident, given that organisms are divided into discrete groups, despite the ample opportunities for potential gene flow and homogenisation. However our ability to consistently determine these divisions is flawed due to our limited understanding of the system and difficulty in recognising and measuring the influences and factors that separate species. The species question is further complicated by a constant evolutionary process that means that organisms are not necessarily static from generation to generation. This process blurs distinctions between related populations to different degrees depending where the species are on the speciation continuum.

A hypothetical ideal species can be considered as a guide for directing taxonomic decisions. This species would have low morphological and genetic intraspecific variation and be well differentiated from other species in these and other traits, such that it forms a monophyletic clade. It also would not form hybrids or contribute with other species, and all individuals would be similar behaviourally and biochemically. All species will violate this ideal in some regard, however it may be a useful concept to keep in mind when dealing with species and speciation problems. This emphasis on conceptualization over delimitation has strong parallels with the "Unified Species Concept" proposed by de Queiroz (2007).

Subspecies

Subspecies have been used in situations where populations of a species are subtly but recognisably and consistently differentiated from each other and

are usually geographically segregated. Subspecies are considered to have low gene flow between them, but retain the ability to breed together. They may also be characterized by hybrid zones in areas where the geographic ranges of the two populations overlap (Hewitt, 1988). They came into popularity with the modern synthesis of evolution, as an attempt to more accurately reflect the continuum of speciation (Mayr & Diamond, 2001). Recently however, the value of subspecies in taxonomy, conservation and biology in general has been questioned (Zink, 2004) and there has been a move toward the elevation of subspecies to full species and the discouragement of subspecific descriptions.

Subspecific populations are, by definition, on the cusp of speciation and as such will always be of scientific interest and a source of debate.

1.4 Aims and objectives

To resolve the taxonomic status of *Carpophilus oculatus* and its subspecies, it is necessary to investigate the dynamics of this species in detail. The relationships and distinctiveness of the subspecies need to be worked out and the taxonomic relationship of *C. oculatus* to *C. maculatus* also needs to be clarified. Samples from throughout the Pacific are required to confirm the extent of the geographic range of *C. oculatus* and the genetic variability within the species. A broad sampling scheme would offer insights into the origin and spread of *C. oculatus*, as well as formation of the different subspecies. The dispersal of the species may also be traced, which would increase our knowledge of dispersal mechanisms and invasion biology.

To assist with the identification of South Pacific *Carpophilus* for taxonomic and biosecurity purposes and to further understand speciation within the Pacific, the following objectives were addressed:

1. Produce a summary of the *Carpophilus* species known from the Pacific and checklists of the species known from each archipelago.

2. Infer a phylogeny of *Carpophilus* using molecular systematics to test the monophyly of *C. oculatus* and determine its sister taxa.

3. Investigate *C. oculatus* genetic data using phylogeographic methods to determine the degree of gene flow and geographic partitioning within the species and subspecies.

4. Conduct colour and outline analysis on *C. oculatus* elytral patterns to determine a method of quantifying the variation within the species.

Chapter 2

An annotated checklist of the *Carpophilus* of the South Pacific.

2.1 Introduction

While it is known that several species of *Carpophilus* are found throughout the Pacific, to date there has been no comprehensive study of the number of species and their ranges. Literature on the subject is scattered and buried in species lists, descriptions and other documents. A major goal of this research was to consolidate these records and provide an up-to-date checklist of the species of *Carpophilus* recorded from the Pacific region.

The Pacific is considered here to consist of Micronesia (including Palau and the Mariana Islands), Melanesia (including mainland New Guinea) and Polynesia (including Hawaii and excluding New Zealand). While a number of *Carpophilus* species are known from the region (Gillogly, 1962; Ewing & Cline, 2005; Dobson, 1993a; Williams *et al.*, 1983), the fauna is poorly

characterised, with comprehensive investigations having been done only in Micronesia (Gillogly, 1962) and Hawaii (Ewing & Cline, 2005). Throughout the rest of the Pacific information is fragmented and difficult to access. Many of these islands, particularly the Melanesian archipelagos, have not been comprehensively surveyed, resulting in incomplete distribution records. Several endemic species are known from Papua New Guinea and the Solomon Islands, but the incidence of cosmopolitan tramp species in these areas is unknown.

In order to provide a context on which to base the remainder of the objectives in this thesis, the aim of this chapter is to summarise our knowledge regarding the diversity and distribution of *Carpophilus* within the Pacific, based on a survey of the available literature and specimens.

2.2 Methods

2.2.1 Data collection

Species were included if specimens were collected from the South Pacific region during the course of this study or if they have been published as being recognised in the region.

Collecting trips to Fiji (September–November 2006; Viti Levu, Vanua Levu and Taveuni), Tahiti (May 2007; Tahiti and Moorea) and Vanuatu (February 2008; Efate and Espiritu Santo) were undertaken by the author for the purpose of collecting specimens for this research. These collecting trips focused on finding specimens of *C. oculatus* and surveyed various species of rotting fruit. Additional specimens from New Britain, Tonga, Rotuma, Cook Islands, Austral Islands, and the Kermadec Islands were collected by colleagues, also searching in rotting fruit. Representative material from the

Pacific has been deposited in the Lincoln University Entomology Research Museum (LUNZ).

Carpophilus specimens from the South Pacific were received from the Bishop Museum (Honolulu, Hawaii) (BHPBM), Oxford University Museum of Natural History (OUMNH), New Zealand Arthropod Collection (Auckland, New Zealand) (NZAC), MAF Biosecurity New Zealand (Auckland, New Zealand) (MAF), Queensland Museum (Brisbane, Australia) (QM), and the Hunterian Museum (Glasgow, Scotland) (HM).

Ron Dobson generously provided unpublished specimen data from his notes taken during his lengthy research on *Carpophilus*.

2.2.2 Collection dates

The occurrence of the species throughout the year is given as the months indicated by specimen label data. As most species and many localities have not had consistent collecting efforts throughout the year, this data should not be taken as evidence of seasonality.

2.2.3 Species associations

The fruit and vegetables that each species has been recorded from is summarised by the species associations. These may or may not be true host species (Martin, 2008), but do provide a guide to occurrence.

2.2.4 Distribution

Countries and islands where specimens have been collected within the Pacific are noted. The Pacific is defined here as including New Guinea and Melanesia, Polynesia including Hawaii but excluding New Zealand and subantarctic

islands, and Micronesia including Palau and the Marianas Islands (Fig. 2.1). Ranges outside this area are described under 'Extralimital Distribution'.

2.3 Checklist

Carpophilus araucariae Dobson, 1995

Comments: Known only from the original description. Was not collected during this research.

Collection dates: May.

Species associations: *Araucaria hunsteinii*.

Distribution: **Papua New Guinea** (Morobe Province) (Dobson 1993a).

Extralimital distribution: None.

References: Dobson (1993a) (description).

Carpophilus bacchusi Dobson, 1995

Comments: A distinctive species known only from the original description. Not collected during this research. Two subspecies have been described (Dobson, 1993a).

Collection dates: January, October.

Species associations: Not known.

Distribution: **Papua New Guinea** (Morobe Province) (Dobson 1993a).

Extralimital distribution: None.

References: Dobson (1993a) (description).

Carpophilus (Ecnomorphus) bakewelli Murray, 1864

planatus Murray, 1864 (Kirejtschuk, 2008)
aterrimus Macleay, 1864 (Kirejtschuk, 2008)

Table 2.1: Summary distribution of *Carpophilus* species within the Pacific region.

	Bismarck Archipelago	Caroline Islands	Cook Islands	Easter Island	Fiji	Gilbert Islands	Guam	Hawaii	West Papua	Kermadec Islands	Kiribati	Marquesas Islands	Mariana Islands	Marshall Islands	Nauru	New Caledonia	Niue	Palau	Papua New Guinea	Samoa	Society Islands	Solomon Islands	Tokelau	Tonga	Tuamotu Archipelago	Austral Islands	Tuvalu	Vanuatu
Carpophilus araucariae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus bacchusi bacchusi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus bacchusi madangensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus bakewelli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus biguttatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-
Carpophilus davidsoni	-	X	-	-	-	X	X	-	-	-	-	-	X	X	-	-	-	X	-	-	-	-	-	-	-	-	-	-
Carpophilus delkeskampi	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carpophilus dimidiatus	-	-	X	-	X	X	X	X	-	-	X	-	X	X	-	X	X	X	X	-	X	X	X	X	-	-	X	X
Carpophilus frivolus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-
Carpophilus fusus	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus hemipterus	-	X	X	-	X	X	-	X	-	-	-	-	X	X	-	-	-	-	X	-	X	-	-	-	X	-	-	-
Carpophilus inconspicuus	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carpophilus leveri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-
Carpophilus littoralis	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carpophilus loriae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus maculatus	X	X	X	X	X	X	X	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Carpophilus marginellus	X	X	X	-	X	-	-	X	-	-	-	-	-	X	-	X	-	-	-	-	X	-	-	X	-	-	X	-
Carpophilus mcnamarai	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus mutabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-
Carpophilus mutilatus	-	X	X	-	X	X	X	X	-	-	-	-	X	X	X	X	-	-	-	X	X	X	-	-	-	-	-	X
Carpophilus nepos	X	-	X	-	X	-	-	X	-	-	-	-	-	-	-	X	-	-	X	X	X	-	-	-	-	-	X	X
Carpophilus obesus	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus obsoletus	-	-	X	-	X	-	-	-	X	-	-	-	-	-	-	X	-	X	X	X	-	X	-	X	-	-	-	X
Carpophilus oculatus cheesmani	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
Carpophilus oculatus indet subsp.	-	X	X	-	X	-	-	X	-	-	-	X	X	X	-	-	X	-	X	X	X	X	X	X	-	X	-	-
Carpophilus oculatus gilloglyi	-	X	X	X	X	-	-	-	-	X	-	-	-	-	-	-	X	-	-	X	X	-	-	X	-	X	X	-
Carpophilus oculatus oculatus	-	-	X	-	X	-	-	X	-	-	-	X	-	-	X	X	X	-	-	X	X	-	X	X	-	-	X	X
Carpophilus pallescens	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	X	-	-	X	-	-	-	-	-	X
Carpophilus schioedtei	-	-	-	-	-	-	-	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carpophilus terminalis	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carpophilus truncatus	-	X	X	-	X	X	X	-	X	-	-	-	X	X	-	-	X	X	X	-	-	X	-	-	-	-	X	X
Carpophilus ustulatus	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus variolosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Carpophilus waterhousei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X	-	-	-	-	-	-
Loriarulus poggi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Urophorus humeralis	X	X	X	-	X	X	X	X	-	-	-	-	X	X	-	X	-	X	-	X	X	X	-	X	-	-	-	X

Figure 2.1: Map of the Pacific delimiting the region for the context of this study.

Comments: The synonymies above are based on inspection of type specimens by Kirejtschuk (2008). This interpretation of *C. aterrimus* is contrary to Hinton (1945), who synonymised it with *C. hemipterus*. This species is pictured in Leschen & Marris (2005) under the name *C. planatus*. Not collected from the Pacific over the course of this research.

Collection dates: January, March, October.

Species associations: Not known.

Distribution: **Papua New Guinea** (Oro (Northern) Province) (Dobson notebooks).

Extralimital distribution: **Australia** (New South Wales, Queensland, Western Australia), **New Zealand** (Whangarei).

References: Murray (1864) (description), Kirejtschuk (2008) (synonymies), Leschen & Marris (2005) (picture).

***Carpophilus biguttatus* Motschulsky, 1858**

Comments: This species is recorded as being found in Tahiti in an online checklist (Nishida, 2008). Recently, Kirejtschuk (2008) placed this species into the genus *Platyarcha*, which is not in the Carpophilinae. Additionally, the type locality of this species is in East India (Murray, 1864). These suggest that this record is in error and may be a misidentification of *C. maculatus*. Not collected during this research.

Collection dates: Not known.

Species associations: Not known.

Distribution: **Society Islands** (Tahiti) (Nishida 2008).

Extralimital distribution: **India**.

References: Murray (1864) (comment on description); Kirejtschuk (2008) (synonymy); Nishida (2008) (French Polynesia record).

Carpophilus (Myothorax) davidsoni Dobson, 1952

Comments: Within the Pacific, this species has only been recorded from Micronesia by Gillogly (1962). Outside the Pacific it is the most economically important species in southern Australian orchards (James *et al.*, 1995, 1997). The aggregation pheromone of this species has been characterised (Bartelt & Weisleder, 1996) and is used extensively for control within orchards (Bartelt & Hossain, 2006; Hossain *et al.*, 2008). Not collected from the Pacific over the course of this research.

Collection dates: January, February, March, April, May, June, July, August, September, October, November, December.

Species associations: *Artocarpus altitis, Carica papaya, Citrus* sp., *Cocos nucifera, Cucumis melo,* to light, *Ficus carica, Lycium* sp., *Malus* sp., *Musa* sp., *Pandanus* sp., *Pithecellobium dulce, Prunus damartica, Prunus persica, Solanum auriculatum, Zea mays.*

Distribution: **Caroline Islands** (Map, Ulithi, Etal, Pulo Anna, Sorol, Ifaluk, Nomwin, Kapingamarangi, Moen (Wena), Moen, Fefan, Toloas (Tonoas), Ponape, Kusaie) (Gillogly 1962), **Gilbert Islands** (Tarawa, Onotoa) (Gillogly 1962), **Guam** (Guam) (Gillogly 1962), **Mariana Islands** (Saipan, Tinian, Agiguan, Rota) (Gillogly 1962), **Marshall Islands** (Eniwetok, Wotho, Kwajalein, Lae, Jemo, Likiep, Jaluit, Arno) (Gillogly 1962), **Palau** (Babelthaup, Angaur) (Gillogly 1962).

Extralimital distribution: **Australia** (New South Wales, Victoria, Queensland, South Australia, Tasmania, Lord Howe Island), **New Zealand** (Whakatane, Hastings, Whangarei, Auckland, North Island), **Philippines**.

References: Leschen & Marris (2005), Gillogly (1962) (description and key), Gillogly (1969) (key), Dobson (1955) (key and genitalia diagrams).

Carpophilus (Carpophilus) delkeskampi Hisamatsu, 1963

Comment: This large-bodied *Carpophilus* species is found in stored products throughout Aisa. Dobson (1993a) described a subspecies from Australia. Not collected during this research.

Collection dates: March, April, December.

Species associations: *Cucumis melo,* stored products.

Distribution: **West Papua** (New Guinea) (Kirejtschuk 2005).

Extralimital distribution: **Australia** (Queensland, Northern Territory), **South India**, **Sierra Leone**, **India**, **India, West Bengal**, **Nepal**, **Sri Lanka**, **Myanmar**, **Thailand**, **Laos**, **Vietnam**, **Malaysia**, **Indonesia**, **Philippines**, **China**, **Japan**, **South Korea**, **East Russia**, **Iran**, **Iraq**, **Jordan**, **Saudi Arabia**, **Turkey**, **Seychelles**, **Taiwan**.

References: Hisamatsu (1963) (description); Dobson (1993a) (description of subspecies); Kirejtschuk (2005) (checklist).

Carpophilus (Myothorax) dimidiatus (Fabricius, 1792)

dimidiatus (Fabricius, 1792) (*Nitidula*)

auropilosus Wollaston, 1854

lewisi Reitter, 1884

pusillus Stephens, 1830

contingens (Walker, 1858)

biguttatus Gemminger and von Harold (non Motschulsky), 1868

dilutus Murray, 1864

limbalis Murray, 1864

nigritus Murray, 1864

testaceus Murray, 1864

hemipterus (Fabricius non Linnaeus, 1792)

bimaculatus (Montrouzier non Linnaeus, 1860)

puberulus (Montrouzier, 1860)

tempestivus Jacquelin-du-Val (non Erichson), 1856

pilosellus Motschulsky, 1858

Comments: *Carpophilus dimidiatus* is a cosmopolitan species that has been recorded throughout the South Pacific. Very few specimens were collected

during this research project; the single specimen found during the course of this study was collected serendipitously. Lack of abundance in these collections is most likely because of biased collecting towards rotting fruit, and not stored products as is its common association. It has been frequently found in and around copra sheds and it is likely that this trade has assisted *C. dimidiatus* in its spread around the Pacific.

This species is an important pest of stored products (Dobson, 1955). It is also known to pollinate Annonaceae (Nagel *et al.*, 1989).

This species can be easily confused with *C. mutilatus*, *C. nepos*, and *C. truncatus*. They can be distinguished from each other by the characters given in Table 2.2.

Collection dates: January, February, March, April, May, June, July, August, September, October, November, December.

Species associations: *Ananas comosus, Arachis hypogaea, Artocarpus altitis, Averrhoa carambola, Capsicum* sp., *Citrus* sp., *Cocos nucifera, Dioscorea* sp., Arecaceae spp., Fabaceae spp., leaf litter and compost, stored products, *Leptospermum scoparium, Macadamia* sp., *Musa* sp., *Ochrosia* sp., *Oryza sativa, Passiflora* sp., *Prunus persica, Tamarindus indica, Triticum* sp., *Zea mays*.

Distribution: **Cook Islands** (Rarotonga, Mauke, Penrhyn, Mangaia) (NZAC, Dobson notebooks), **Fiji** (Fiji Islands, Viti Levu, Taveuni, Wakaya, Ovalau) (Dobson notebooks, NZAC), **Gilbert Islands** (South Tarawa) (NZAC), **Guam** (Guam) (Gillogly 1962), **Hawaii** (Kauai, Oahu, Hawaii, Midway Island) (Ewing & Cline 2005, Nishida & Beardsley 2002), **Kiribati** (Tarawa) (NZAC), **Mariana Islands** (Tinian, Rota, Saipan) (Gillogly 1962), **Marshall Islands** (Arno, Jaluit, Wake Island) (Gillogly 1962), **New Caledonia** (Grande Terre) (QM, OUMNH), **Niue** (Niue) (NZAC), **Palau** (Babelthaup) (Gillogly 1962), **Papua New Guinea** (Morobe Province, Madang Province, Morobe District) (Dobson notebooks), **Society Islands** (Tahiti) (Dobson notebooks, LUNZ, Nishida 2008), **Solomon Islands** (Guadalcanal, Gizo, Sikaiana) (Dobson notebooks), **Tokelau** (Nukunono) (NZAC), **Tonga** (Niuatoputapu, Tongatapu, Falehau, Hakake, Tonga Is.) (NZAC, Dobson notebooks), **Tuvalu** (Funafuti) (NZAC), **Vanuatu** (Efate) (Dobson notebooks, OUMNH).

Extralimital distribution: Cosmopolitan species. **Australia** (Queensland, Victoria, Torres Strait, New South Wales), **New Zealand** (Greymouth, Auck-

land), **Britain**, **Ethiopia**, **Bonin Islands**, **England**, **Nigeria**, **India**, **Kenya**, **Botswana**, **Namibia**, **South Africa**, **Ivory Coast**, **Cameroon**, **Eithiopia**, **Zaire**, **Madagascar**, **United States of America** (Texas, Arizona, Florida).

References: Leschen & Marris (2005); Gillogly (1962); Audisio (1993) (descriptions); Hayashi (1978) (larval description); Gorham (1987) (key to adults and larvae); Ewing & Cline (2005); Connell (1977) (keys); Dobson (1955) (key and genitalia diagrams).

Carpophilus (Ecnomorphus) frivolus Murray, 1864

Comments: Originally described from Melbourne, Australia, this species was recorded by Gillogly (1962) from Palau. A picture of this species is found in Leschen & Marris (2005). Not collected during this research.

Collection dates: August, October, November.

Species associations: Not known.

Distribution: **Palau** (Peleliu) (Gillogly 1962).

Extralimital distribution: **Australia** (Victoria), **Philippines**.

References: Gillogly (1969) (key), Gillogly (1962) (description), Murray (1864) (description), Leschen & Marris (2005) (picture).

Carpophilus fusus Murray, 1864

Comments: Nothing further is known about this species beyond its original description (Murray, 1864). Not collected during this research.

Collection dates: January, June, August, October, November.

Species associations: *Careya australis*.

Distribution: **West Papua** (Vogelkop) (Murray 1864), **Papua New Guinea** (Madang Province, Morobe Province, Oro (Northern) Province) (Dobson notebooks).

Extralimital distribution: **Australia** (Queensland, North Queensland, Torres Strait, Northern Territory), **Indonesia**.

Table 2.2: Characteristic features of four easily confused members of the *Myothorax* subgenus.

	C. dimidiatus	*C. mutilatus*	*C. nepos*	*C. truncatus*	*C. maculatus*
Hypomeron punctation	Coarsely and distinctly punctured	Lightly granulate	Lightly granulate	Indistinctly punctured	Smooth and impunctate
Prosternum puncatation	Densely punctate	Punctate	Punctate	Densely punctate	Impunctate except for area just anterior of prosternal process
Relative lengths of second and third antennal segments	Third longer than second	Second longer than third	Roughly equal	Roughly equal	Third longer than second
Male hind tibia	Evenly expanded along length	Evenly expanded along length	Evenly expanded along length	Abruptly expanded from $\frac{1}{3}$ length	Evenly expanded along length
Pronotal punctation	Moderately punctate (punctures separated by 1–2 diameters)	Closely punctate (<1 diameter separation). Medial impunctate strip at base of pronotum	Moderately punctate. No impunctate medial strip	Moderately punctate	Moderately punctate

Connell 1977; Gillogly 1962; Audisio 1993

References: Murray (1864) (description).

Carpophilus (Carpophilus) hemipterus (Linnaeus, 1758)

hemipterus) (Linnaeus, 1758) (*Dermestes*)
bimaculatus (Linnaeus, 1797) (*Silpha*)
brevipennis Germain, 1856
cadaverinus (Fabricius, 1801) (*Nitidula*)
dimidiatus (Heer non Fabricius, 1841) (*Cateretes*)
ficus (Fabricius, 1801) (*Stenus*)
flexuosus (Herbst, 1841) (*Nitidula*)
pictus (Heer, 1841) (*Cateretes*)
quadriguttatus (Thunberg, 1794) (*Nitidula*)
circumdatus Ragusa, 1892
quadratus (Fabricius, 1758) (*Nitidula*)

Comments: This is a cosmopolitan species that is frequently found in stored products and rotting fruit. It is also known to play an important role in the pollination of Annonaceae species (Blanche & Cunningham, 2005; Klein *et al.*, 2007) and has some forensic significance, as it has been found inside bone marrow of corpses (Oliva, 2001). Yeasts growing on rotting fruit form an important part of its diet and some of these yeast species have been characterised (Miller & Mrak, 1953). This species is susceptible to entomopathogenic nematodes (Glazer *et al.*, 1999) and is parasitised by *Cerchysiella utilis* (Noyes) (Hymenoptera: Encyrtidae) (Johnson *et al.*, 2000). Its larval development has been studied by James & Vogele (2000).

Collection dates: January, February, March, April, May, June, July, August, September, October, November, December.

Species associations: *Ananas comosus, Annona squamosa, Artocarpus altitis, Capsicum* sp., *Carica papaya, Eleocharis dulcis,* to light, *Ficus carica, Malus* sp., *Mangifera indica, Metrosideros polymorpha, Passiflora* sp., *Prunus persica, Spondias dulcis, Zea mays.*

Distribution: **Caroline Islands** (Moen) (Gillogly 1962), **Cook Islands** (Mauke, Rarotonga) (NZAC, LUNZ), **Fiji** (Fiji Islands) (Evenhuis 2009), **Gilbert Islands** (Tarawa) (Gillogly 1962), **Hawaii** (Kauai, Oahu, Lanai, Maui) (Ewing & Cline

2005), **Mariana Islands** (Saipan, Tinian) (Gillogly 1962), **Marshall Islands** (Eniwetok) (Gillogly 1962), **Papua New Guinea** (Rossel Island) (Dobson notebooks), **Society Islands** (Tahiti, Moorea) (LUNZ), **Tuamotu Archipelago** (Tuamotu Islands) (Nishida 2008).

Extralimital distribution: Cosmopolitan. **Australia** (South Australia, Victoria, New South Wales, Northern Territory, Queensland), **New Zealand** (South Island, Nelson, Dunedin, Auckland, Shannon, Whangarei, Christchurch), **Botswana**, **Sudan**, **N Rhodesia**, **Morocco**, **South Africa**, **Senegal**, **Gambia**, **Cameroon**, **Equatorial Guinea**, **Congo**, **Zaire**, **Eithiopia**, **Uganda**, **Kenga**, **Rwanda**, **Burundi**, **Tanzania**, **Madagascar**, **Seychelles**.

References: Leschen & Marris (2005); Gillogly (1962); Audisio (1993) (descriptions and keys), Hayashi (1978) (larval description); Jr (1963) (pupa description); Ewing & Cline (2005); Connell (1977) (keys); Gorham (1987) (key to adults and larvae); Dobson (1955) (key and genitalia diagrams).

Carpophilus (Ecnomorphus) inconspicuus Murray, 1864

Comments: Known only from the original description, with the exception of a record from the Bismarck Islands by Gillogly (1969). Not collected during this research

Collection dates: June.

Species associations: None.

Distribution: **Bismarck Archipelago** (Manus) (Gillogly 1969).

Extralimital distribution: **Indonesia**.

References: Murray (1864) (description), Gillogly (1969) (key).

Carpophilus leveri Dobson, 1995

Comments: Known only from the original description. Not seen during the course of this research. Very similar in appearence to *C. obesus*. Not collected during this research.

Collection dates: April, July, November.

Species associations: Not known.

Distribution: **Solomon Islands** (Guadalcanal) (Dobson 1993a).

Extralimital distribution: None.

References: Dobson (1993a) (description).

Carpophilus littoralis (Eschscholtz, 1822)

Comments: The original description of this species, and a translation from the German original, is available in Appendix A. The description is based on a female and may be conspecific with *C. mutilatus*, based on the contrasting colours of the legs and the undersurface. Williams *et al.* (1983) gives the distribution of this species as "Tahiti", which is incorrect according to the original description. Not collected during this research.

Collection dates: Unknown.

Species associations: Rotting fruit (species unknown).

Distribution: **Marshall Islands** (Ratack Chain) (Eschscholtz 1822).

Extralimital distribution: None.

References: Eschscholtz (1822) (description), Williams *et al.* (1983) (checklist).

Carpophilus (Ecnomorphus) loriai (Grouvelle, 1906)

Comments: This species was recently synonymised with *C. luridipennis* Macleay, 1873 by Kirejtschuk (2008). Pictures of these two species published in Leschen & Marris (2005) show some profound differences between these two taxa. Further revision of the original designations and subsequent usage of these names is clearly necessary. Not collected during this research.

Collection dates: February, May, September.

Species associations: Not known.

Distribution: Not known **Papua New Guinea** (New Guinea) (Williams *et al.* 1983).

Extralimital distribution: **Australia** (Northern Territory, Queensland, New South Wales, East Australia).

References: Leschen & Marris (2005) (pictures), Kirejtschuk (2008) (synonymies).

***Carpophilus (Myothorax) maculatus* Murray, 1864**

vittiger Murray, 1864

Comments: This species is ubiquitous throughout the South Pacific Islands and was the most common species collected during the course of this research. It is extremely variable in colouration and the various morphs can be mistaken for most species in the *Myothorax* subgenus. Some of its defining characteristics are given in Table 2.2.

Little work has been done on the biology of this species, but it is known to pollinate Annonaceae (Nagel *et al.*, 1989) and was found on a wide range of host fruit in high numbers (pers. obs.)

This species has been recorded as being present in the Kermadec Islands (Leschen & Marris, 2005; Broun, 1910). However, as all *Carpophilus* specimens seen by the author from that group have been *C. oculatus*, this is potentially a misidentification and the species may not be present in the Kermadecs.

The combination of great intraspecific variability, wide host and geographical range, and high numerical abundance make this species a potential biosecurity risk.

Collection dates: January, February, March, April, May, June, July, August, September, October, November, December.

Species associations: *Anacardium occidentale, Ananas comosus, Annona muricata, Antiarus toxicaria, Artocarpus altitis, Artocarpus heterophylla, Averrhoa bilimbi,*

Averrhoa carambola, Capsicum sp., *Carica papaya, Chrysophyllum cainito, Citrus* sp., *Citrus limon, Cocos nucifera, Coffea* sp., *Dioscorea* sp., Arecaceae spp., leaf litter and compost, to light, *Hibiscus* sp., *Inocarpus* sp., *Ipomoea batatas, Lycopersicon esculentum, Mangifera indica, Momordica charantia, Morinda citrifolia, Musa* sp., *Nerium oleander, Pandanus* sp., *Piper methysticum, Pithecellobium dulce, Psidium guajava, Saccharum* sp., *Sechium edule, Solanum melongena, Spondias dulcis, Zea mays.*

Distribution: **Bismarck Archipelago** (New Ireland, Mussau, East New Britain) (Gillogly 1969, LUNZ), **Caroline Islands** (Map, Yap, Pulo Anna, Ulithi, Sorol, Woleai, Faraulep, Ifaluk, Lamotrek, Losap, Kapingamarangi, Tol (Ton), Moen (Wena), Fefan I, Ponape, Lelu (Lele) I) (Gillogly 1962), **Cook Islands** (Rarotonga, Aitutaki, Pukapuka Atoll, Atui, Avarua, Mangaia) (NZAC, Dobson notebooks, LUNZ), **Easter Island** (Easter Island) (HM), **Fiji** (Taveuni, Viti Levu, Vanua Levu) (MAF, Dobson notebooks, NZAC, LUNZ), **Gilbert Islands** (Tebanga, Tarawa, Onotoa, Abemama) (Dobson notebooks, Gillogly 1962), **Guam** (Guam) (Gillogly 1962), **Hawaii** (Oahu, Hawaii, Midway Island) (Ewing & Cline 2005, Murray 1864, Nishida & Beardsley 2002), **West Papua** (Waigeo, Aru Islands) (Dobson notebooks), **Kiribati** (Tarawa) (NZAC), **Marquesas Islands** (Tahuata, Hiva Oa, Nuku Hiva, Fatu Hiva) (Dobson notebooks), **Mariana Islands** (Saipan, Tinian, Rota) (Gillogly 1962), **Marshall Islands** (Wotho, Lae, Kwajalein, Likiep, Jemo, Jaluit, Arno) (Gillogly 1962), **Nauru** (Nauru) (LUNZ), **New Caledonia** (Grande Terre, Kavatch) (NZAC, LUNZ, Dobson notebooks, QM), **Niue** (Niue) (NZAC, Dobson notebooks, HM), **Palau** (Angaur, Peleliu, Garakayo (Ngergoi)) (Gillogly 1962), **Papua New Guinea** (Morobe Province, Madang Province) (Dobson notebooks), **Samoa** (Upolu) (NZAC, LUNZ, Dobson notebooks, HM), **Society Islands** (Tahiti, Raiatea, Moorea) (LUNZ, Dobson notebooks), **Solomon Islands** (Guadalcanal, San Cristobal, Choiseul, Ontong Java) (Dobson notebooks), **Tokelau** (Nukunono) (NZAC, HM), **Tonga** (Tongatapu, Vava'u, Eua, Lifuka, , Niuafo'ou) (NZAC, MAF, Dobson notebooks), **Tuamotu Archipelago** (Fakarava, Napuka, Nabuka) (Dobson notebooks), **Austral Islands** (Rurutu, Rimatara) (Dobson notebooks, LUNZ), **Tuvalu** (Funafuti, Wallis, Rotuma) (NZAC, LUNZ, HM), **Vanuatu** (Mai Island, Malekula, Efate, Aneityum, Erromango, Espiritu Santo) (Dobson notebooks, OUMNH, NZAC, LUNZ).

Extralimital distribution: **Christmas Is.**, **Australia** (Queensland, , Northern Territory, Thursday Island), **Philippines**, **Cuba**, **Cocos-Keeling Is**, **Indonesia**, **Nicobar Islands**, **Mollucas**.

References: Murray (1864) (original description); Leschen & Marris (2005), Gillogly

(1962) (descriptions and keys); Connell (1977); Gillogly (1969); Gorham (1987), Ewing & Cline (2005) (keys), Dobson (1955) (key and genitalia diagrams).

Carpophilus (Semocarpolus) marginellus Motschulsky, 1858

nitens Fall, 1910

Comment: This species is cosmopolitan, and is widespread throughout the Pacific region. It has been found from rotting fruit, stored products (Audisio, 1993) and is known to pollinate Annonaceae species (Blanche & Cunningham, 2005).

Collection dates: January, February, March, April, May, June, July, August, October, November, December.

Species associations: *Artocarpus heterophylla, Averrhoa carambola, Capsicum* sp., *Carica papaya, Citrus* sp., *Cocos nucifera, Dahlia* sp., leaf litter and compost, to light, *Inocarpus* sp., *Lycopersicon esculentum, Mangifera indica, Metrosideros excelsa, Musa* sp., *Solanum melongena, Zea mays.*

Distribution: **Bismarck Archipelago** (East New Britain) (LUNZ), **Caroline Islands** (Wena (Moen)) (Gillogly 1962), **Cook Islands** (Rarotonga) (NZAC), **Fiji** (Viti Levu, Moturiki) (LUNZ, NZAC, Dobson notebooks), **Hawaii** (Oahu) (Ewing & Cline 2005, LUNZ), **Marshall Islands** (Eniwetok) (Gillogly 1962), **New Caledonia** (Grande Terre) (OUMNH), **Society Islands** (Tahiti) (LUNZ, Nishida 2008), **Tonga** (Tongatapu, Eua) (NZAC), **Tuvalu** (Rotuma) (LUNZ).

Extralimital distribution: **Australia** (New South Wales, Queensland, Victoria), **New Zealand** (Mayor I., Nelson, Auckland), **Philippines**, **Bonin Islands**, **Singapore**, **Taiwan**, **South Africa**, **Guinea**, **Ghana**, **Seychelles**.

References: Murray (1864), Leschen & Marris (2005); Audisio (1993); Gillogly (1962) (descriptions); Hayashi (1978) (larval description); Gorham (1987) (key to adults and larvae); Gillogly (1969); Ewing & Cline (2005); Connell (1977) (keys); Dobson (1955) (key and genitalia diagrams).

Carpophilus (Ecnomorphus) mcnamarai Dobson, 1995

Comment: Known only from the original description. Not seen during this research.

Collection dates: Not known.

Species associations: Not known

Distribution: **Papua New Guinea** (Oro (Northern) Province) (Dobson 1993a).

Extralimital distribution: None.

References: Dobson (1993a)(original description).

Carpophilus mutabilis Fairmaire, 1849

Comment: Based solely on the description, Murray (1864) considered this species to be very similar to his *C. vittiger*, but because he had not seen specimens he left it for future workers to determine. Dobson (pers comm.) has dissected specimens of *C. mutabilis* and considers them identical to *C. maculatus*. This synonym has not been formalised. As the name *C. mutabilis* would have priority, it is considered best to leave this situation until such a time as a thorough revision is undertaken of the genus. Not collected during this research.

Collection dates: Not known.

Species associations: "oranges and citrons".

Distribution: **Society Islands** (Tahiti) (Murray 1864, Nishida 2008).

Extralimital distribution: None.

References: Murray (1864) (description), Dobson (1993b) (confusion with *C. oculatus*).

Carpophilus (Myothorax) mutilatus Erichson, 1843

luridus Murray, 1864

Comment: Collected throughout the region. This species is a serious pest in crops including maize in the United States (Arbogast & Throne, 1997) and stone fruit orchards in southern Australia (James *et al.*, 1995; Hossain *et al.*, 2008), and is implicated in the severe damage of cycads in Micronesia (Marler & Muniappan, 2006). It is also a key pollinator species, particularly of Annonaceae (Klein *et al.*, 2007; Blanche & Cunningham, 2005). The aggregation pheromone has been synthesised (Bartelt *et al.*, 1993) and has been used for management of the species (Hossain *et al.*, 2008). Its larval development has been studied by James & Vogele (2000).

This species is very similar to *C. nepos*, *C. dimidiatus*, and *C. truncatus*, but can be distinguished from them by the characters shown in Table 2.2. In several publications, it is stated that the male mandibles are grossly asymmetrical (Leschen & Marris, 2005). Within the series available to me, while in some cases this is true, it is by no means a consistent characteristic of the species.

Collection dates: January, February, March, April, May, June, July, August, September, October, November, December.

Species associations: *Artocarpus altitis, Calophyllum* sp., *Capsicum* sp., *Carica papaya, Citrus* sp., *Citrus limon, Cocos nucifera, Cucumis melo, Cycas* sp., *Cydonia oblonga,* to light, *Helianthus annuus, Hibiscus* sp., *Malus* sp., *Mangifera indica, Pandanus* sp., *Passiflora* sp., *Prunus persica, Saccharum* sp., *Solanum auriculatum, Zea mays.*

Distribution: **Caroline Islands** (Ponape, Kusaie) (Gillogly 1962), **Cook Islands** (Rarotonga) (NZAC), **Fiji** (Viti Levu) (NZAC), **Gilbert Islands** (South Tarawa) (NZAC), **Guam** (Guam) (Gillogly 1962), **Hawaii** (Kauai, Oahu, Molokai) (Ewing & Cline 2005, LUNZ), **Mariana Islands** (Saipan) (Gillogly 1962), **Marshall Islands** (Eniwetok, Jemo) (Gillogly 1962), **Nauru** (Nauru) (LUNZ), **New Caledonia** (Grande Terre, Lifu) (LUNZ, NZAC, OUMNH), **Samoa** (Upolu) (NZAC), **Society Islands** (Moorea, Tahiti) (LUNZ), **Solomon Islands** (Guadalcanal, Honiara) (Dobson notebooks), **Vanuatu** (Erromango, Malekula, Espiritu Santo, Epi) (Dobson notebooks, OUMNH).

Extralimital distribution: **Australia** (Northern Territory, New South Wales),

Philippines, **South India**, **Cyprus**, **Honduras**, **Morocco**, **United States of America** (Florida, California).

References: Murray (1864); Leschen & Marris (2005); Audisio (1993); Gillogly (1962) (descriptions and keys); Hayashi (1978) (larval description); Jr (1963) (pupa description); Gorham (1987) (key to adults and larvae); Ewing & Cline (2005); Connell (1977); Gillogly (1969) (keys); Dobson (1955) (key and genitalia diagrams).

***Carpophilus (Myothorax) nepos* Murray, 1864**

freemani Dobson, 1956 (Kirejtschuk, 1996)

Comment: This species has been recorded as being a pest on corn (Arbogast & Throne, 1997) and pollinator of Annonaceae (Blanche & Cunningham, 2005). It has also been used in laboratory experiments as it is easy to culture (Brandhorst *et al.*, 2000). Research has been conducted on the aggregation pheromones of this species under the name *C. freemani* (Bartelt & Weisleder, 1996).

This species was found much more extensively through the Pacific than was previously recorded. Though very similar to *C. mutilatus* in particular, it can be differentiatied from other similar species using the characters in Table 2.2. On average, it is the smallest *Carpophilus* specimens commonly encountered in the Pacific.

Collection dates: January, February, March, April, May, June, July, August, September, October, December.

Species associations: *, Artocarpus altitis, Averrhoa carambola, Avicennia marina, Capsicum* sp.*, Carica papaya, Citrus* sp.*, Cocos nucifera,* Arecaceae spp.*, Ipomoea batatas, Mangifera indica, Manihot esculenta, Musa* sp.*, Saccharum* sp.*, Solanum melongena, Spondias dulcis, Veitchia joanna, Zea mays.*

Distribution: **Bismarck Archipelago** (East New Britain) (LUNZ), **Cook Islands** (Rarotonga) (LUNZ), **Fiji** (Viti Levu, Quisenberg) (LUNZ, NZAC, Dobson notebooks), **Hawaii** (Oahu) (Ewing & Cline 2005, LUNZ), **New Caledonia**

(Grande Terre) (LUNZ, OUMNH), **Papua New Guinea** (Morobe Province, Morobe District) (Dobson notebooks), **Samoa** (Tutuila) (NZAC), **Society Islands** (Tahiti) (LUNZ), **Tuvalu** (Funafuti) (NZAC), **Vanuatu** (Efate, Espiritu Santo) (LUNZ).

Extralimital distribution: **Australia** (Queensland, Northern Territory), **French Guiana**, **Morocco**, **Angola**, **United States of America** (Florida, California, Delaware, Illinois).

References: Murray (1864) (description); Dobson (1956) (description of *C. freemani*); Hayashi (1978) (larval description); Gorham (1987) (key to adults and larvae); Ewing & Cline (2005); Connell (1977); Audisio (1993) (keys); Kirejtschuk (1996); Jelínek & Audisio (2007)(synonymy).

Carpophilus (Carpophilus) obesus Murray, 1864

Comment: This species was originally described from New Guinea. Although it is widespread through South Asia and Australia, little is known of its biology. It is figured in (Leschen & Marris, 2005). Not collected in this research.

Collection dates: February, April, May, June, October.

Species associations: Not known.

Distribution: **West Papua** (Waigeo, Vogelkop, Aru Islands) (Dobson notebooks, Murray 1864), **Papua New Guinea** (Oro (Northern) Province, Madang Province) (Dobson notebooks).

Extralimital distribution: **India**.

References: Murray (1864) (original description), Leschen & Marris (2005) (picture).

Carpophilus (Carpophilus) obsoletus Erichson, 1843

cribellatus Motschulsky, 1858
funereus Reitter (non Murray), 1884
immaculatus Lucas, 1849

sericeus Motschulsky, 1858
strigipennis Motschulsky, 1858

Comment: This species is cosmopolitan and has been recorded from a number of Pacific Islands. It is found frequently in stored products, and has been known to cause severe damage to goods such as rice, wheat and maize (Hinton, 1945). In Taiwan, five to six generations per year have been recorded, with adults living between 150 and 200 days on average (Hinton, 1945). The aggregration pheromone of this species has been characterised and synthesised (Petroski *et al.*, 1994).

Collection dates: January, February, March, April, May, June, July, August, September, October, November, December.

Species associations: *Allium sativum, Ananas comosus, Arachis hypogaea, Bixa orellana, Camellia sinensis, Cannabis* sp., *Capsicum* sp., *Carica papaya, Cocos nucifera, Coffea* sp., *Colocasia esculenta, Dioscorea* sp., stored products, *Ficus carica, Gossypium* sp., *Litchi chinensis, Musa* sp., *Oryza sativa, Phoenix dactylifera, Theobroma cacao, Triticum* sp., *Vitis vinifera, Zea mays, Zizyphus* sp..

Distribution: **Bismarck Archipelago** (East New Britain) (LUNZ), **Cook Islands** (MAF), **Fiji** (MAF), **West Papua** (New Guinea) (Dobson notebooks), **New Caledonia** (MAF), **Palau** (Koror) (Gillogly 1962), **Papua New Guinea** (Madang Province) (Dobson notebooks), **Samoa** (MAF), **Solomon Islands** (Russell Is., Guadalcanal, Isabel, Gizo) (Hinton 1945, Dobson notebooks), **Tonga** (MAF), **Vanuatu** (Aneityum) (Dobson notebooks).

Extralimital distribution: **Australia** (Queensland), **Israel**, **Iraq**, **Sudan**, **Sheadi**, **Malaysia**, **Hong Kong**, **Italy**, **Nigeria**, **Sicilia**, **Indonesia**, **Tanzania**, **Morocco**, **Taiwan**, **Sri Lanka**, **Thailand**, **Namibia**, **Ghana**, **Guinea**, **Equatorial Guinea**, **Gambia**, **Eithiopia**, **United States of America**.

References: Murray (1864), Leschen & Marris (2005); Gillogly (1962); Audisio (1993); Hinton (1945) (descriptions and keys); Connell (1977); Gorham (1987) (key); Dobson (1954) (characterisation of male and female genitalia); Dobson (1955) (key and genitalia diagrams).

***Carpophilus (Myothorax) oculatus* Murray, 1864**

Comment: Dobson (1993b) described three subspecies of this species based largely on male genitalia and subtle differences in punctation of the pronotum. The following are further characters observed during this study that serve to differentiate the subspecies.

C. o. oculatus: Pronotal punctures sparse, round and fine on disc, becoming reniform and coarser toward margin, no impunctate medial line; microsculpture minutely reticulate coriarious; usually less pubescent. Female pygidium truncate.

C. o. gilloglyi: Pronotal punctures on vertex and disc of pronotum close and reniform, impunctate median line anterior of scutellum; microsculpture reticulate coriarious, coarser than in other subspecies. Female pygidium emarginate medially.

C. o. cheesmani: Pronotum coarsely and densely punctured with reniform punctures, becoming almost contiguous basally, creating obliquely transverse lines; microsculpture minutely reticulate coriarious. Female pygidium truncate.

C. o. oculatus and *C. o. gilloglyi* have broad distributions through the central and eastern Pacific, while *C. o. cheesmani* is restricted to Vanuatu. In Dobson's notebooks there are some records of specimens from Papua New Guinea and the Solomon Islands. However they were not mentioned in his 1993 publication on the species, suggesting these records may be erroneous.

Prior to this study, *C. o. cheesmani* was the only subspecies known from Vanuatu. However, the results of field work for this research showed that *C. o. oculatus* is very common on Espiritu Santo and Efate, while

C. o. cheesmani was rarely found. A difference in abundance between the two subspecies was also noted in localities where *C. o. oculatus* and *C. o. gilloglyi* exist in sympatry. On Viti Levu, Vanua Levu and Tahiti, *C. o. gilloglyi* was found much more abundantly than *C. o. oculatus*, whereas on Taveuni, *C. o. oculatus* was more commonly collected.

Collection dates: indet subspecies : January, February, March, April, May, June, July, August, September, October, November, December.

C. o. oculatus: January, February, March, April, May, June, July, August, September, October, November.

C. o. gilloglyi: January, February, March, April, May, June, July, August, September, October, November, December.

C. o. cheesmani: January, February, March, April, August, September.

Species associations: indet subspecies : *Ananas comosus, Capsicum* sp., *Citrus* sp., *Colocasia esculenta, Dioscorea* sp., stored products, to light, *Inocarpus* sp., *Lycopersicon esculentum, Mangifera indica, Nerium oleander, Ochrosia* sp., *Pandanus* sp., *Pithecellobium dulce.*

C. o. oculatus: *Artocarpus altitis, Averrhoa bilimbi, Averrhoa carambola, Barringtonia butonica, Chrysophyllum cainito, Citrus* sp., *Cocos nucifera, Colocasia esculenta, Dioscorea* sp., leaf litter and compost, stored products, to light, *Inocarpus* sp., *Ipomoea batatas, Leptospermum scoparium, Lycopersicon esculentum, Mangifera indica, Musa* sp., *Ochrosia* sp., *Passiflora* sp., *Persea americana, Spondias dulcis, Veitchia joanna, Zea mays.*

C. o. gilloglyi: *Anacardium occidentale, Artocarpus altitis, Artocarpus heterophylla, Averrhoa bilimbi, Barringtonia butonica, Carica papaya, Chrysophyllum cainito, Citrus* sp., *Citrus limon, Cocos nucifera, Coffea* sp., *Colocasia esculenta,* leaf litter and compost, to light, *Entada phaseoides, Freycinetia* sp., *Inocarpus* sp., *Mangifera indica, Manihot esculenta, Musa* sp., *Myoporum laetum, Puffinus assimilis, Puffinus pacificus, Schinus* sp., *Zea mays, Zingiber officinale.*

C. o. cheesmani: *Artocarpus altitis, Citrus* sp., *Ipomoea batatas, Psidium guajava.*

Distribution: indet subspecies : **Caroline Islands** (Wena (Moen), Fefan, Nama, Ponape, Kusaie, Pohnpei) (Gillogly 1962, Ewing & Cline 2005), **Cook Islands** (Rarotonga, Rorotonga) (MAF, Dobson notebooks), **Fiji** (Koro, Taveuni, Ovalau, Wakaya) (Dobson notebooks), **Hawaii** (Oahu) (MAF, Ewing & Cline 2005, Dobson notebooks), **Marquesas Islands** (Fatu Hiva, Tahuata, Hiva Oa) (Dobson notebooks), **Mariana Islands** (Saipan) (Gillogly 1962), **Marshall Is-**

lands (Likiep) (Gillogly 1962), **Niue** (Niue) (Dobson notebooks), **Papua New Guinea** (Morobe District) (Dobson notebooks), **Samoa** (Upolu) (MAF, Dobson notebooks), **Society Islands** (Tahiti, Raiatea) (MAF, Dobson notebooks), **Solomon Islands** (Guadalcanal) (Dobson notebooks), **Tokelau** (Nukunona) (Dobson notebooks), **Tonga** (Tongatapu) (MAF, Dobson notebooks), **Austral Islands** (Rapa) (Dobson notebooks).

C. o. oculatus: **Cook Islands** (Rarotonga) (Dobson 1993b, NZAC), **Fiji** (Taveuni, Kadavu, Viti Levu, Vanua Levu) (Dobson 1993b, LUNZ, MAF, USP, NZAC), **Hawaii** (Dobson 1993b), **Marquesas Islands** (Fatu Hiva) (Dobson 1993b), **Nauru** (Nauru) (LUNZ), **New Caledonia** (Grande Terre) (QM, NZAC), **Niue** (Niue) (NZAC), **Samoa** (Upolu, Tutuila) (HM, NZAC), **Society Islands** (Bora Bora, Tahiti) (Dobson 1993b, LUNZ), **Tokelau** (Nukunono) (NZAC), **Tonga** (Tongatapu) (Dobson 1993b, NZAC, MAF, HM, LUNZ), **Tuvalu** (Rotuma) (LUNZ), **Vanuatu** (Espiritu Santo, Efate) (LUNZ).

C. o. gilloglyi: **Caroline Islands** (Ponape Island, Truk) (Dobson 1993b), **Cook Islands** (Rarotonga, Atiu) (Dobson 1993b, NZAC, MAF), **Easter Island** (Easter Island (Rapa Nui)) (NZAC), **Fiji** (Vanua Levu, Taveuni, Viti Levu, Kadavu, Ovalau, Lakeba) (LUNZ, Dobson 1993b, USP, NZAC), **Kermadec Islands** (Meyer Island, Raoul Island, Chanters Islets) (Dobson 1993b, NZAC, LUNZ), **Niue** (Niue) (Dobson 1993b, NZAC), **Samoa** (Upolu, Savai'i) (NZAC, HM), **Society Islands** (Bora Bora, Moorea, Tahiti) (Dobson 1993b, LUNZ), **Tonga** (Tongatapu, Vavau Island, Vava'u, Eua, Niuafo'ou) (Dobson 1993b, NZAC, MAF, HM, LUNZ), **Austral Islands** (Rapa, Rimatara) (LUNZ), **Tuvalu** (Rotuma) (LUNZ).

C. o. cheesmani: **Vanuatu** (Malekula, Ounua, Tanna, Erromango, Efate) (Dobson 1993b, LUNZ).

Extralimital distribution: None.

References: Murray (1864) (Original description); Dobson (1993b) (description of subspecies); Leschen & Marris (2005); Gillogly (1962) (descriptions and keys); Ewing & Cline (2005) (key)

Carpophilus (Myothorax) pallescens Murray, 1864

Comment: Known only from the original description, with the exception of some specimens recorded by Dobson. According to the original description, this species is very similar to *C. dimidiatus*, differing from it by being nearly

impunctate. Not collected during this research.

Collection dates: January, February, May, July, September.

Species associations: Not known.

Distribution: **West Papua** (Moluccas, Waigeo) (Dobson notebooks, Murray 1864), **Papua New Guinea** (Central Province) (Dobson notebooks), **Solomon Islands** (Guadalcanal) (Dobson notebooks), **Vanuatu** (Malekula, Tanna) (Dobson notebooks).

Extralimital distribution: None.

References: Murray (1864) (original description).

Carpophilus (Myothorax) schioedtei Murray, 1864

Comment: A little known species known only from South East Asia, with the exception of a record from Hawaii by Kirejtschuk (2005). This record was not mentioned by Ewing & Cline (2005), and Kirejtschuk (2005) gave no further details. Therefore, until further specimens are collected from Hawaii, this record is considered to be doubtful. Not collected during this research.

Collection dates: July, November.

Species associations: Not known.

Distribution: **Hawaii** (Hawaii) (Kirejtschuk 2005), **West Papua** (New Guinea) (Kirejtschuk 2005).

Extralimital distribution: **Philippines**, **Taiwan**, **Vietnam**, **Laos**, **Thailand**, **India**, **Malaysia**, **Indonesia**.

References: Murray (1864) (Original description); Gillogly (1969) (key).

Carpophilus (Ecnomorphus) terminalis (Murray, 1864)

terminalis Murray, 1864 (*Stauroglossicus*)
gentilis Murray, 1864 (Kirejtschuk, 2008)

Comments: This species has been recorded from Fiji as *C. gentilis*, which has been recently synonymised by Kirejtschuk (2008). Very little is known

of its biology. No specimens were sighted during the course of this research. This species is pictured in Leschen & Marris (2005).

Collection dates: January.

Species associations: Not known.

Distribution: **Fiji** (Fiji Islands) (Evenhuis 2009).

Extralimital distribution: **Australia** (New South Wales, Victoria).

References: Murray (1864) (description); Evenhuis (2009) (Fiji record); Leschen & Marris (2005) (picture).

***Carpophilus (Myothorax) truncatus* Murray, 1864**

floridanus Fall, 1910
halli Dobson, 1954

Comment: This species is very similar to *C. dimidiatus*, being most easily distinguished by the shape of the hind tibia (Table 2.2). Unfortunately, this character is restricted to the male and very few characters separate females from other species in the *Myothorax* subgenus.

A widespread species, and one that is known to pollinate Annonaceae (Nagel *et al.*, 1989).

Specimens identified and published as being *C. pilosellus* belong to this species. It is not a synonym however, as the type specimen of *C. pilosellus* is actually considered to be a member of *C. mutilatus* (Kirejtschuk, 1996; Jelínek & Audisio, 2007; Leschen & Marris, 2005). Not collected during this research.

Collection dates: January, February, March, April, May, June, July, August, September, October, November, December.

Species associations: *Cocos nucifera, Coffea* sp., stored products, to light, *Litchi chinensis, Musa* sp., *Oryza sativa, Prunus persica, Triticum* sp..

Distribution: **Caroline Islands** (Yap, Kapingamarangi, Ifaluk, Kusaie) (Gillogly 1962), **Cook Islands** (MAF), **Fiji** (Rotuma, Ovalau, Taveuni) (MAF, Dobson notebooks), **Gilbert Islands** (Onotoa) (Gillogly 1962), **Guam** (Guam) (Gillogly 1962), **West Papua** (Biak Is) (Dobson notebooks), **Mariana Islands** (Saipan, Tinian, Rota) (Gillogly 1962), **Marshall Islands** (Eniwetok, Jaluit) (Gillogly 1962), **Niue** (Niue) (MAF, Dobson notebooks, NZAC), **Palau** (Kayangel, Koror) (Gillogly 1962), **Papua New Guinea** (Morobe Province, Madang Province) (Dobson notebooks, MAF), **Solomon Islands** (Guadalcanal, New Georgia, Gizo, Santa Cruz, Malaita) (Dobson notebooks, MAF), **Tuvalu** (Funafuti) (NZAC), **Vanuatu** (Malekula) (Dobson notebooks).

Extralimital distribution: **Australia** (New South Wales, Queensland, Northern Territory, Victoria), **New Zealand** (South Island), **Sudan**, **China**, **Indonesia**, **Italy**, **Bonin Islands**, **Botswana**, **Taiwan**, **South Africa**, **Madagascar**, **Seychelles**, **Morocco**.

References: Murray (1864) (original description), Leschen & Marris (2005); Gillogly (1962); Audisio (1993) (description and key); Hayashi (1978) (larval description); Gorham (1987) (key to adults and larvae); Connell (1977) (key); Kirejtschuk (1996); Jelínek & Audisio (2007) (synonymy of *C. pilosellus*).

***Carpophilus (Ecnomorphus) ustulatus* Murray, 1864**

Comment: Originally described from New Guinea, this species is also found in Australia. A photograph of this species was published in Leschen & Marris (2005).

Collection dates: Not known.

Species associations: Not known.

Distribution: **West Papua** (Vogelkop) (Murray 1864), **Papua New Guinea** (New Guinea) (Williams *et al.* 1983).

Extralimital distribution: None.

References: Murray (1864) (original description), Leschen & Marris (2005) (picture).

Carpophilus (Carpophilus) variolosus Murray, 1864

Comment: Originally described from Sarawak (Murray, 1864) but is stated as being in Papua New Guinea by Williams *et al.* (1983). These two publications contain all that is known about this species. Further collecting is required to confirm that this species is present in PNG.

Collection dates: Not known.

Species associations: Not known.

Distribution: **Papua New Guinea** (New Guinea) (Williams *et al.* 1983).

Extralimital distribution: **Indonesia**.

References: Murray (1864) (original description).

Carpophilus (Ecnomorphus) waterhousei Dobson, 1995

Comment: The description of this species closely matches that of *C. inconspicuus* and *C. frivolus*.

Collection dates: May, July, August.

Species associations: leaf litter and compost.

Distribution: **Papua New Guinea** (Oro (Northern) Province) (Dobson 1993a, Dobson notebooks), **Solomon Islands** (Guadalcanal, San Cristobal) (Dobson 1993a, Dobson notebooks).

Extralimital distribution: None.

References: Dobson (1993a) (original description).

Loriarulus poggi (Kirejtshuk, 1987)

poggi Kirejtshuk, 1987 (*Carpophilus*)

Comment: This species is only known from the original description. Nothing is known of its biology.

Collection dates: Not known.

Species associations: Not known.

Distribution: **Papua New Guinea** (Gulf Province) (Kirejtschuk 1987).

Extralimital distribution: None.

References: Kirejtschuk (1987) (original description); Kirejtschuk (1997) (Comparison with *Carpophilus* subgenera).

***Urophorus humeralis* (Fabricius, 1798)**

picinus Boheman, 1851
punctatus Fleutiaux, 1887
*ricksecker*i Fall, 1910

Comment: This widespread species is easily distinguished from the other carpophiline species in the region by having three heavily sclerotised tergites visible dorsally. It is very commonly found in rotting fruit and vegetables (Connell, 1981), attracted to yeast volatiles (Nout & Bartelt, 1998), and is known to assist in the pollination of Annonaceae (Blanche & Cunningham, 2005).

This species is susceptible to entomopathogenic nematodes of the genus *Heterorhabditus* (Glazer *et al.*, 1999) to the extent that they are being investigated as a possible biological control agent for the species (Glazer *et al.*, 2007). Its larval development was studied by James & Vogele (2000).

The genus *Urophorus* in the past has been considered to be a subgenus of *Carpophilus*. Gillogly (1962) first elevated *Urophorus* to a full genus, on the basis of the abdominal structure. This placement has not been accepted by all workers however, and the species often appears in the literature as *C. humeralis* Connell (1981).

Collection dates: January, February, March, April, May, June, July, August, September, October, November, December.

Species associations: *Ananas comosus, Annona muricata, Annona squamosa, Artocarpus altitis, Artocarpus heterophylla, Averrhoa bilimbi, Averrhoa carambola, Capsicum* sp., *Citrus* sp., *Cocos nucifera, Durio* sp., leaf litter and compost, to light, *Freycinetia* sp., *Ipomoea batatas, Lycopersicon esculentum, Malus* sp., *Mangifera indica, Manihot esculenta, Metrosideros polymorpha, Musa* sp., *Musa popoulu, Passiflora* sp., *Persea americana, Plumeria rubra, Prunus persica, Psidium guajava, Solanum tuberosum, Spondias* sp.

Distribution: **Bismarck Archipelago** (New Britain) (LUNZ), **Caroline Islands** (Yap, Map, Ulithi, Wena (Moen)) (Gillogly 1962), **Cook Islands** (Rarotonga) (LUNZ), **Fiji** (Vanua Levu, Taveuni, Viti Levu) (LUNZ, NZAC), **Gilbert Islands** (Tarawa) (Gillogly 1962, NZAC), **Guam** (Guam) (Gillogly 1962), **Hawaii** (Kauai, Oahu, Molokai, Lanai, Maui, Hawaii) (Ewing & Cline 2005), **Mariana Islands** (Saipan, Tinian, Rota) (Gillogly 1962), **Marshall Islands** (Eniwetok) (Gillogly 1962), **New Caledonia** (Grande Terre) (LUNZ), **Palau** (Koror, Peleliu) (Gillogly 1962), **Samoa** (Upolu) (LUNZ), **Society Islands** (Tahiti) (Nishida 2008), **Solomon Islands** (Guadalcanal, Rendova, Isabel, Malaita) (Dobson notebooks), **Tonga** (Tongatapu) (LUNZ), **Vanuatu** (Efate) (NZAC).

Extralimital distribution: **Australia** (Victoria, New South Wales, Thursday Island, Queensland, Northern Territory, South Australia, Torres Strait), **Bonin Islands**, **Indonesia**, **Nigeria**, **Cocos-Keeling Is**, **Britain**, **India**, **Morocco**, **United States of America** (California).

References: Gillogly (1962) (key, description and elevation to genus); Hayashi (1978) (larval description); Gorham (1987) (key to adults and larvae); Dobson (1955) (key and genitalia diagrams); Connell (1977); Ewing & Cline (2005) (keys); Connell (1981) (bibliography and discussion of nomenclature).

Indeterminate species

Specimens representing at least four other species that have not been able to be identified with certainty were collected during the course of this study. Two of these species were sequenced as part of the molecular systematics research detailed in Chapter 3. These were found to be sufficiently distinct and collected with enough specimens to be named and described in a future paper. Other species are represented by short series and require further

specimens to be collected before they can be described. In particular, a single specimen of a very distinctive species with superficial similarities to *C. marginellus* was collected from leaf litter in Fiji.

The presence of these unidentified specimens present in collections show that the diversity of *Carpophilus* in the Pacific is not yet fully known. The collecting efforts conducted for the purpose of this research did not recover all species known from the region, as the primary focus was on economically important and widespread species of fruit with importance for biosecurity. Further collecting efforts should investigate a range of other habitats, particularly leaf-litter and non-commercial fruits in less disturbed habitats.

2.4 Distribution

Most island regions in the Pacific have between six and ten species of Carpophilinae. The most widespread species was *C. maculatus* being found in all island regions with the exception of the Kermadec Islands. The second most widespread is *C. dimidiatus* which is frequently found on smaller islands, possibly as a result of copra trading. Many of the widespread species (*C. dimidiatus*, *C. hemipterus*, *C. marginellus*, *C. mutilatus*, *C. nepos*, *C. obsoletus*, *C. truncatus* and *U. humeralis* are cosmopolitan and are associated with stored products and commercial crops suggesting that these species may be adventive to the region.

Papua New Guinea (PNG) has the greatest species diversity with 19 species recorded. This is not surprising as it is the largest landmass in the Pacific and is the closest to South East Asia which is the centre of diversity for the genus. Not as many species have been recorded from West Papua, most likely because it has not been explored entomologically to the same extent. Interestingly the widespread and common species *C. marginellus*,

C. mutilatus, and *U. humeralis* have not been recorded from mainland PNG as well as *C. davidsoni* known from Micronesia and Australia, north and south of the country respectively. It is expected that further collecting will reveal these species also.

Fiji has the next highest number of species in the region, with 12 species. This high diversity may reflect Fiji's status as the 'hub of the Pacific' with high volumes of trade and produce being moved through its borders.

Species lists per region are given in Appendix B, and are summarised in Table 2.1.

2.5 Natural History

During field collections in Fiji, Tahiti and Vanuatu, the following observations were noted. *Carpophilus* in rotting fruit are often found along with the nitidulid species *Phenolia* spp., *Epuraea ocularis* and an unidentified aleocharine staphylinid beetle; the latter is characterised by a distinctive yellow and black colouration with the male possessing projections of the posterior angles of the elytra and medial edges of tergites. Less commonly found are *Stelidota* spp., other *Epuraea* spp. (both Nitidulidae) and some species of Hydrophilidae.

Field observations suggest that there may be some degree of succession in the beetle community found in decaying fruit. However, the variability of species composition and abundance in these fruit is very high and a study investigating this succession would require very high sample sizes to detect the change. There may also be a partitioning of the fruit between the different nitidulid species. *Carpophilus* has frequently been observed in the outer layer of the fruit, between the flesh and skin or pith. *Phenolia* on the other hand are frequently found inside the flesh of the fruit.

Several specimens of *C. maculatus*, *C. mutilatus*, *C. hemipterus* and *C. nepos* from Tahiti and Rarotonga collected in May and August respectively were found to have phoretic mite deuteronymphs of the family Uropodidae (Bajerlein & Błoszyk, 2004) attached to the legs and abdomen. These were sometimes found in relatively high abundance, with up to four being found on one specimen.

2.6 Morphology

The shapes of the prosternal process and the abdominal intercoxal process between the metacoxae were seen to vary between species; these may be important characters to develop in future work on *Carpophilus*. More detailed investigation of the male median lobe and female genitalia, though endorsed by Dobson (Dobson, 1954), has not yet been investigated at a broad scale. The male median lobe is very membranous, but cursory examination during this research indicates that there is variation which needs to be characterised. The 3-dimensional structure of the lateral lobes is also important but difficult to quantify; however advances in techniques such as laser-scanning confocal microscopy (Polilov & Beutel, 2010; Lee *et al.*, 2009) may provide more accurate methods description of these important structures.

2.6.1 Sexual dimorphism

There is significant sexual dimorphism in *Carpophilus*. As well as the very obvious differences in the structure of the terminal abdominal segments, a number of other structures also vary according to sex; in particular the legs and mandibles. The tibiae of male *Carpophilus* tend to be more expanded than in females, and tend to have stouter spines on the apical margin. Tarsi also tend to be expanded in comparison with females and are much more

hirsute, presumably to assist with grasping the female during copulation. The mandibles of some male *Carpophilus* species are asymmetrical, with one being larger and/or a different shape to the other. As a general rule, males also tend to be hairier than females. This is particularly so for *C. hemipterus*, where the males have long, obvious, golden pubescence on the prosternal process and gular region; in females this pubescence is much shorter and less conspicuous. Female *C. hemipterus* also have a vague, impunctate area posteriorly on the disc of the pronotum, which is lacking in the males.

2.7 Summary and conclusions

The taxonomy of *Carpophilus* in general, and in the South Pacific in particular, remains in dire need of attention. A number of species described from the region are known only from the original descriptions, which in many cases are inadequate for accurate identification of unknown specimens. There also remain a number of species which are undescribed. The high morphological variation in *Carpophilus* is a major reason for the lack of taxonomic work on the group. This variation is caused by sexual dimorphism that is present in most species to a greater or lesser degree, and which is further confounded by high levels of variation between individuals of the same sex and species. This individual variation reaches its fullest extent in *C. maculatus*, individuals of which can be easily mistaken for specimens of *C. oculatus*, *C. mutilatus*, *C. nepos* and *C. dimidiatus*, as well as being very similar to a number of undescribed species. Details of the male genitalia, in particular the parameres (lateral lobes), are of most use for unequivocal determination of species, however a number of other characters including the shapes of the abdominal inter-coxal and prosternal processes, and details of the male and

female genitalia may be uncovered if studied in depth. Unfortunately, it was not possible in the time frame of this research to make an extensive study on morphological methods for the identification of *Carpophilus*. However, a LUCID key is intended to be produced subsequent to this thesis to assist in the identification of the more common species of *Carpophilus*. This has the benefits of being able to be extended in the future to encompass more species and characters, should the opportunity arise.

The collecting effort for this research primarily targeted known habitats of *C. oculatus*, and was heavily biased towards collecting from freshly rotting fruit and vegetables. These do not represent the typical habitats for some species, such as *C. dimidiatus*. Therefore, although *C. dimidiatus* is known to be present in essentially all South Pacific nations, it was collected only once during the course of this research. No doubt a number of other species will have likewise been missed by this biased sampling. Further research is necessary to determine the host specificity of *Carpophilus* species.

A number of the widespread species are cosmopolitan and associated with commercially important products suggesting that these species may be adventive to the region. Further research would be of interest to determine if this is the case and if so, what are the impacts these species are having in the region.

Chapter 3

Molecular systematics of *Carpophilus*

3.1 Introduction

The species, *C. oculatus* and *C. maculatus* are two of the most frequently intercepted species of *Carpophilus* found in fresh produce imported into New Zealand from the Pacific. The two can be difficult to distinguish from each other as they are similar in size, colouration, and both have variably-shaped colour patterns on the elytra. This similarity makes identification of these two species difficult, and misidentifications are common.

While morphological evidence for monophyly of the *C. oculatus* subspecies include the distinctive ring-shaped colour pattern and the pronotal L/W ratio (See Chapter 5), there are no clear and unambiguous synapomorphies that define the species. A sister taxon relationship is suggested between *C. oculatus* and *C. maculatus* by the sculpturing of the prosternum and by the shape of the male genitalia, which are very similar between these two species—more so than between other species in the same group. This

similarity is particularly striking between *C. maculatus* and *C. o. gilloglyi*, and has led some to surmise whether these two species form a single, hyper-variable species (Leschen & Marris, 2005).

There has been no formal systematic study of the genus and the relationships within *Carpophilus* remain largely unknown. Subgeneric classifications have remained in a state of flux, with differing placements proposed by Murray (1864), and Kirejtschuk (2008). However, the exact limits of these subgenera have not been expanded on in the context of an overall review of *Carpophilus* systematics.

This research also gives us the opportunity to investigate the utility of DNA barcoding (Hebert *et al.*, 2003a) for the identification of species within the genus. A number of other *Carpophilus* species, particularly within the *Myothorax* subgenus, are difficult to identify morphologically as they are only subtly different from each other.

3.1.1 Molecular systematics: an overview

Willi Hennig and Walter Zimmerman from 1930 to 1950 pioneered the formation of explicit criteria and principles in the inference of phylogeny. These criteria have been widely accepted and used from the 1960s until today. Over this same period, advances in molecular biology led to the development of protein sequencing and allozyme electrophoresis. From their inception, these molecular techniques were recognised to contain data of systematic interest. Subsequently, molecular biology and systematics have enjoyed much reciprocal illumination, with molecular data being of primary importance in the development of many of the algorithmic methods used to infer phylogenies (Felsenstein, 2004). The use of DNA sequences in molecular systematics was revolutionised by the development of Sanger DNA sequencing in 1977, and

the invention of the polymerase chain reaction (PCR) in 1985 (Beebee & Rowe, 2008).

The DNA barcoding concept (Hebert *et al.*, 2003a) advocates using a single gene (the mitochondrial gene cytochrome *c* oxidase subunit I, COI)) across all animal life, which would be of use for identification in cases where morphological identification was difficult or impossible. While the original papers emphasised the utility of barcoding primarily for identification purposes (Hebert *et al.*, 2003a,b) for which it has been applied to biosecurity (Armstrong & Ball, 2005), other papers purport to demonstrate its utility for DNA taxonomy (Hajibabaei *et al.*, 2006; Hebert *et al.*, 2004). This has made the concept a controversial one, as the analytical methods used in barcoding are based on pure distance measures that do not differentiate between homologous and homoplastic characters (Cognato, 2006). While most workers now believe that barcoding is a useful addition in an integrative taxonomic framework (Vences *et al.*, 2005; Mengual *et al.*, 2006), extreme views still do exist (Tautz *et al.*, 2003; Rubinoff *et al.*, 2006; Packer *et al.*, 2009).

In contrast to the non-recombining, matrilineal inheritance of mitochondrial DNA, nuclear markers are biparental and have the potential for recombination, thus reflecting a greater portion of the evolutionary history of the species. Many nuclear regions evolve more slowly than mitochondrial genes making them more suitable for higher-level systematics; however the ribosomal-encoding regions tend to evolve at a rate closer to that of the mitochondria and are regularly used for study on the species level and below (Caterino *et al.*, 2000). In particular, the nuclear markers Internal Transcribed spacer 2 (ITS2) and the D1-D2 region of the 28S ribosomal RNA unit have been proposed as being of use in determining species boundaries as nuclear equivalents of COI Sonnenberg *et al.* (2007); Coleman (2009), how-

ever they have not been characterised as fully as COI and have not attained as widespread use.

In this objective, the monophyly of *C. oculatus* and its relationship with *C. maculatus* was tested using molecular systematic techniques. The opportunity was also taken to provide a preliminary glimpse into the systematics of *Carpophilus*, by sampling a range of species within the genus and with an emphasis on species within the subgenus *Myothorax*. A molecular phylogeny of *Carpophilus* was constructed based on sequences of mitochondrial and nuclear gene regions from as many species as it was possible to obtain. This was used primarily to test the monophyly of *C. oculatus* and its subspecies, and to test for a sister-taxon relationship between *C. oculatus* and *C. maculatus*. It also provides a basis for the systematics of *Carpophilus* in general and will be useful for guiding future taxonomic work on the genus.

3.2 Methods

3.2.1 Taxon coverage and specimens

To test the monophyly of *C. oculatus*, the three subspecies of this species were collected preferentially. The relationship of *C. oculatus* with *C. maculatus* was also targeted, given the morphological similarity between them. Beyond this, other *Carpophilus* species were collected serendipitously by the author and colleagues, primarily from the Pacific, but from other localities also.

Specimens were collected by hand from rotting fruit and vegetables and preserved in propylene glycol in the field for transporting back to New Zealand. In the laboratory, specimens were sorted and stored in 100% ethanol.

A single leg was removed from each specimen to be used for molecular analysis and the remainder card-mounted as a voucher specimen.

3.2.2 Molecular methods

DNA was extracted using the prepGEM DNA extraction kit (ZyGEM Ltd, Hamilton, New Zealand). Incubation consisted of 30 min at 75℃ and 5 min at 95℃. This longer incubation period ensured that dehydrated tissues had sufficient time to rehydrate and lyse. DNA was amplified using a 10 µl PCR reaction containing 0.25 U Expand High-Fidelity Taq (Roche Applied Science, Indianapolis, IN, USA), 0.2 mM dNTPs, 2 mM $MgCl_2$ and 0.3 µM of both forward and reverse primers. To encourage DNA amplification in difficult specimens, 2 µl GC Rich mixture (Roche) was added to the reaction when necessary. The $5'$ end of the cytochrome *c* oxidase subunit I (COI) mitochondrial gene, the D1-D2 region of the 28S ribosomal RNA gene and the internal transcribed spacer 2 (ITS2) region in the nuclear ribosomal encoding cistrons were amplified using the primers listed in Table 3.1. For COI the combination LCO1490/HCO2198 was used preferentially, with TY-J-1460/C1-N-2191 used when these amplifications were unsuccessful. ITS2 amplifications required a primer concentration of 0.5 µM and 2 mM $MgCl_2$. Reactions were run on a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) with an initial denature of 94℃ for 2 min, followed by 40 cycles of 94℃ (15 s), 45℃ (30 s) and 72℃ (75 s), and with a final extension at 72℃ for 7 min. An annealing temperature of 54℃ was used for 28S reactions. Success was confirmed by running PCR products in 1.0% agarose gels made using a NaOH/borate buffer with a pH of 8.0; these were run at 170 V and 50 mA for 15 min. Gels were stained during casting with SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA)

Table 3.1: Markers and PCR primer combinations used in this research

Marker	Primer name	Primer sequence	Reference
COI	LCO1490	5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′	Folmer *et al.* 1994
	HCO2198	5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′	Folmer *et al.* 1994
	TY-J-1460	5′-TAC AAT TTA TCG CCT AAA CTT CAG CC-3′	Simon *et al.* 1994
	C1-N-2191	5′-CCC GGT AAA ATT AAA ATA TAA ACT TC-3′	Simon *et al.* 1994
28S	LSUfw2	5′-ACA AGT ACC DTR AGG GAA AGT TG-3′	Sonnenberg *et al.* 2007
	LSUrev1	5′-TAC TAG AAG GTT CGA TTA GTC-3′	Sonnenberg *et al.* 2007
ITS	CAS5p8sFc	3′-TGAA CAT CGA CAT TTY GAA CGC ACA T-5′	Ji *et al.* 2003
	CAS28sB1d	3′-TTC TTT TCC TCC SCT TAY TRA TAT GCT TAA-5′	Ji *et al.* 2003

and viewed with a GeneWizard Gel imaging system (SynGene, Cambridge, England). PCR products were sequenced in 10 µl reactions containing 0.3 µl of PCR product, 0.5 µl BigDye® Terminator (v3.1) (Applied Biosystems), 2 µl BigDye® Sequencing buffer (v1.1/3.1) and 0.8 µM of the primers used for amplification. Products were sequenced in both directions. PCR cleanup was done with CleanSEQ® Dye-Terminator Removal kit (Agencourt Bioscience Corporation, Beverly, MA, USA). Sequences were read using a Long Read Sequencing Protocol on an ABI Prism® 3100-Avant Genetic Analyzer (Applied Biosystems).

3.2.3 Analysis

Sequences were aligned by eye using the manual aligning software BioEdit (Hall, 1999). Neighbour-joining trees based on Kimura 2-parameter (K2P) distances were created using APE version 2.3-2 (Paradis *et al.*, 2004; R Development Core Team, 2008). Parsimony analysis was conducted using DNAPars of the PHYLIP package (Felsenstein, 2005). Maximum likelihood analyses were run using PHYML ALRT (Anisimova & Gascuel, 2006; Guindon & Gascuel, 2003), and the topology tested using both SH-like likelihood ratio tests and parametric bootstrap procedures with 100 replicates.

Bayesian analyses were run in MRBAYES (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) with 1 000 000 generations, sampling every 1000 generations. Other settings and priors were kept at default values. Results from the first 250 000 generations were discarded as a burnin, based on a plot of the average standard deviation of split frequencies (Fig. 3.1). A majority-rule consensus tree was calculated from the remaining trees to get the posterior probability of each clade. Models for the ML and Bayesian analyses were chosen using the function `phymltest()` in APE. Saturation plots of the number of pairwise transitions and transversions plotted against K2P distances were constructed in R (Fig. 3.2).

All gene regions were initially analysed separately before a combined analysis of the nuclear genes. COI was excluded from this analysis, due to the differing history of the nuclear and mitrochondrial genomes (Bull *et al.*, 1993). Partitioned analyses were run in MRBAYES with a partition for each gene, both running a GTR + Γ model. Analyses ran for 5 000 000 generations, sampling every 1000 generations. To investigate the probability of trees based on *a priori* expectations (i.e. monophyly of *C. oculatus* and *Carpophilus*), SH-tests (Shimodaira & Hasegawa, 1999) were conducted using the `SH.test()` function in the phangorn package for R (Schliep, 2009).

3.3 Results

3.3.1 Taxon sampling

Specimens of 17 species of *Carpophilus* were sequenced. These represented four subgenera, as proposed by Kirejtschuk (2008). *Semocarpolus* was represented by a single species, *C. marginellus*; *Carpophilus* by three species (*C. lugubris*, *C. obsoletus* and *C. hemipterus*); *Ecnomorphus* by four (*C.*

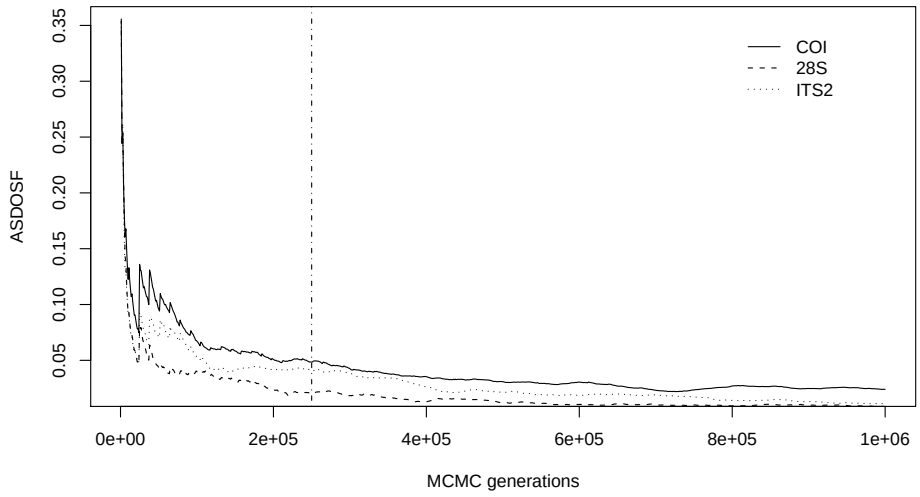


Figure 3.1: Average standard deviation of split frequencies (ASDOSF) plotted against MCMC generation to calculate burn-in.

antiquus, *C. discoideus*, *C. bakewelli* and *C. corticinus*); and *Myothorax* by eight species (*C. dimidiatus*, *C. mutilatus*, *C. davidsoni*, *C. gaveni*, *C. nepos*, *C. maculatus*, *C. oculatus* and two undescribed species). A single species of *Urophorus* (*U. humeralis* was also included in the dataset. COI and 28S were sequenced from all species, with the exception of *C. bakewelli* and *C. obsoletus* for which 28S was unable to be amplified. ITS2 was only sequenced from selected species within the *Myothorax* subgenus. Outgroups were selected from available specimens and previously published sequences of other species within the Nitidulidae. These represented three subfamilies outside of the Carpophilinae and included *Epuraea signata* and *E. ocularis* (Epuraeinae), unidentified *Conotelus* and *Brachypeplus* species (Cillaeinae), and *Aethina concolor*, *Omosita discoidea*, three Meligethes species and unidentified specimens of *Phenolia*, *Stelidota* (Nitidulinae).

3.3.2 Alignments and variation

A total of 200 sequences over the three markers were obtained. Sequences have been submitted to Genbank with accession numbers GU217433–GU217530 for COI, GU217391–GU217432 for 28S and GU217338–GU217390 for ITS2. Specimen details are available in Appendix C. Sequence polymorphism data for each gene are presented in Table 3.2. Minimum pairwise divergences for all markers was 0.

28S and ITS2 alignments required the addition of alignment gaps because of the presence of indels. While there are no indels in the COI dataset, missing values from paricularly short sequences are present. The majority of missing values were primarily from two specimens, the removal of which decreased the number of missing values by 103. Base frequencies of the three markers are typical for insects (Lin & Danforth, 2004).

Although COI and 28S had roughly equivalent levels of variable sites (Table 3.2), COI had more parsimony-informative sites than 28S, and was better at distinguishing between species of *Carpophilus* (Fig. 3.3 c.f. Fig. 3.8). Distribution of parsimony-informative sites in COI across first, second and third codon positions respectively was 37, 5, and 159, a result consistent with most protein-coding genes (Xia, 1998). Saturation plots (Fig. 3.2) for all markers were essentially linear, and did not show significant evidence of plateauing.

3.3.3 Phylogenetic analyses

COI

Maximum likelihood of the COI region inferred a single tree of -ln likelihood 7800.61611 from a GTR + Γ model with nucleotide frequencies and rate

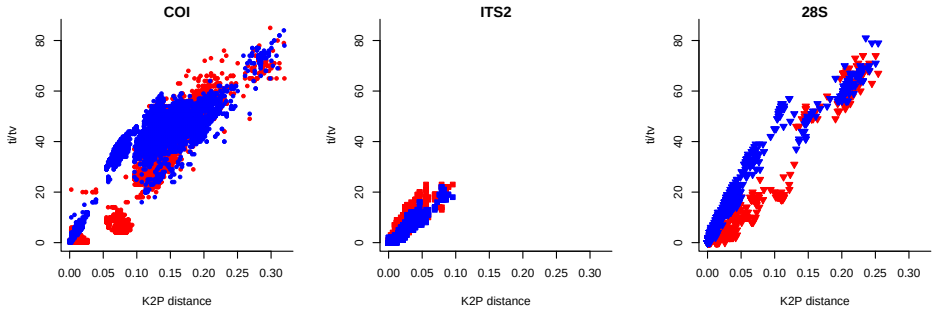


Figure 3.2: Saturation plots of transition/transversion frequencies against K2P genetic distances. Red: transversions, blue: transitions.

Table 3.2: Sequence polymorphism data.

	COI	28S	ITS
Sequences	104	43	53
Species	29	22	6
Length	576	734	504
Gaps & missing values	159/56	135	169
Variable sites	219	226	49
Percentage	38%	38%	15%
Parsimony informative	201	133	27
Base frequencies			
A	28.0	20.8	24.9
C	19.6	25.5	20.2
G	16.9	31.5	23.2
T	35.5	22.2	31.7
Pairwise divergences			
Mean	0.0234	0.1102	0.0514
Maximum	0.0896	0.2095	0.2134

Table 3.3: Rate parameters of models used in analyses.

Analysis	f(A–C)	f(A–G)	f(A–T)	f(C–G)	f(C–T)	f(G–T)	Γ
COI	1.58377	7.30570	4.48997	0.38926	11.96949	1.0 (fixed)	0.246 (4 cats)
28S	0.58770	2.09683	1.48477	0.46896	3.41398	1.0 (fixed)	0.326 (4 cats)
ITS	0.89063	2.24518	2.55499	0.71150	1.93285	1.0 (fixed)	–

parameters as shown in Tables 3.2 & 3.3. 934 most parsimonious trees were inferred with length of 1708.

COI ML trees (Fig. 3.3) grouped conspecific taxa together with very high bootstrap values. The monophyly of *C. o. oculatus* and *C. o. gilloglyi* is supported with both high ML bootstrap values (75%) and Bayesian posterior probabilities (0.99). Higher relationships amongst species within and between genera are not resolved with any degree of certainty, resulting in a large polytomy.

Subspecific pairwise differences are given in Table 3.4.

Carpophilus o. gilloglyi was shown to have a very deep split (7.93%) between western specimens collected in Fiji (including Rotuma) and eastern populations from the Kermadec Islands to French Polynesia and including Tonga. Within these clades, mean pairwise distances were 0.75% for the western clade and 0.95% for the eastern clade. The relationships of *C. o. cheesmani* are completely unresolved in the ML analyses. A deep split was also seen between Australian and Pacific populations of *C. maculatus*, with a mean K2P pairwise distance between the clades of 6.36%. There was also significant structuring within the Pacific clade, with an average distance of 1.75%. Within a single, small island (Wallis), a specimen was 2.68% different from its conspecifics from the same island.

Bayesian analyses (Fig. 3.4) perform little better in resolving higher relationships, with the sister group of the *C. o. oculatus*–*C. o. gilloglyi* clade remaining unresolved. A relationship between *C. dimidiatus* and *C. o. cheesmani* is very weakly supported with a posterior probability of 0.68, but was not supported by ML. Results also suggest that the sister group of *C. maculatus* is not *C. oculatus*, but a clade including *C. gaveni*, and two undescribed *Carpophilus* species. Their relationship with *C. maculatus*

is reasonably well supported (0.95) although the clade is not (0.60) (Fig. 3.4). Relationships are also supported for *C. corticinus* and *C. hemipterus* (0.92), and between *C. obsoletus* and *U. humeralis* (0.96). A *Carpophilus* + *Urophorus* + *Epuraea* (CUE) clade was supported by a posterior probability of 1.0.

Parsimony (Fig. 3.5) showed many of the same trends, including poor support for deeper relationships. The east–west split in *C. o. gilloglyi* is present, with 100% bootstrap supports for the clades. The two are supported as sister groups, though with rather lower (74% bootstrap) support. *Carpophilus o. gilloglyi* and *C. o. oculatus* were also supported as sister taxa, and is the deepest relationship with greater than 50% support.

Neighbour-joining trees (Fig. 3.6) correctly grouped conspecific specimens. As with the other analyses, deeper relationships were not supported with any confidence by bootstrapping procedures. Mean pairwise distances between species are shown in Table 3.4. Interspecific distances ranged between 0.196 and 0.085, averaging 0.141. When the subspecies of *C. oculatus* are included, the minimum distance between species becomes 0.071. Minimum mean intraspecific distances were an order of magnitude lower, ranging from 0.000 for species represented by a single specimen to a maximum of 0.056 within *C. oculatus*, averaging 0.006. When the subspecies of *C. oculatus* were included, the most diverse species was *C. o. gilloglyi* with 0.043.

Mapping the morphology of male parameres onto a cladogram based on COI ML and Bayesian topologies of the *Myothorax* subgenus (Fig. 3.7). provides a method of judging the most useful of the two topologies. While *Myothorax* is not resolved as being monophyletic in ML, the topology inferred appears more congruent with these genitalic characters. Despite the distinct similarity between the genitalia of *C. maculatus* and *C. o. gilloglyi*,

these two taxa are not closely related (Fig. 3.7). This result suggests convergence or plesiomorphy and rejects synapomorphy as being an explanation for this similarity. Also worthy of note is the likeness between the parameres of *C. gaveni* and *Carpophilus* sp. 2.

28S rDNA

Parsimony analysis of the 28S region produced two most parsimonious trees of length 692, differing in their topology within the *C. o. oculatus* and *C. o. gilloglyi* clade. Maximum likelihood inferred a single tree with a -ln likelihood of 3603.59084 from a GTR + Γ model with nucleotides frequencies and rate parameters as shown in Table 3.3.

The 28S ML analysis showed resolution at the subgeneric level, but did not adequately resolve lower relationships (Fig. 3.8). The monophyly of the *C. oculatus* group was supported by a posterior probability of 1 and 85% bootstrap values; *C. o. cheesmani* was sister to the other two subspecies, while *C. o. gilloglyi* was paraphyletic with respect to *C. o. oculatus* and did not show the geographic structure found in the mitochondrial data. Beyond this, resolution within the genus was extremely poor. *C. gaveni*, *C. maculatus* and the two undescribed *Carpophilus* species were identical in their 28S sequences and were barely different from *C. davidsoni*, *C. mutilatus* and *C. nepos*. Surprisingly, *C. dimidiatus* was shown to have an extremely divergent 28S sequence, and a relationship with *Epuraea signata* was supported in both ML (73%) and Bayesian (1.0) analyses. The CUE clade was also well supported (100% ML, 0.89 BPP).

28S results were much more congruent with subgeneric classification than was COI, though subgenera were not strictly monophyletic in all cases. Species within *Carpophilus s. str.* came out as monophyletic in both ML

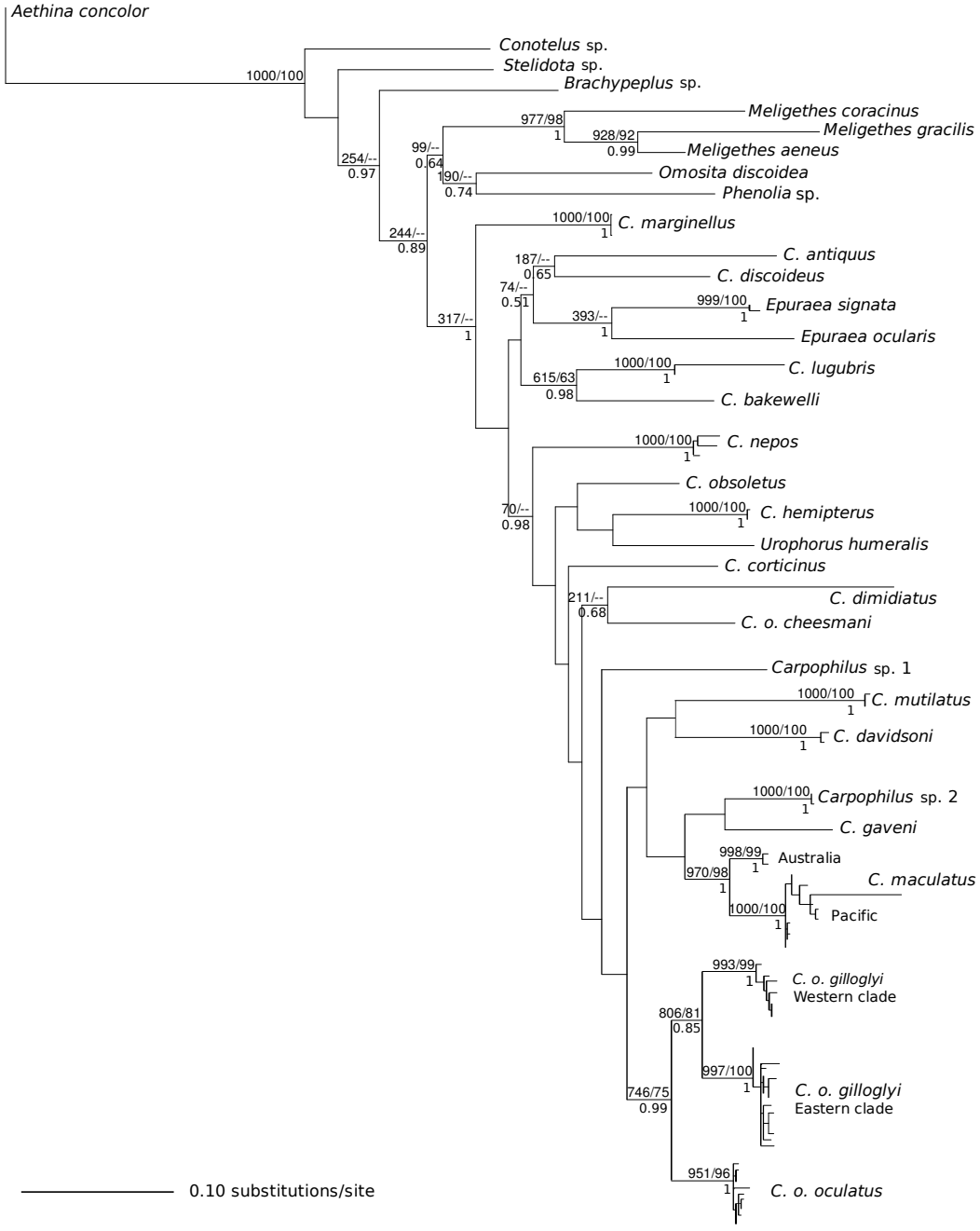


Figure 3.3: Maximum likelihood tree based on COI sequence data. Values above nodes are SH-like approximate Likelihood Ratio Test values. Values below nodes are Bayesian posterior probabilities. Support values less than 50/0.5 not shown.

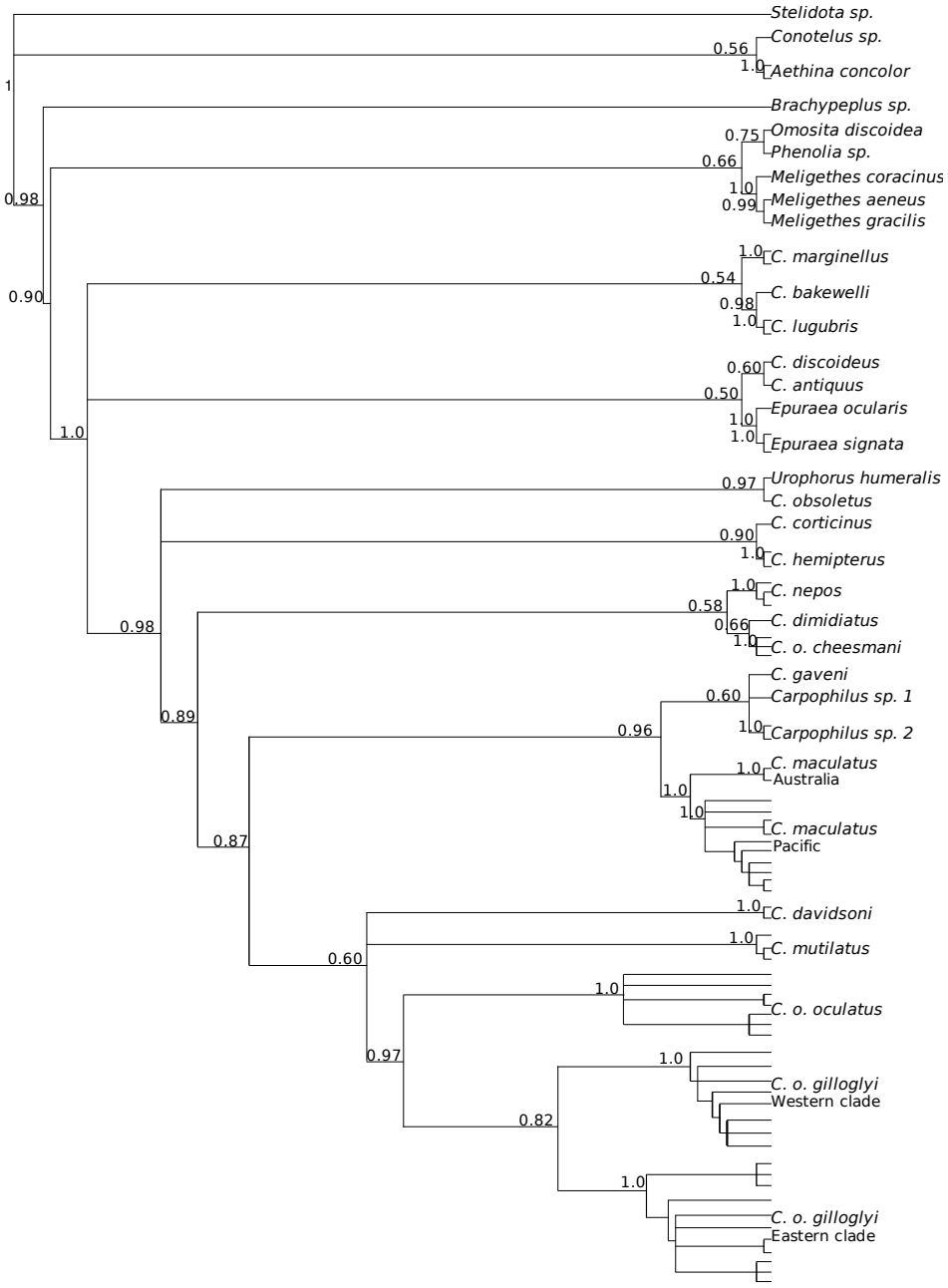


Figure 3.4: COI Bayesian tree. Nodal supports given as Bayesian posterior probabilities.

Figure 3.5: Majority-rule consensus of 934 most parsimonous trees based on COI sequence data. Values above nodes are consensus proportions. Values below nodes are boostrap percentages.

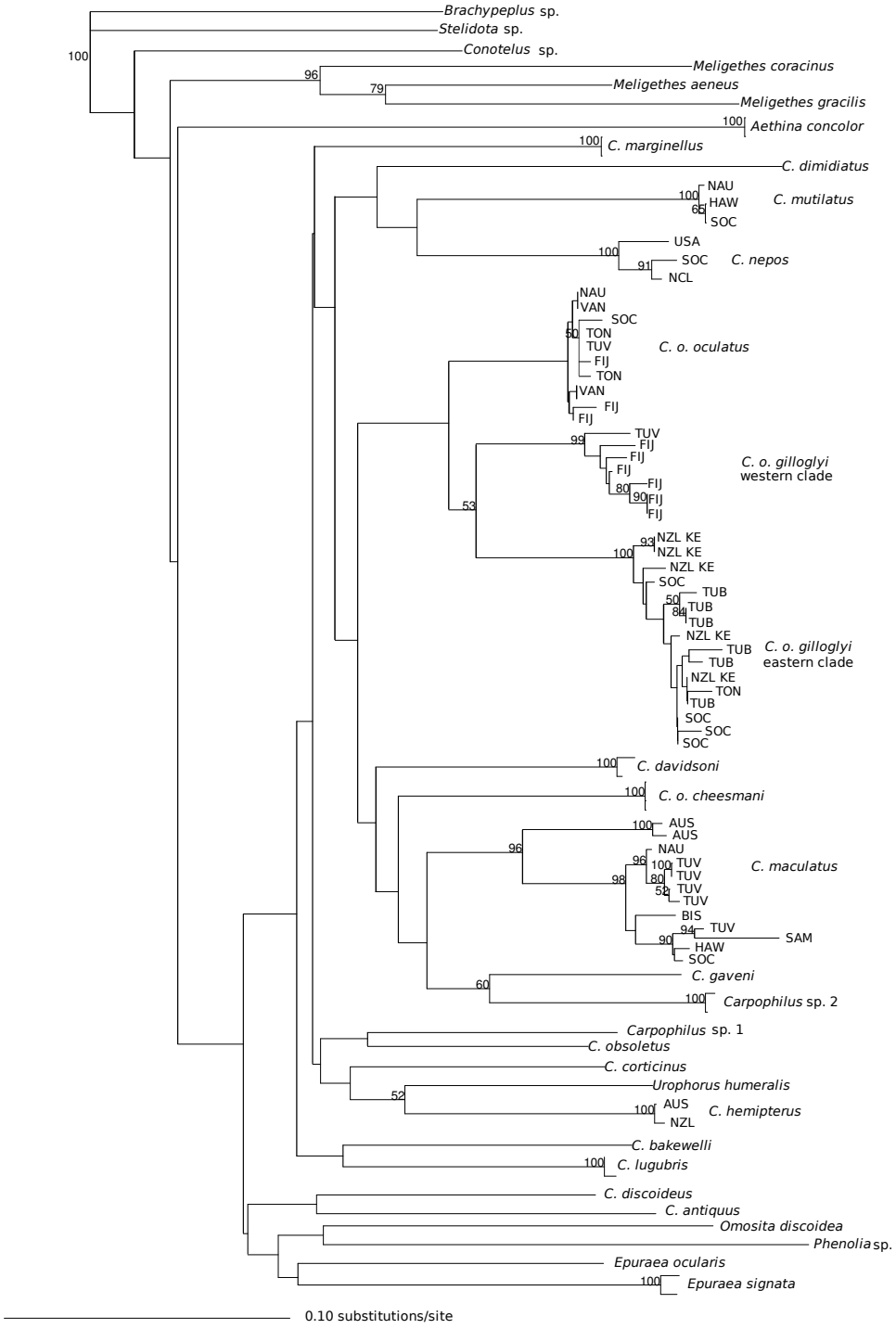


Figure 3.6: Neighbour-joining tree based on Kimura 2-parameter distances of COI sequence data. Bootstrap percentages greater than 50 displayed.

Table 3.4: Mean pairwise K2P distances of COI sequences within and between *Carpophilus* species. n=number of sequenced specimens. Species indicated by asterisks had only a single specimen available for sequencing.

	n	*C. maculatus*	*C. davidsoni*	*C. hemipterus*	*C. marginellus*	*C. mutilatus*	*C. nepos*	*C.* sp. 1	*C.* sp. 2	*C. obsoletus**	*C. lugubris*
C. maculatus	12	0.0299									
C. davidsoni	2	0.1337	0.0024								
C. hemipterus	2	0.1346	0.1564	0.0012							
C. marginellus	2	0.1379	0.1147	0.1342	0.0000						
C. mutilatus	3	0.1610	0.1449	0.1789	0.1510	0.0011					
C. nepos	3	0.1348	0.1328	0.1527	0.1338	0.1111	0.0108				
C. sp. 1	1	0.1334	0.1511	0.1619	0.1358	0.1455	0.1353	0.0000			
C. sp. 2	2	0.1095	0.0981	0.1637	0.1303	0.1397	0.1125	0.1206	0.0012		
*C. obsoletus**	1	0.1158	0.1388	0.1050	0.1073	0.1364	0.1148	0.0969	0.1144	0.0000	
C. lugubris	2	0.1448	0.1329	0.1232	0.1176	0.1593	0.1463	0.1436	0.1510	0.1176	0.0012
C. corticinus	1	0.1470	0.1557	0.1094	0.1217	0.1636	0.1587	0.1336	0.1469	0.1049	0.1181
C. antiquus	1	0.1635	0.1930	0.1562	0.1473	0.1709	0.1635	0.1790	0.1650	0.1300	0.1470
*C. bakewelli**	1	0.1579	0.1700	0.1359	0.1503	0.1338	0.1483	0.1533	0.1673	0.1354	0.1145
*C. discoideus**	1	0.1547	0.1564	0.1400	0.1305	0.1436	0.1416	0.1546	0.1717	0.1359	0.1378
C. gaveni	1	0.1086	0.1248	0.1536	0.1532	0.1499	0.1112	0.1137	0.0851	0.1216	0.1432
*C. dimidiatus**	1	0.1641	0.1551	0.1640	0.1475	0.1531	0.1431	0.1590	0.1589	0.1472	0.1644
C. oculatus	54	0.1173	0.1124	0.1343	0.1240	0.1353	0.1290	0.1238	0.1267	0.1195	0.1306
C. o. oculatus	21	0.1215	0.1024	0.1195	0.1137	0.1229	0.1276	0.1171	0.1280	0.1196	0.1274
C. o. cheesmani	3	0.1155	0.1023	0.1351	0.1214	0.1503	0.1259	0.1279	0.1035	0.0993	0.1522
C. o. gilloglyi	30	0.1145	0.1205	0.1445	0.1315	0.1425	0.1302	0.1280	0.1281	0.1215	0.1306

	C. corticinus	*C. antiquus*	*C. bakewelli**	*C. discoideus**	*C. gaveni*	*C. dimidiatus**	*C. oculatus*	*C. o. oculatus*	*C. o. cheesmani*	*C. o. gilloglyi*
C. corticinus	0.0000									
C. antiquus	0.1536	0.0000								
*C. bakewelli**	0.1506	0.1363	0.0000							
*C. discoideus**	0.1451	0.1274	0.1565	0.0000						
C. gaveni	0.1218	0.1716	0.1596	0.1739	0.0000					
*C. dimidiatus**	0.1747	0.1958	0.1894	0.1619	0.1622	0.0000				
C. oculatus	0.1214	0.1647	0.1318	0.1500	0.1187	0.1509	0.0564			
C. o. oculatus	0.1157	0.1702	0.1121	0.1600	0.1150	0.1380	0.0464	0.0036		
C. o. cheesmani	0.1192	0.1806	0.1415	0.1570	0.1107	0.1446	0.1075	0.0986	0.0000	
C. o. gilloglyi	0.1255	0.1593	0.1447	0.1424	0.1221	0.1607	0.0583	0.0712	0.1245	0.0427

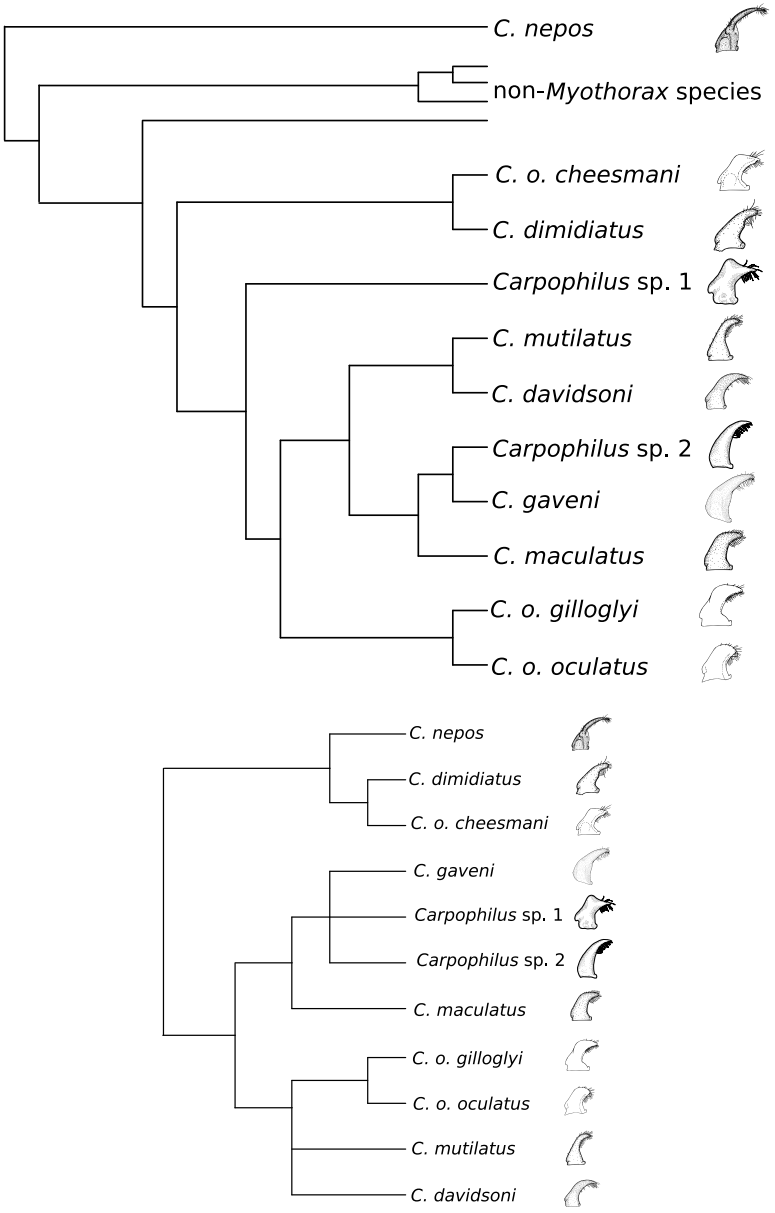


Figure 3.7: Cladograms showing the diversity of male genitalia (parameres) within the subgenus *Myothorax*. Topologies based on COI ML (top) and Bayesian (bottom) trees. Figures taken from Dobson (1955, 1956, 1964) with the exception of the two undescribed species.

and parsimony analyses. ML included *U. humeralis* in this clade while parsimony positioned it as a sister taxon to *Carpophilus s. str.*. The single species within *Semocarpolus*, *C. marginellus*, was sister to, but strongly differentiated from the *Urophorus–Carpophilus s. str.* clade. *Ecnomorphus* was paraphyletic with respect to a monophyletic *Myothorax* (excluding *C. dimidiatus*).

Parsimony trees were largely congruent with ML and Bayesian analyses, with the exception that *Epuraea* emerged as sister to a *Carpophilus* + *Urophorus* clade.

ITS2

Analysis of 53 ITS2 sequences produced a single most parsimonious tree with a length of 328, and a tree with a maximum likelihood of -ln = 1478.17930 using a GTR model (Table 3.3). Adding a gamma distribution to the model did not result in a different topology.

ITS2 data was only gathered for select taxa within the *Myothorax* subgenus. At this level it showed good variation between species. *Carpophilus o. cheesmani* was again shown to be a sister taxon to the other *C. oculatus* subspecies. In contrast to the 28S data there was sufficient variation in the marker to show intra-specific genetic structure. Maximum likelihood (Fig. 3.10) analyses revealed a lot of variation within *C. o. oculatus*. These specimens were resolved as being paraphyletic with respect to a monophyletic and very homogeneous *C. o. gilloglyi*. No geographic structuring within *C. o. gilloglyi* is shown by ML analyses. Unexpectedly, *Carpophilus* sp. 2 came out within *C. o. oculatus* also. It sits on a reasonably long branch within the group and may be a case of homoplasy, possibly combined with insufficient taxon sampling.

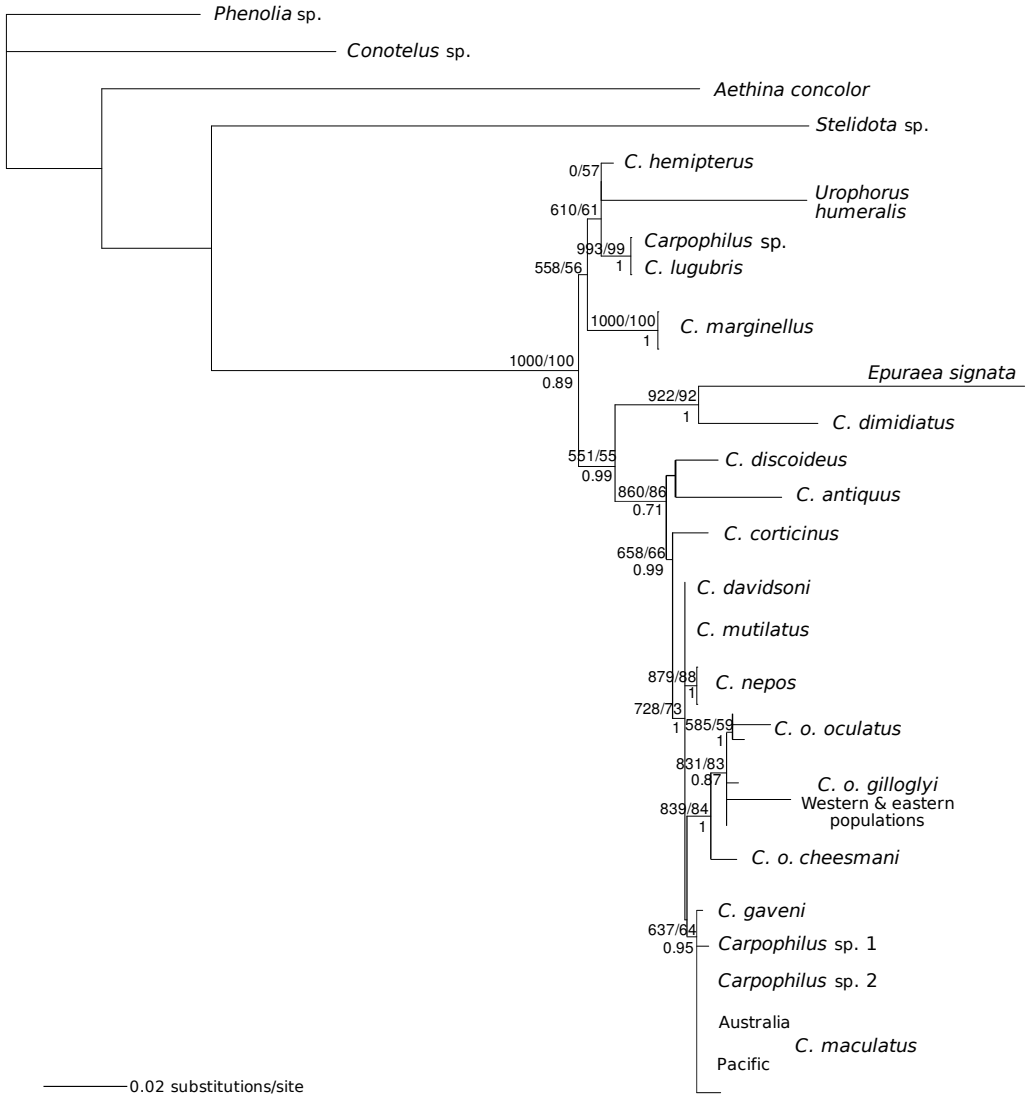


Figure 3.8: Maximum likelihood tree based on 28S D1-D2 region sequence data. Values above nodes are SH-like approximate Likelihood Ratio Test values/bootstrap percentages. Values below nodes are Bayesian posterior probabilities.

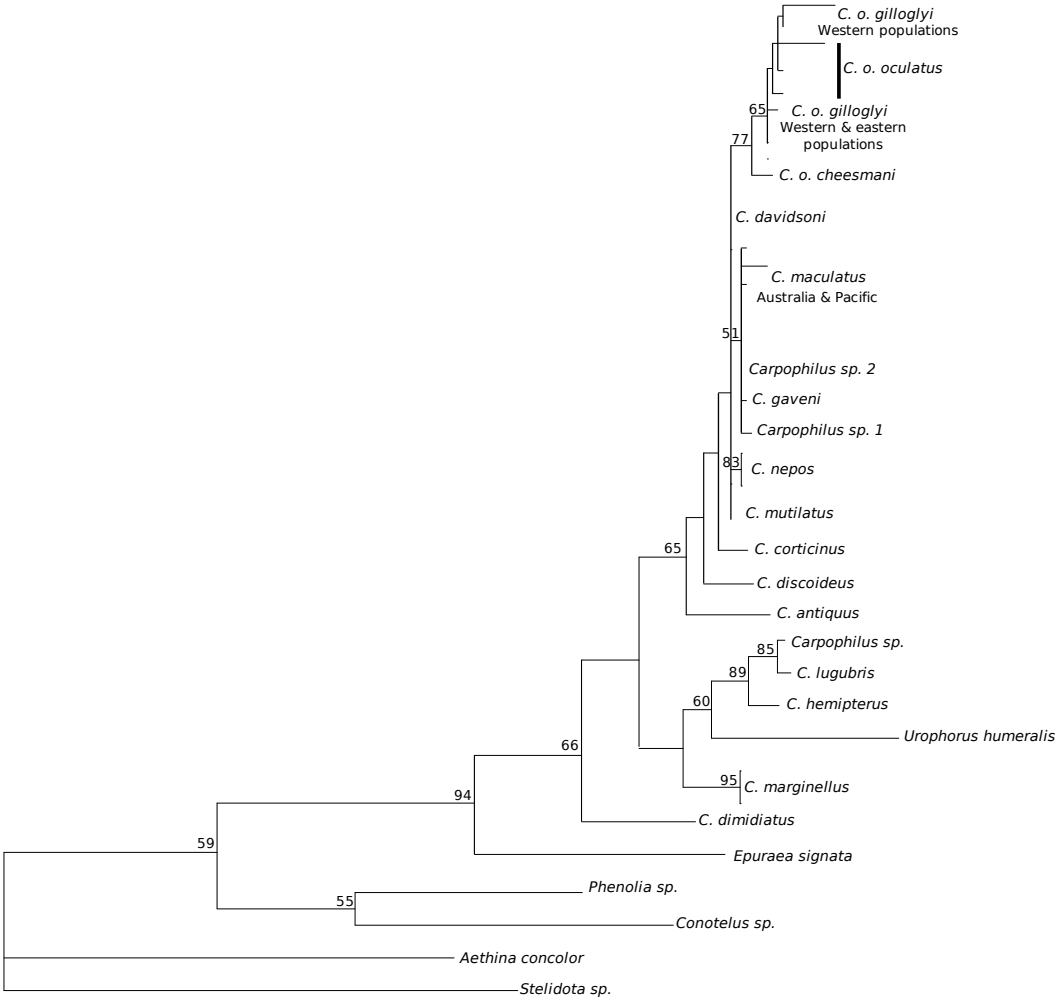


Figure 3.9: One of two most parsimonious trees based on 28S D1-D2 region sequence data. Bootstrap percentages over 50 are shown above nodes.

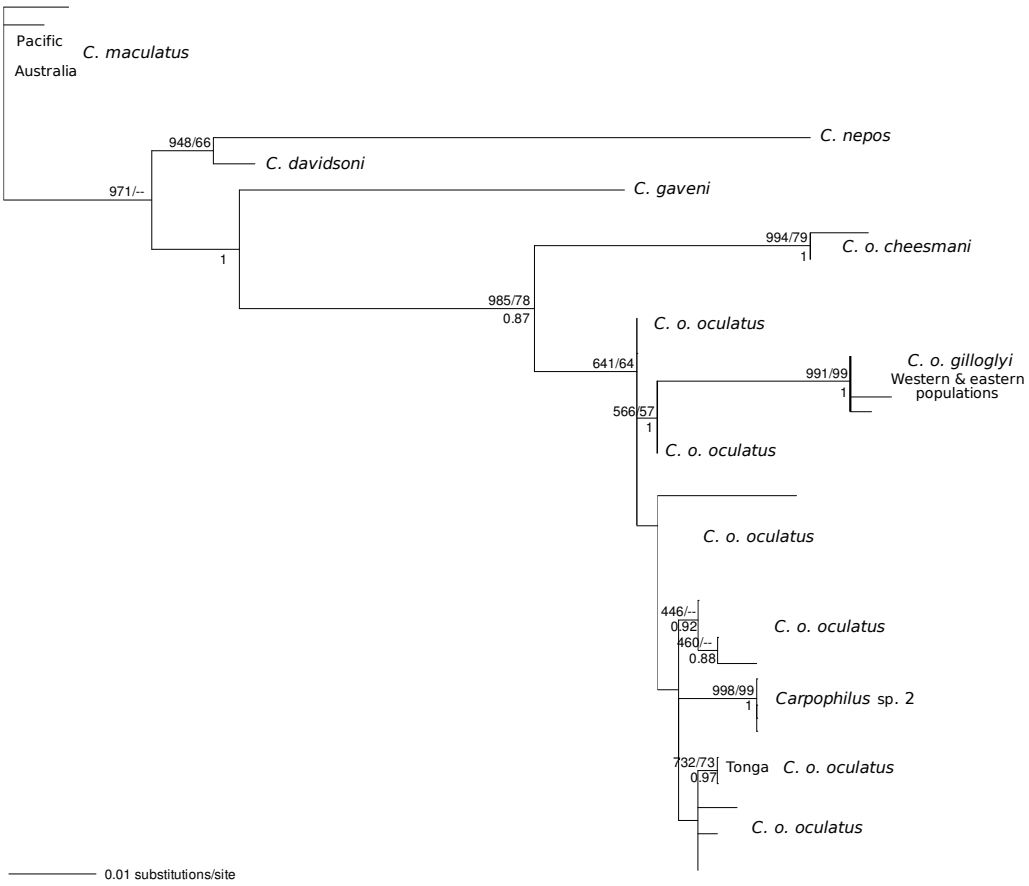


Figure 3.10: Maximum likelihood tree based on Internal Transcribed Spacer 2 sequence data. Values above nodes are SH-like approximate Likelihood Ratio Test values/bootstrap percentages. Values below nodes are Bayesian posterior probabilities.

In contrast to maximum likelihood, parsimony reveals some separation between the western and eastern *C. o. gilloglyi* populations (Fig. 3.11), as revealed in COI sequences. The separation is not as clear-cut, with neither population being monophyletic. It also resolves *C. o. oculatus* and *C. o. gilloglyi* as being reciprocally monophyletic, though with *Carpophilus* sp. 2 still being placed within *C. o. oculatus*.

Bayesian partitioned analyses of the ITS2 and 28S datasets provided no extra resolution of the *Myothorax* group.

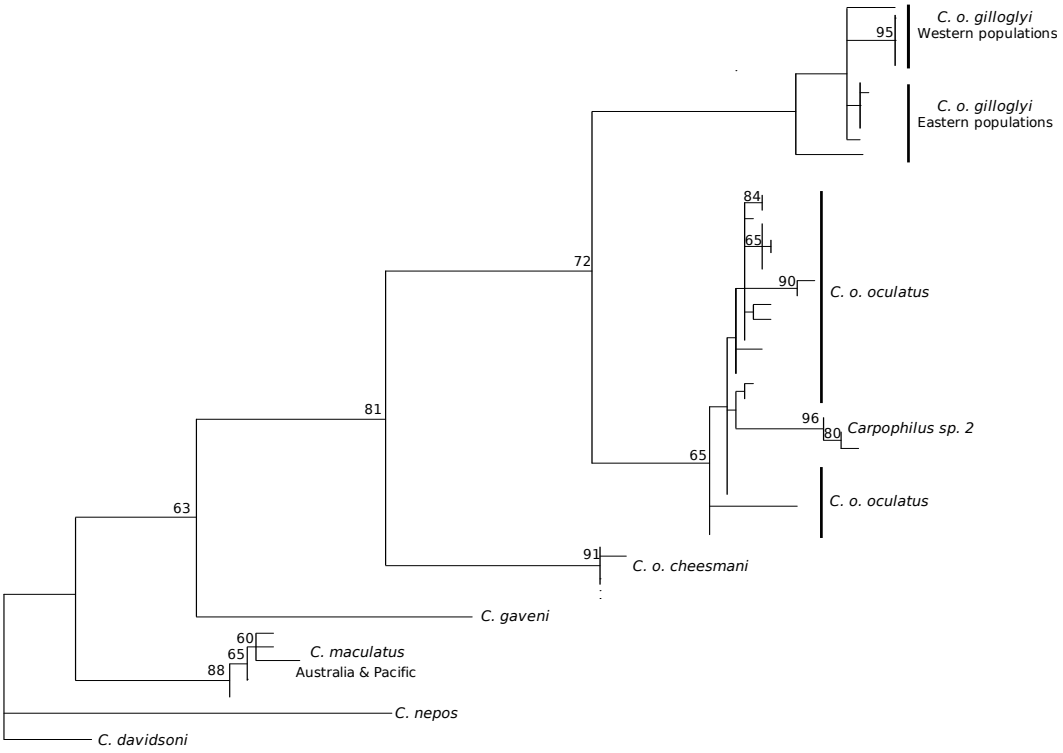


Figure 3.11: Single most parsimonious tree based on Internal Transcribed Spacer 2 sequence data. Bootstrap percentages over 50 are shown above nodes.

Table 3.5: SH test results for COI and ITS2 datasets.

Trees	log likelihood (ln L)	Difference in ln L	*p*-value
COI:			
Most likely	-7788	0	0.5483
C. oculatus monophyly	-9118	1330	<0.0001
Carpophilus monophyly	-9125	1337	<0.0001
Both	-9120	1332	<0.0001
ITS:			
Most likely	-1491	0	0.4870
C. oculatus monophyly	-1529	38	0.0046

3.3.4 SH tests

SH-tests of topology were conducted on the COI dataset to determine the individual and combined significance of *Carpophilus* and *C. oculatus* non-monophyly. ITS2 was tested for *C. oculatus* monophyly only. Results show that coercion of monophyly resulted in a significantly worse likelihood score for both markers (Table 3.5). A relatively small difference was found between the two ITS2 test trees, but very large differences were found between COI test trees. The change in likelihood score of enforcing *C. oculatus* monophyly was little different from that of forcing *Carpophilus* monophyly, despite being the result of a single change in the tree. Likewise, the combined changes to the tree were substantially different from the optimal, but little different from the individual changes.

3.4 Discussion

Data presented here demonstrate that the subspecies of *C. oculatus* are genetically well differentiated, with divergences ranging between 8–14%. In all analyses, *C. o. oculatus* and *C. o. gilloglyi* emerged as sister taxa. Beyond that however, relationships are unclear. *Carpophilus o. cheesmani* was indicated to be closely related to the other two subspecies in the nuclear

datasets, but were placed well outside the *oculatus–gilloglyi* clade in COI analyses. Non-comprehensive taxon sampling and the use of a relatively quickly evolving gene are considered to have caused this lack of resolution.

In the light of the morphological and genetic differences between the subspecies of *C. oculatus*, considering them as full species in their own right is justifiable. They often occur sympatrically but retain very large genetic differences between the taxa, suggesting that gene flow is very limited. They are easily separated by the shape of male genitalia, and by the differences in pronotal punctation. These subspecies appear to be persisting, despite sympatry—a diagnostic trait of "good" species according to Mallet (2008). The elevation of these subspecies to full species follows the precedent of a Californian tiger beetle, *Cicindela lunalonga* Schaupp which was elevated from being a subspecies of *Ci. terricola* following molecular evidence of strict monophyly and a high pairwise divergence in phylogenetic analyses (Woodcock *et al.*, 2007).

Despite having genitalia and colouration very similar to *C. o. gilloglyi*, *C. maculatus* was not inferred as being the sister taxon to *C. oculatus*. In COI Bayesian analyses, a sister-group relationship was inferred with a group consisting of *C. gaveni* and two undescribed *Carpophilus* species. This was concordant with 28S results, as *C. maculatus* had sequences that were essentially identical with the sequences from these same species. However, the ITS2 tree shows *C. gaveni* as being sister to the *C. oculatus* clade. Mapping morpological variation onto the tree suggests that the similarity in genitalia between *C. maculatus* and *C. o. gilloglyi* may be a symplesiomorphy. This morphological data also supports the topology of the ML tree over that of the Bayesian tree, despite that many of these nodes in the ML tree are not statistically supported by the COI data.

Species assigned to the subgenus *Myothorax* also formed a monophyletic group in most analyses, though in COI data it was present with low support. 28S was reasonably congruent with subgeneric classifications, but did not resolve subgenera as being strictly monophyletic. These preliminary results suggest that the classification of Kirejtschuk (2008) does represent the natural clades, however more comprehensive species sampling within these subgenera is required before this can be confirmed.

Urophorus humeralis is consistently resolved within *Carpophilus sensu lato*. *Urophorus* was originally described as a subgenus of *Carpophilus*. Although it was elevated to a full genus by Gillogly (1962), later workers have debated the validity of this elevation, and have continued to consider it as a subgenus (e.g. Ewing & Cline, 2005). Though the structure of the male terminalia in *U. humeralis* looks similar to that in *Carpophilus*, closer investigation shows that the 8th tergite of the former species is larger in *U. humeralis*, making up the pygidium. A round patch with significantly different sculpture found ventrally in the same location as the 8th tergite in *Carpophilus* completes the illusion. This morphological feature provides significant evidence against the placement of *Urophorus* within *Carpophilus* as inferred from the molecular data. However, more specimens of *Urophorus* and more extensive sampling of other *Carpophilus* subgenera would be needed to investigate this further.

COI is highly variable within *Carpophilus* and does not provide any reliable hypotheses of higher relationships within the genus. While saturation plots did not show evidence of saturation in any of the genes, the model of evolution used in their construction was a simple two parameter model. The model of evolution has been shown to severely influence the shape of these plots, with more complex models of evolution detecting saturation when

simpler models cannot (Sullivan & Joyce, 2005).

Despite being proposed as a nuclear equivalent of the COI region for the identification of species (Sonnenberg *et al.*, 2007), the 28S D1-D2 region proved to be unreliable for distinguishing between the taxa in this research. This was particularly the case within the *Myothorax* subgenus, members of which were distinctly different in their COI sequences. However, 28S did show promise for the detection of higher-level phylogenetic relationships. The placement of *U. humeralis* and *Epuraea* within *Carpophilus* may be explained by insufficient taxon sampling or long branch attraction. The level of divergence of *C. dimidiatus* is very high. More specimens of this species are required to confirm whether this divergence is not an error. As a member of the *Myothorax* subgenus, it would have been expected that the species would have been placed with the remainder of species in that subgenus.

This research also demonstrates that the COI barcode region provides a useful tool for the identification of *Carpophilus* specimens to species. A neighbour-joining tree accurately groups the conspecific sequences together to form individual clades. Given an accurate database based on correctly identified voucher specimens, this would enable the identification of eggs, immature stages, teneral adults and damaged specimens to species level. Such specimens are unable to be identified using current morphological methods.

A DNA taxonomy based on the COI barcoding region would recognise the both the Australian & Pacific clades of *C. maculatus* and the eastern & western clades of *C. o. gilloglyi* as separate species. In the case of *C. maculatus*, nuclear genes do not detect a difference between these two clades and the specimens do not appear to show any morphological or ecological differences. Because of the uniparental mode of mitochondrial DNA inheri-

tance, phylogenies based exclusively on this class of data do not accurately show the degree of connectivity and gene flow within and between populations. Furthermore, a concept of species as being explanatory hypotheses (Fitzhugh, 2005; de Queiroz, 2007) results in a classification that is based on the weight of evidence for there being different species. Current knowledge of both the biology and morphology of these populations do not require them to be explained as being different species.

There may be some justification for the species determination of the *C. o. gilloglyi* clades, as ITS2 also supports these clades (considered in greater depth in Chapter 4). Once again however, no lines of evidence outside of these two genes currently point to species-level differences between the two clades. Specimens from these areas appear to be identical morphologically, have been found in the same habitat and appear to have the same ecological niche. It is possible that these populations are in the early stages of allopatric speciation. It is surprising that these two clades are so distinct considering the high frequency of human movement between Fiji and Tonga. It is even more puzzling when the large distances between islands inhabited by the eastern clade are considered, over which it appears to have maintained recent gene flow. Because of the limited sampling in Tonga, more focused collecting throughout Tonga and the Lau archipelago would be needed to determine whether the apparent geographic separation of the two *C. o. gilloglyi* clades is real, or if there is some overlap in the ranges of these clades and to what extent that overlap occurs. More detailed specimen sampling, particularly with the use of quickly-evolving genetic markers such as microsatellites, may provide clues as to the creation and maintenence of this deep divergence within *C. o. gilloglyi*. Should the two clades be found to exist in sympatry, the proportion of individuals belonging to each clade plus genetic diversity

of these two populations and extent to which the distributions overlap may offer clues as to the processes (e.g. invasion) that currently influence the distribution of each clade.

Chapter 4

Phylogeography of *Carpophilus oculatus*

4.1 Introduction

Carpophilus oculatus has a large range throughout the central Pacific, on a large number of islands, many of which are geologically young (e.g. Tahiti, Rapa, Raoul Island). Possible explanations for this extremely wide range include unassisted dispersal via wind and ocean currents, or by human-mediated dispersal. The latter explanation can be divided further into relatively ancient dispersal by the Austronesian expansion through the Pacific, and effects from the recent increase in trade and movement of produce and associated commensals since European discovery and colonisation.

The initial colonisation of the central Pacific by the Austronesian peoples proceeded in a West-East direction originating in South-East Asia, settling in coastal regions in New Guinea and Melanesia, and reaching Fiji, Tonga and Samoa around 1000 BC (Kirch, 2000). A second expansion through the remainder of the Eastern Pacific began with the colonisation of the

Cook, Marquesas and Society Islands around 200 BC. By 1000 AD this had reached the remote archipelagoes of Hawaii, Easter Island, New Zealand and the Kermadec Islands. There is also evidence that the South American coast was reached at least occasionally (Kirch, 2000). The colonisation of the islands had a profound impact on the biota of the region, the extent of which is only now starting to be realised. It is becoming clear that colonisation of islands was deliberate, with settling parties carrying crops for their destination with them. These include important host plants for *C. oculatus* including breadfruit (*Artocarpus altitus*), coconut (*Cocos nucifera*) and pandanus (*Pandanus* spp.). Other flora and fauna thought to have been distributed through the South Pacific at this point in the history of the islands include dogs, pigs, chickens, the Pacific Rat (*Rattus exulans*) and paper mulberry trees (*Broussonetia papyrifera*).

The European colonisation of the Pacific proceeded in the opposite direction. Although the Solomon Islands were among the first Pacific Islands to be discovered, by Alvaro de Mendana in 1595, it was not until the voyages of James Cook and Bougainville in 1768–1769 that lasting contact was made, primarily in the Society Islands and the Marquesas. For several decades, these two archipelagoes were the first stopping point for further exploration of the Pacific.

Human movement in the Pacific is not confined to these two major time periods however, as there is evidence for post-settlement interactions between Polynesian communities for centuries prior to European influence in the region. Basalt adzes originating from Tutuila in American Samoa have been discovered in Mangaia (Cook Islands), and stone from Eiao in the Marquesas in archaeological sites in Moorea and Mangareva (French Polynesia) (Kirch, 2000). Another major movement in the Pacific occurred during

World War II, when huge amounts of cargo were carried between South Pacific archipelagos with little regard for biosecurity. The invasion of Guam by the brown tree snake (*Boiga irregularis*) (Sherley, 2000), and the spread of the ship rat (*Rattus rattus*) (Sherley, 2000) have been attributed to this period. Current trade in the Pacific is primarily with nations outside of the region, primarily Australia, New Zealand and the United States. Fiji, Papua New Guinea, Tonga and French Polynesia are the major exporters in the Pacific, each exporting over $10 million NZD of agricultural products in 2005 (McGregor, 2007) with this amount predicted to increase. The phytosanitary and quarantine measures of Pacific nations have been identified as an area of concern (McGregor, 2007). Much less trade occurs between and within island nations, however the quarantine measures regulating these movements are even less strict than international exports, and provide a potential pathway for insect colonisation of new islands.

A natural spread of *C. oculatus* through the Pacific should follow patterns of diversity that would be somewhat consistent with the prevailing wind and currents present in the region. Populations are also be likely to be much older, and show more genetic structuring between island groups. If the species evolved *in situ*, the sister species of *C. oculatus* is likely to be found in the region, assuming it is still extant.

If *C. oculatus* was spread across the Pacific exclusively by human mediated founder dispersal processes, we would expect to see a highly diverse source population that is paraphyletic to multiple daughter populations. If the rate of molecular evolution was extremely fast, a stepwise branching pattern as people moved through the Pacific could be expected. The site of the diverse, paraphyletic source population may indicate the mode of dispersal, being sited in the West if it was dispersed by Austronesians, or in the

East if it were dispersed from the time of European colonisation. Finding *C. oculatus* in South America, particularly if there were genetic links to South Pacific populations, may provide evidence of both Polynesian contact with South America, and that dispersal of *C. oculatus* was mediated during that period of history.

Distinguishing between the influence of these two patterns of human settlement on the dispersal of *C. oculatus* would be difficult. Both would be obscured by subsequent dispersal between island groups, which could be attributed to both natural and human-mediated movement. The timeframes over which these human-mediated movements would've occurred are also too recent to have influenced all but the fastest evolving genetic markers.

4.1.1 Phylogeography and its utility

Data from Chapter 3 support the subspecies of *C. oculatus* as distinct entities that should be elevated to the status of species. However, the question remains as to whether or not gene flow occurs, both between subspecies and between populations of the subspecies inhabiting different archipelagos. Inferring the degree and direction of gene flow is important from both a speciation and biosecurity point of view. Most species concepts require, or assume, low to no gene flow between putative species. Biosecurity operations desire knowledge as to invasion pathways, the extent to which species dispersal is encouraged by human activity and whether or not they are dealing with distinct species, as different species may have different impacts on biosecurity. The widespread distribution of *C. oculatus* gives us an interesting opportunity to take a phylogeographic approach to investigate the incidence of genetic structuring and gene flow within this species, and to study this in light of the presence of subspecies with respect to biosecurity.

Phylogeography is the juncture between population genetics as influenced by geography, and molecular systematics. From the genesis of the discipline by Avise *et al.* (1987), it has become extremely popular and useful for inferring within-species history and evaluating hypotheses of speciation and changes in climate and species distributions within the past two million years (Beheregaray, 2008). It has gained huge popularity, particularly with the advent of easy-to-use methods such as Nested Clade Phylogeographic Analysis (Templeton, 1998). Although this method has recently come under scrutiny for regularly and falsely inferring phylogeographic structure in simulations of panmictic populations (Petit 2008; Knowles 2008 but see rebuttal Templeton 2008), the development of other techniques and theory has kept the discipline at the forefront of molecular ecology.

Phylogeography has traditionally been based on data from mitochondrial DNA, particularly COI and the rRNA regions (Beheregaray, 2008). However, it has been increasingly realised that to get an accurate picture of species history nuclear genes should also be included in the analysis (Hare, 2001). While microsatellites are commonly used in phylogeographic studies, the development and characterisation of species-specific microsatellite regions is time-consuming, expensive and often requires cloning. The ribosomal internal transcribed spacers (ITS) 1 & 2 are relatively quickly evolving regions that have been shown to be of use for inferring intra-specific population structure (Coleman, 2003). ITS2 has also recently been put forward as a species-delimiting gene based on observations that certain changes in its secondary structure are correlated with a lack of interbreeding between species (Coleman, 2009).

Despite the dynamic nature of the Pacific environment and the historical interest of the region to evolutionary biology, there has been relatively little

phylogeographic study done on Pacific taxa. The exception to this trend is the fauna of the Hawaiian Islands, which have been extensively studied (e.g. Cowie & Holland, 2008; Rubinoff, 2008; Bird *et al.*, 2007). The majority of previously reported phylogeographic analysis of South Pacific biota are concerned with marine species (e.g. Drew & Barber, 2009; Plaisance *et al.*, 2008; Benzie & Williams, 1997; Schultz *et al.*, 2007; Thacker *et al.*, 2008; Ragionieri *et al.*, 2009; Schultz *et al.*, 2008), with fewer studies on terrestrial plants and animals (e.g Garb & Gillespie, 2006; Kirchman & Franklin, 2007; Butaud *et al.*, 2005; Pulvers & Colgan, 2007; Takayama *et al.*, 2006). Undoubtedly this partially has been due to the logistical difficulties of field work in the islands and obtaining fresh specimens suitable for DNA extraction. While there have been some molecular systematic studies of Pacific Dytiscidae (Balke *et al.*, 2004, 2007), none of these studies have been done in a phylogeographic context.

The goal of this research was to investigate the genetic diversity of *C. oculatus*, infer possible gene flow between populations, and determine the influence of geography on the genetic structuring of this species.

4.2 Methods

4.2.1 Data collection

COI and ITS2 sequence data collected for *C. oculatus* specimens in Chapter 3 were analysed in a phylogeographic framework. Within the scope of this study, only a subsample of the specimens that had COI sequenced were also sequenced for ITS2.

4.2.2 Data preparation

Sequences were aligned by eye using the manual alignment program BioEdit (Hall, 1999). ITS2 sequences with multiple copies differing by the presence of insertion/deletions (indels) were aligned manually in most cases, but in extreme circumstances, the program CHAMPURU (Flot, 2007) was used to elucidate the position of the indel.

4.2.3 Data analysis

Analyses were conducted similarly for both mitochondrial and nuclear data sets. The degree of genetic structuring within populations was measured using Analysis of Molecular Variance (AMOVA) as implemented in Arlequin (Excoffier *et al.*, 2005), which was also used for the calculation of population pairwise F_{ST}. Negative values of F_{ST} are to be interpreted as F_{ST}=0, due to vagaries in the way the statistic is calculated (Long, 1986). Population summary statistics were calculated using DnaSP (Librado & Rozas, 2009). Minimum spanning networks were inferred using SplitsTree (Huson, 1998) using the default options of 1000 spring embedder iterations and collapsing identical haplotypes. Statistical parsimony networks were inferred using TCS (Clement *et al.*, 2000) with a connection limit of 95%.

4.3 Results

4.3.1 mtDNA

For the COI region, 54 sequences of 548 bp were obtained for which the populations statistics are summarised in Table 4.1. A total of 30 haplotypes were found, the majority of which were only detected as single specimens. There were a number of non-synonymous substitutions. One *C. o. gillo-*

Table 4.1: Population summary statistics for *Carpophilus oculatus* COI sequences: sample size(n), number of haplotypes(N), haplotype diversity($H_D \pm SD$), nucleotide diversity ($\pi \pm SD$), Tajima's D statistic (D) and Fu's F_S statistic (F_S).

Population	n	N	$H_D \pm SD$	$\pi \pm SD$	D	F_S
C. o. oculatus	21	10	0.800 ± 0.079	0.00377 ± 0.00056	-0.88525	-4.321
Fiji and Rotuma	4	4	1.000 ± 0.177	0.00517 ± 0.00140	0.37186	-1.322
Tonga	2	2	1.000 ± 0.500	0.00182 ± 0.00091	–	0
Vanuatu	13	3	0.564 ± 0.112	0.00271 ± 0.00047	1.70214	1.668
Society Is	1	1	–	–	–	–
Nauru	1	1	–	–	–	–
C. o. gilloglyi	30	19	0.936 ± 0.032	0.03900 ± 0.00251	1.3750	0.365
Fiji and Rotuma	12	6	0.682 ± 0.148	0.00683 ± 0.00187	-1.4545	0.262
Austral Is	7	5	0.857 ± 0.137	0.01060 ± 0.00253	-0.28253	0.585
Society Is	4	3	0.833 ± 0.222	0.00547 ± 0.00185	-0.80861	0.731
Kermadec Is	6	4	0.800 ± 0.172	0.00681 ± 0.00181	-0.31472	0.633
Tonga	1	1	–	–	–	–
C. o. cheesmani	3	1	–	–	–	–
Total *C. oculatus*	54	30	0.949 ± 0.017	0.05987 ± 0.00412	0.5324	1.336

glyi specimen from Rapa showed a single polymorphic peak in both forward and reverse reads of the COI PCR product. Both haplotypes were incorporated into subsequent analyses. This individual appears to be heteroplasmic, possibly due to either paternal leakage or recombination, which are being increasingly detected in animals (White *et al.*, 2008). It is also possible that this sequence may be a mitochondrial pseudogene, however the quality of the sequence and its homology with other *C. oculatus* sequences make that possibility less likely.

Carpophilus o. oculatus was found to have a reasonably high haplotype diversity (all populations: 0.800 ± 0.079), however, this subspecies had a rather low nucleotide diversity (all populations: 0.00377 ± 0.00056), particularly when compared with the very high nucleotide diversity of *C. o. gilloglyi* (all populations: 0.03900 ± 0.00251), which had a correspondingly

Table 4.2: COI AMOVA results.

	Source of variation	d.f.	Percentage of variation	Fixation indices	P values
Subspecies	Among subspecies	2	68.49	Φ_{CT}: 0.6849	<0.001
	Among islands within subspp.	14	26.49	Φ_{SC}: 0.8405	<0.001
	Within islands	37	5.03	Φ_{ST}: 0.9497	<0.001
Subspecies and Fiji	Among subspecies	3	91.59	Φ_{CT}: 0.9460	<0.001
	Among islands within subspp.	13	3.01	Φ_{SC}: 0.3580	<0.001
	Within islands	37	5.40	Φ_{ST}: 0.9159	<0.001
Archipelagos	Among archipelagos	7	18.58	Φ_{CT}: 0.1858	0.13
	Among subspp. within arches	9	74.58	Φ_{SC}: 0.9160	<0.001
	Within populations	37	6.84	Φ_{ST}: 0.9316	<0.001

AMOVA structure as follows, using population numbers as defined in Table 4.3:
Subspecies: (populations 1–7)(populations 8–16)(population 17)
Subspecies and Fiji: (1–7)(12–16)(8–11)(17)
Archipelago: (Rotuma: 1,10)(Fiji: 5,8,9,11)(Nauru: 2)(Society Islands: 3,12) (Vanuatu: 6,7,17)(Austral Islands: 13,14)(Tonga: 4,15)(Kermadec Islands: 16)

d.f., degrees of freedom.
Significance tests using 1023 permutations.

higher haplotype diversity (all populations: 0.936 ± 0.032). A single haplotype was found in *C. o. cheesmani*, preventing the calculation of diversity indices.

AMOVA results show that the greatest level of molecular differentiation occurs between the subspecies (68%) with a relatively minor contribution by geography (26%) (Table 4.2). The geographical component can primarily be attributed to the east/west split in *C. o. gilloglyi*, as evidenced by the major decrease in the geographical contribution (down to 3%) when the two groups of *C. o. gilloglyi* are elevated into the highest AMOVA grouping (Table 4.2). When the data are rearranged to form primary groups by geography, the resulting Φ_{CT} value (0.1858) is not significant, suggesting the observed variation is no different than that expected by chance. Current

sampling suggests that the geographic range of these two populations of *C. o. gilloglyi* are clearly defined (Fig. 4.1). However, this conclusion is based on a single *C. o. gilloglyi* specimen from Tonga, with no specimens obtained from other island groups in the region between Fiji and French Polynesia.

Pairwise F_{ST} values (Table 4.3) were greater than 0.8 between subspecies and between the eastern and western *C. o. gilloglyi* clades. Lowest values were betwen *C. o. oculatus* populations in Taveuni and Espiritu Santo (F_{ST}=0.0149), and between *C. o. gilloglyi* populations in Taveuni and Vanua Levu (F_{ST}=0.0158) and Tonga and Rapa (F_{ST}=0.1878).

Neutrality tests (Tajima's D and Fu's F_S) were fairly congruent with each other when applied at the subspecies and species level, but differed when considering populations. In general, all tests at the population level were low ($|D$ & $F_S| < 1.0$), with the exception of Vanuatu *C. o. oculatus* populations (D=1.70214), *C. o. gilloglyi* overall (D=1.3750) and Fijian *C. o. gilloglyi* populations (D=-1.4545). The positive values of the former two suggest secondary contact between previously divergent groups, while the negative value of Fijian *C. o. gilloglyi* suggests high numbers of low frequency haplotypes and is commonly inferred to suggest recent population expansion. All tests were statistically insignificant.

Haplotype networks (Figs 4.4 & 4.6b) reveal that only one haplotype is shared between archipelagos. This haplotype is shared between populations of *C. o. oculatus* found in Vanuatu (both Espiritu Santo and Efate) and Nauru. Although no eastern *C. o. gilloglyi* haplotypes are shared between archipelagos, they are 'scattered' amongst each other, with haplotypes from the Kermadecs forming nodes in the middle of the networks and being quite distant from each other. There is a reasonable amount of within-archipelago structuring within the western clade of *C. o. gilloglyi*.

Table 4.3: F_{ST} values. Populations 1–7: *C. o. oculatus*, 8–11: Western *C. o. gilloglyi*, 12–16: Eastern *C. o. gilloglyi*, 17: *C. o. cheesmani*. Above diagonal: ITS2 values. Below diagonal: COI values.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	–	1.0000	1.0000	-0.1579	0.2308	0.3600	0.4348	1.0000	1.0000	1.0000	0.8880	1.0000	1.0000	1.0000	–	1.0000	0.9738
2	1.0000	–	1.0000	-0.5714	-0.6667	-0.6000	-0.4444	1.0000	1.0000	1.0000	0.8880	1.0000	1.0000	1.0000	–	1.0000	0.9738
3	1.0000	1.0000	–	-0.2571	-0.4286	0.2615	0.4583	1.0000	1.0000	1.0000	0.8931	1.0000	1.0000	1.0000	–	1.0000	0.9751
4	0.3333	0.6000	0.7143	–	0.1952	0.3120	0.3145	0.7027	0.8514	0.7027	0.8027	0.6901	0.7250	0.8768	–	0.8483	0.8952
5	-0.4286	-0.6667	0.2308	0.3551	–	0.0938	0.1748	0.8830	0.9302	0.8830	0.8853	0.8773	0.8931	0.9401	–	0.9286	0.9452
6	-0.2000	-0.6000	0.5200	0.4857	0.0149	–	-0.1478	0.9256	0.9473	0.9256	0.9148	0.9207	0.9319	0.9527	–	0.9461	0.9561
7	-0.2593	-0.4167	0.5073	0.4686	-0.0033	-0.1656	–	0.9390	0.9595	0.9390	0.9219	0.9347	0.9442	0.9644	–	0.9586	0.9632
8	1.0000	1.0000	1.0000	0.9740	0.9123	0.9568	0.9564	–	0.0000	0.0000	-1.0000	1.0000	1.0000	1.0000	–	1.0000	0.9795
9	0.8675	0.8640	0.8740	0.8991	0.8894	0.9241	0.9277	0.0159	–	0.0000	0.0000	1.0000	1.0000	1.0000	–	1.0000	0.9877
10	1.0000	1.0000	1.0000	0.9765	0.9206	0.9610	0.9607	1.0000	0.3542	–	-1.0000	1.0000	1.0000	1.0000	–	1.0000	0.9795
11	1.0000	1.0000	1.0000	0.9960	0.9765	0.9800	0.9780	1.0000	0.3193	1.0000	–	0.3636	0.7500	0.7098	–	0.6500	0.9618
12	0.9322	0.9306	0.9351	0.9456	0.9284	0.9511	0.9521	0.9205	0.8925	0.9137	0.9708	–	1.0000	1.0000	–	1.0000	0.9795
13	1.0000	1.0000	1.0000	0.9802	0.9315	0.9669	0.9665	1.0000	0.8805	1.0000	1.0000	0.6471	–	1.0000	–	1.0000	0.9779
14	0.9011	0.8989	0.9053	0.9175	0.9079	0.9318	0.9343	0.8809	0.8734	0.8705	0.9420	0.2699	0.5127	–	–	0.0000	0.9896
15	1.0000	1.0000	1.0000	0.9794	0.9286	0.9655	0.9650	1.0000	0.8732	1.0000	1.0000	0.3333	1.0000	0.1879	–	–	–
16	0.9132	0.9111	0.9170	0.9270	0.9153	0.9370	0.9389	0.8991	0.8850	0.8902	0.9509	0.2411	0.5520	0.3103	0.2000	–	0.9875
17	1.0000	1.0000	1.0000	0.9950	0.9748	0.9827	0.9816	1.0000	0.9541	1.0000	1.0000	0.9746	1.0000	0.9552	1.0000	0.9627	–

Location of populations: 1: Rotuma, 2: Nauru, 3: Society Islands, 4: Tonga, 5: Taveuni (Fiji), 6: Espiritu Santo (Vanuatu), 7: Efate (Vanuatu). 8: Taveuni, 9: Vanua Levu (Fiji), 10: Rotuma, 11: Viti Levu (Fiji). 12: Society Islands, 13: Rimatara (Austral Islands), 14: Rapa (Austral Islands), 15: Tonga, 16: Kermadec Islands. 17: Efate.

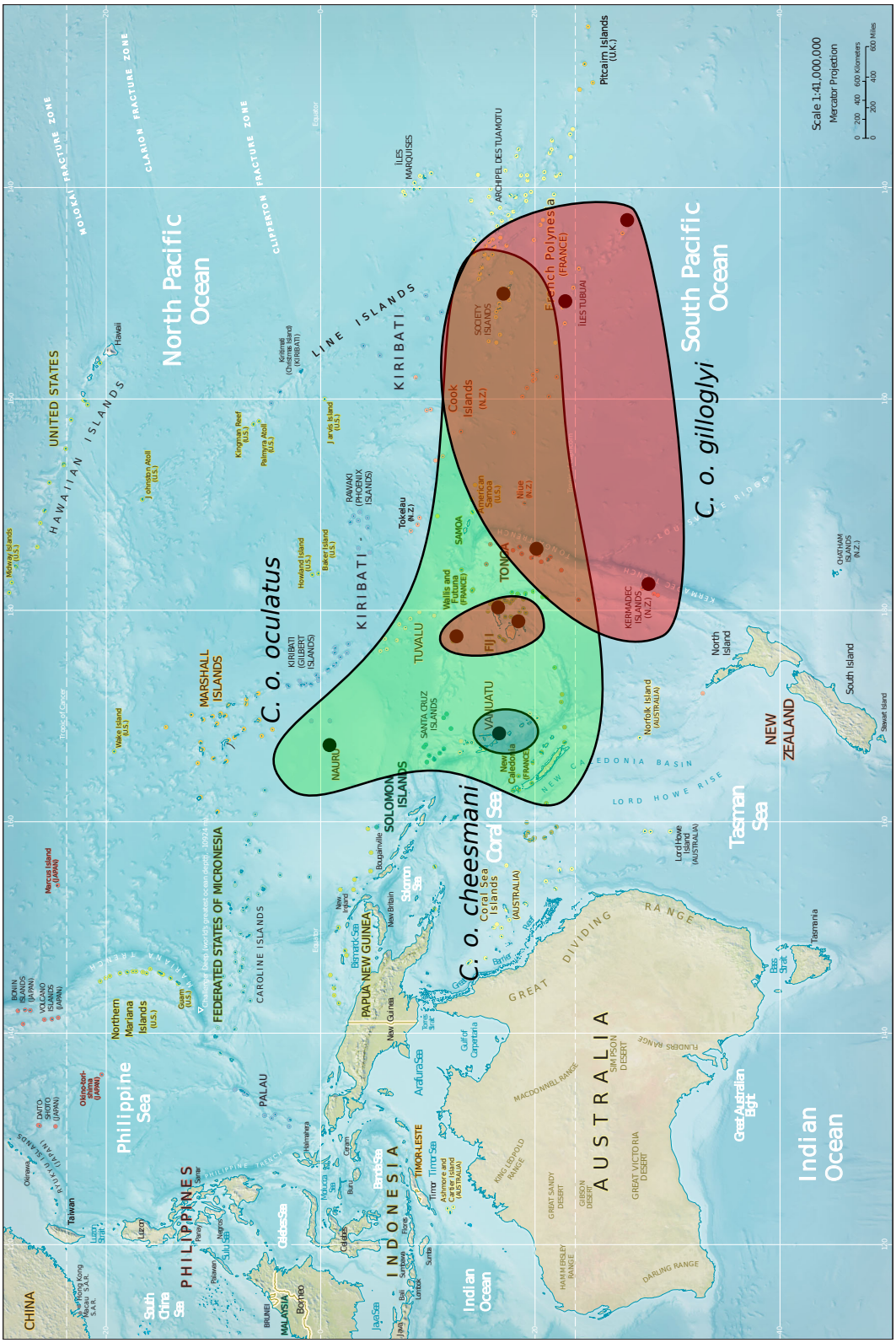


Figure 4.1: Distribution of *C. oculatus* subspecies based on specimens collected in this study. Green: *C. o. oculatus*, blue: *C. o. cheesmani*, red: *C. o. gilloglyi*, black dots: sampling localities.

Table 4.4: Population summary statistics for *Carpophilus oculatus* ITS sequences: number of individuals(n), number of haplotypes(N), % of specimens with multiple copies, haplotype diversity($H_D \pm SD$), nucleotide diversity ($\pi \pm SD$), Tajima's D statistic (D) and Fu's F_S statistic (F_S).

Population	n	N	%	$H_D \pm SD$	$\pi \pm SD$	D	F_S
C. o. oculatus	12	11	58	0.905 ± 0.040	0.00639 ± 0.00155	-1.44691	-4.183
Fiji and Rotuma	3	5	67	1.000 ± 0.126	0.00851 ± 0.00135	0.49788	-1.901
Tonga	2	2	50	0.667 ± 0.314	0.01580 ± 0.00745	–	3.473
Vanuatu	5	4	80	0.764 ± 0.006	0.00248 ± 0.00049	0.10123	-0.627
Society Is	1	1	0	–	–	–	–
Nauru	1	1	0	–	–	–	–
C. o. gilloglyi	17	3	0	0.228 ± 0.129	0.00061 ± 0.00036	-1.50358	-1.680
Fiji and Rotuma	8	2	0	0.250 ± 0.180	0.00062 ± 0.00045	-1.05482	-0.182
Austral Is	5	1	0	–	–	–	–
Society Is	1	1	0	–	–	–	–
Kermadec Is	3	1	0	–	–	–	–
C. o. cheesmani	4	2	0	0.500 ± 0.265	0.00350 ± 0.00185	-0.75445	1.716
Total *C. oculatus*	33	15	21	0.848 ± 0.002	0.01810 ± 0.00205	-0.40407	-0.436

4.3.2 Nuclear DNA

For ITS2, 33 specimens were sequenced revealing 15 unique alleles 482 bp in length, including alignment gaps. Unaligned, sequences ranged from 396 to 432 bp, averaging 418 bp. Although fewer specimens were sampled than COI, all populations included in the mitochondrial dataset were included in ITS2 with the exception of the Tongan *C. o. gilloglyi* population.

In contrast to the mitochondrial data, the ITS2 region revealed much greater nucleotide diversity in *C. o. oculatus* (0.00659 ± 0.00155) than in *C. o. gilloglyi* (0.00061 ± 0.00045). *Carpophilus o. cheesmani* had greater nuclear variation than mitochondrial and had two ITS2 alleles. Within *C. o. oculatus*, populations showed similar trends to the mitochondrial results, with Vanuatu populations displaying relatively lower diversity than those in Fiji; in contrast, Tongan populations showed the greatest nucleotide di-

versity. Within *C. o. gilloglyi*, there was very little diversity with only five alleles shared throughout the range.

Intragenomic ITS2 differences were found in seven specimens of *C. o. oculatus*. Usually two variants were detected in equal frequencies; the most variants detected in any one individual was three (Fig. 4.2). Copies usually differed from each other by microsatellite loci formed of 2–3 repeats of a 2 bp unit. The exception was a specimen from Fiji, which differed by an insertion within a microsatellite region (Table 4.5). The position of microsatellites differed between specimens, and were correlated with geographic locality (Table 4.5). Comparison with *C. o. gilloglyi* and *C. o. cheesmani* sequences suggested that the extension of these microsatellite regions was a characteristic of *C. o. oculatus*. Overall, 58% of *C. o. oculatus* specimens had more than one ITS2 sequence. The archipelago with the greatest proportion of individuals possessing multiple ITS2 variants was Vanuatu having 80% of specimens with intragenomic differences. Multiple copies were not detected in either of the other two subspecies, despite having similar repeat regions to "Copy one" of the microsatellite loci. After recording the percentage of specimens that possessed multiple copies, the resolved gene sequences were treated as haplotypic data and analyses were conducted in the same way as for mitochondrial sequences. As ITS2 is repeated several hundred times in the genome, and is not believed to be chromosome-specific, it was considered incorrect to treat these data as diploid genotypes (Excoffier *et al.*, 2006).

Lowest positive F_{ST} values were between Taveuni and Espiritu Santo *C. o. oculatus* populations (0.0938 and 0.1748 respectively) (Table 4.3). Values of 0 indicating identical sequences occured within Fijian *C. o. gilloglyi*. High F_{ST} values were calculated between the Eastern and Western *C. o. gilloglyi* mitochondrial clades, and within the Eastern population F_{ST} values were

Table 4.5: Summary of *C. o. oculatus* intragenomic ITS2 differences. "Copy one" is the hypothesised ancestral sequence based on comparison with other *C. o. oculatus* ITS2 sequences. "Copy two" is the derived form of the sequence. "Specimen symbol" indicates which specimens share that locus.

Type	Position	Copy one	Copy two	Specimen symbol	Locality
Microsatellite	484	$(TA)_2$	$(TA)_3$	★ ✽	Vanuatu
Microsatellite	449	$(TA)_2$	$(TA)_3$	✸ ✽	Vanuatu
Microsatellite	428	$(CG)_2$	$(CG)_3$	✚	Tonga
Microsatellite	118	$(T)_7$	$(T)_5$	✳	Fii
Insertion	199	TTTT	TTGTT	⅄	Fiji

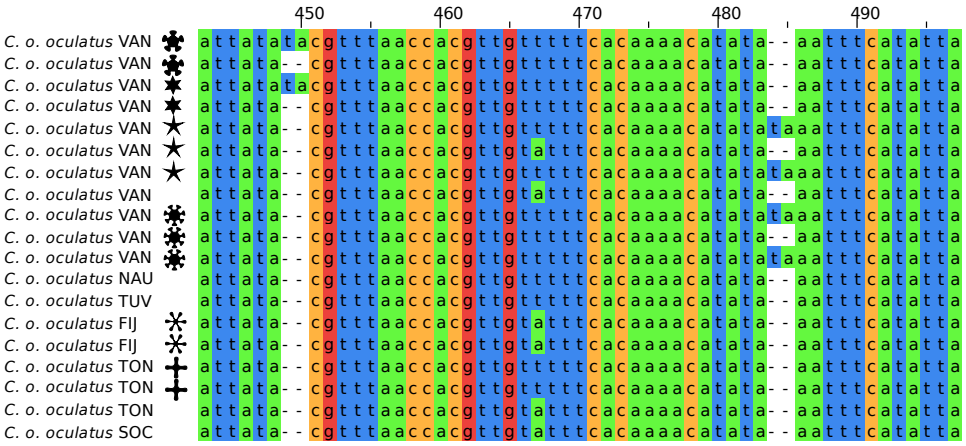


Figure 4.2: Portion of ITS2 sequence alignment showing different haplotypes within individual specimens (shared symbols) within and between localities. Sequences without symbols were derived from specimens without intragenomic variation. Note microsatellites at alignment positions 449 and 484.

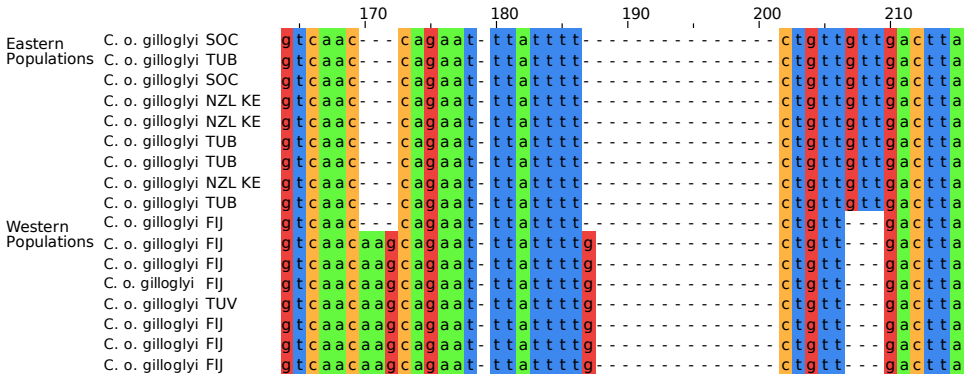


Figure 4.3: Portion of ITS2 sequence alignment showing indels between alignment positions 170 and 220 that differentiate eastern and western *C. o. gilloglyi* clades

primarily 1.000. This may be symptomatic of the computational challenges presented by the data, particularly the presence of indels.

Neutrality tests showed a similar story as for the mitochondrial dataset (Table 4.4). The major difference between the two is the behaviour of the *C. o. gilloglyi* populations; ITS2 sequences suggest there are more alleles present than expected and that this subspecies is expanding (D=-1.5038, F_S=-1.680) . F_S tests also suggest that *C. o. cheesmani* and Tongan *C. o. oculatus* populations have less alleles than expected.

ITS2 AMOVA results (Table 4.6) also show that the greatest differentiation between *C. oculatus* specimens occurs at the subspecies level, with 88% of variation explained by this grouping alone. Geography had very little influence explaining only 5% of the variation. When *C. o. gilloglyi* was split into its Eastern and Western components according to COI differences, the geographic component dropped to 2%, but the Φ_{SC} value decreased dramatically and the barely significant p-value of 0.044 is not convincing. Geographical restructuring resulted in a very inadequate model, with geography only explaining 6% of the data and having a non-significant Φ_{CT}

Table 4.6: ITS AMOVA results.

	Source of variation	d.f.	Percentage of variation	Fixation indices	P values
Subspecies	Among subspecies	2	88.27	Φ_{CT}: 0.8827	<0.001
	Among islands within subspp.	13	5.21	Φ_{SC}: 0.4445	<0.001
	Within islands	26	6.51	Φ_{ST}: 0.9349	<0.001
Subspecies and Fiji	Among subspecies	3	90.35	Φ_{CT}: 0.9035	<0.001
	Among islands within subspp.	12	2.43	Φ_{SC}: 0.2518	0.044
	Within islands	26	7.22	Φ_{ST}: 0.9278	<0.001
Archipelagos	Among archipelagos	7	5.54	Φ_{CT}: 0.0554	0.418
	Among subspp. within arches	8	84.88	Φ_{SC}: 0.8986	<0.001
	Within populations	26	9.57	Φ_{ST}: 0.9043	<0.001

AMOVA structure similar to Table 4.2, with the exception that population 15 (Tongan *C. o. gilloglyi*) was not included.
d.f., degrees of freedom.
Significance tests using 1023 permutations.

value of 0.0554.

The calculation of haplotypes in DnaSP (Tables 4.1 & 4.4) and Splitstree (Figs. 4.4 & 4.5) differ from TCS (Fig. 4.6) in that the former two either consider gaps as missing data, or ignore positions where gaps are present. This difference reveals some interesting things in the data. The statistical parsimony network inferred three separate networks and two other alleles that did not join to any other network (4.6a). *Carpophilus o. gilloglyi* was calculated to have five alleles, four of which were connected by the network. Using this method of analysis, there is a distinct correlation with the Eastern and Western clades as previously revealed by mitochondrial analyses. In contrast, the minimum spanning network only recognises three *C. o. gilloglyi* alleles, and no geographical structuring is evident (Fig. 4.5). An inspection of the alignment shows that there is a difference between

eastern and western populations (Fig. 4.3), and that this difference is due to differing patterns of indels between the two clades.

4.4 Discussion

The primary aim of this part of the thesis was to determine the genetic variability of *C. oculatus* and to discover where in the Pacific this diversity was highest with the assumption that this high diversity would represent the source of the radiation of the species. It was found that *C. o. gilloglyi* had greater haplotypic and nucleotide diversity in COI than *C. o. oculatus* by an order of magnitude. Surprisingly only a single haplotype of *C. o. cheesmani* was found shared by three specimens. Fijian populations of *C. o. oculatus* displayed the most diversity, as was expected by being in the geographical centre of the species' range. *Carpophilus o. gilloglyi* was most diverse in the Austral Islands.

In the nuclear gene, the situation was reversed with *C. o. oculatus* being more diverse than *C. o. gilloglyi* in both haplotype and nucleotide diversity. *Carpophilus o. cheesmani* had more nuclear than mitochondrial diversity. Fijian *C. o. oculatus* showed the greatest haplotype diversity, while Tongan populations showed the greatest nucleotide diversity.

The population of *C. o. gilloglyi* found in the Kermadecs does not appear to be a recent introduction. From six specimens sampled, a total of four different COI haplotypes were detected. The statistical parsimony haplotype network, also placed specimens from the Kermadecs in the middle of the relevant haplotype network, suggesting that the Kermadecs may have been colonised early on in the diversification of this subspecies.

The lower COI nucleotide diversity of *C. o. oculatus* compared with *C. o. gilloglyi* is surprising considering the larger range inhabited by *C.*

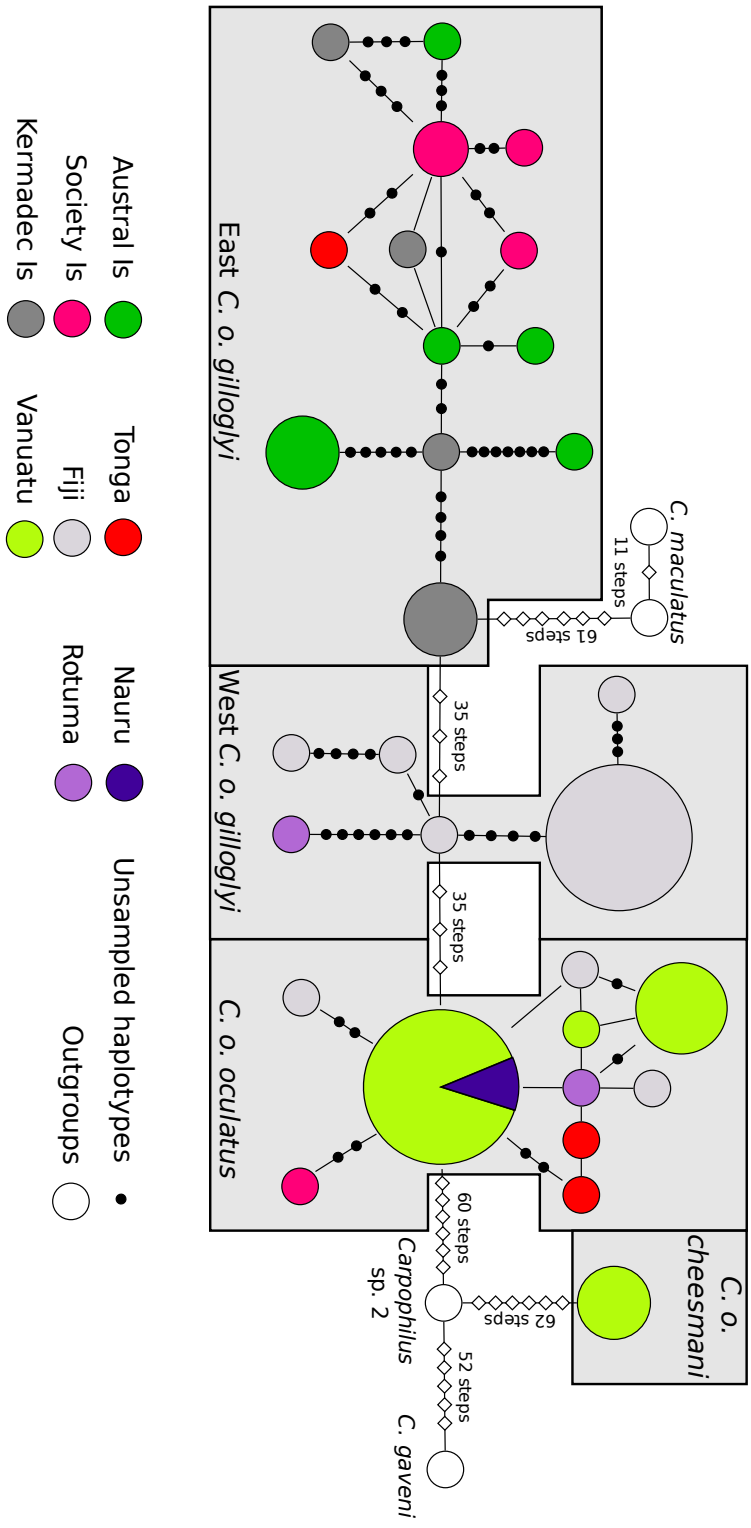


Figure 4.4: Minimum spanning COI haplotype network of *C. oculatus*.

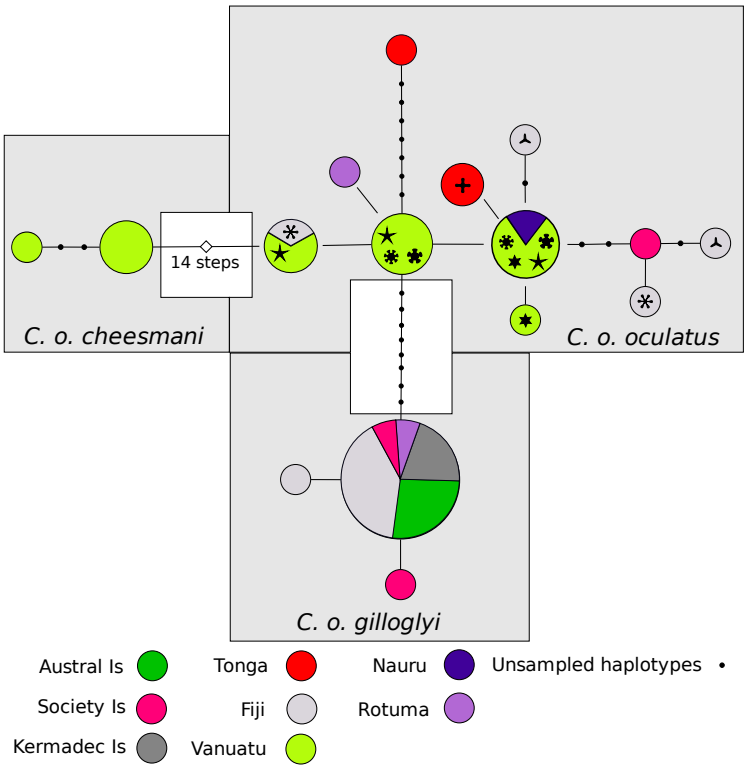
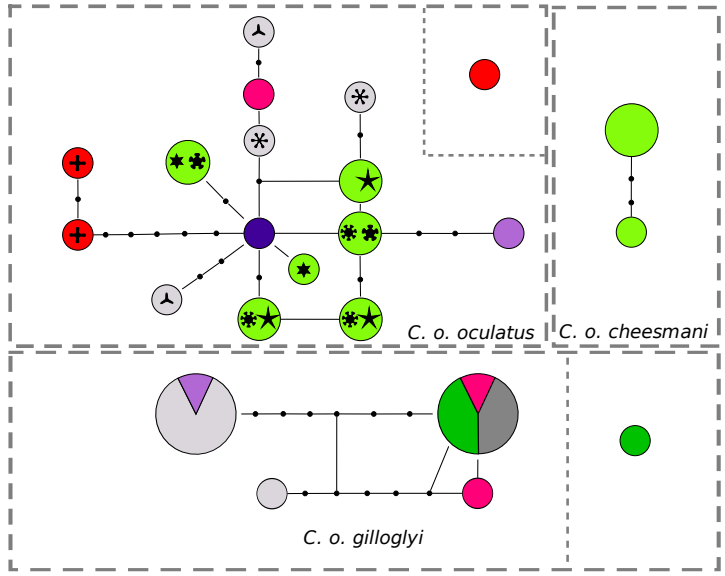
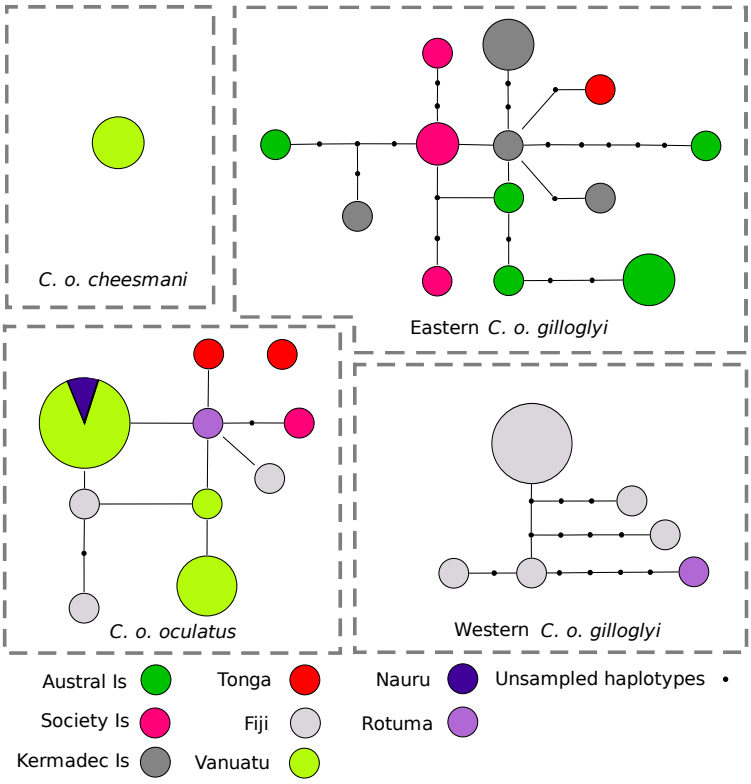


Figure 4.5: Minimum spanning ITS2 allele network of *C. oculatus*. Circles sharing the same symbol denote alleles shared by the same specimen.



(a)



(b)

Figure 4.6: Statistical parsimony networks of *C. oculatus*. Size of circles correspond to the number of specimens sharing those haplotypes. (a) ITS2 copies. Circles sharing the same symbol denote alleles shared by the same specimen. (b) COI haplotypes.

o. oculatus. One would consider that genetic diversity would be greater in species with larger geographic ranges because of the greater distances that would need to be overcome to allow gene flow and thus having a greater likelihood of forming distinct, localised populations. This lower diversity may be indicative of a recent range expansion by *C. o. oculatus*.

AMOVA results show that separation by subspecies reveals the greatest degree of structuring, with geographic variation being much lower, especially within ITS2. All comparisons between populations are therefore best made within subspecific boundaries.

Taken together, the COI and ITS2 results indicate that *C. o. gilloglyi* may be in the process of incipient speciation into a Western clade found in Fiji and Rotuma, and an Eastern clade found from the Kermadec Islands and Tonga to French Polynesia (Fig. 4.1). This is most clear in COI, with strongly defined clades in previous tree analyses (Figs 3.3–3.6), unconnected statistical parsimony networks (Fig. 4.6b), and a large proportion of total variance in AMOVA (Table 4.2). The evidence from ITS2 is less clear cut, shown by patterns in parsimony-based analyses (Figs 3.11 & 4.6a), and a small but informative portion of variation in AMOVA (Table 4.6), that amounts to half of the geographical variance component. This separation in ITS2 is not picked up in likelihood or distance-based analyses, because the variation between the two groups is primarily found in indels possessed differently by the different populations (Fig. 4.3). These alignment gaps are treated differently by the different analytical methods, and when treated as missing data, the geographic structure disappears.

The influences directing this speciation are unclear. Both populations appear identical morphologically, including in details of male genitalia, and both are found in identical host and habitat situations. The geographic

separation occurs between Fiji and Tonga, two archipelagos that have had significant human contact in both modern and pre-European time periods. As such, our current understanding of the system reveals no clear barriers to panmixture, whether morphological, ecological or dispersive. Obviously, further research and greater number of specimens are required to determine if this geographic separation is actually occurring, and that gene flow between these populations really is undetectable across a number of genetic markers. Once this has been confirmed, the factors that maintain this distinction between populations also need to be ascertained.

The inference of secondary contact between the two clades of *C. o. gilloglyi* is intruiging and worthy of future investigation. This conclusion is certainly reasonable from the current data at hand. Secondary contact in Vanuatu *C. o. oculatus* is less apparent and is possibly biased by high representation of only two haplotypes. These haplotypes are not particularly divergent however, and so the possibility of continually panmictic populations, genetic drift or other processes cannot be ruled out. The inference of expansion of the Fijian *C. o. gilloglyi* populations is also interesting. Highly negative D values are correlated with 'star'-like patterns in haplotype networks, formed from a single very common haplotype surrounded by several rarer haplotypes, usually with only one or two mutations differentiating the rarer haplotypes from the common one. These patterns are frequently seen in temperate phylogeographic studies and are often considered to be evidence of refugia during ancient climate cycles (Aoki *et al.*, 2008; Gratton *et al.*, 2008; Rich *et al.*, 2008) This expectation may also be true in situations where populations have arisen from founder effects. Aoki *et al.* (2008) found differences in haplotype network patterns between weevil populations on the Japanese mainland and in the Ryukyu Islands. Mainland populations

displayed 'star' patterns while island populations showed a much looser network with more missing haplotypes, similar to the networks inferred from *C. oculatus*. Aoki *et al.* (2008) attributed this difference to the milder climate of the Ryukyus and the absence of bottlenecks due to severely contracted populations by glaciation during the last ice age. The lack of significance of the fixation index values reported here suggests that these populations should best be considered to be stable and constant until further evidence is brought to bear on the system.

The positive F_S statistic for *C. o. cheesmani* suggests that the population has either gone through a bottleneck, or is currently declining. Interestingly, in the Vanuatu collections undertaken for this study, *C. o. cheesmani* was found to be uncommon on Efate and was not collected on Espiritu Santo.

The ribosomal encoding region consists of several hundred copies repeated throughout the genome. These copies are maintained as identical through a poorly known mechanism of concerted evolution (Liao, 1999; Nei & Rooney, 2005). Intragenomic variation of ITS2 generally indicates that the population is a hybrid (Coleman, 2003), leading to the intriguing possibility that *C. o. oculatus* may have originated through hybridisation. However, this research does not offer any clear indication as to the possible parent species of this taxon, were this the case. Based on morphological similarity and the sister-group relationship between the species as revealed by analysis of COI, it would be assumed that *C. o. gilloglyi* would be a parent species. Interestingly, *C. o. oculatus* ITS sequences are paraphyletic with respect to *C. o. gilloglyi*, which would normally suggest that *C. o. oculatus* is the ancestral species. Previous studies have used coalescent-based methods, such as that implemented in the software STRUCTURE (Hub-

lisz *et al.*, 2009; Pritchard *et al.*, 2000) to determine hybrids (Arif-Ul-Hasan *et al.*, 2009). Studies on mosquitoes (Arif-Ul-Hasan *et al.*, 2009), lice (Leo & Barker, 2002), blackflies (LaRue *et al.*, 2009) and other taxa (Harris & Crandall, 2000) have also revealed multiple copies of ITS2, and it may prove to be a relatively common occurrence. Further research into the ITS2 region of *C. o. oculatus*, and the differing behaviour between *C. o. oculatus* and *C. o. gilloglyi*, two closely related taxa, may provide some insights into the evolution of ITS2 and the mechanisms of concerted evolution.

It is widely believed that elongation of small microsatellites (less than 20 repeats) is more common than contraction (Calabrese & Sainudiin, 2004). These data are consistent with this theory, though there is the exception at position 118. This microsatellite is shorter than other *C. o. oculatus*, but is the same length as the homologous region of *C. o. gilloglyi*. There are two possible explanations for this case. The first is that the microsatellite has been shortened from the usual *C. o. oculatus* state. The second, and potentially more likely explanation, is that it is an ancestral polymorphism from the common ancestor of the two species. Research on the ITS2 region of the confamilial genus *Meligethes* showed that slippage-derived sequences showed important phylogenetic information, however they did not find intragenomic variation within individuals (Trizzino *et al.*, 2009).

Recent developments in phylogeography draw heavily on coalescent theory (Nielsen & Beaumont, 2009). Consequently, a raft of much more sophisticated analytical techniques have come to the fore as phylogeography changes from a primarily inductive discipline to embracing much more of a hypothesis forming and testing paradigm. These analyses provide a method for relating models of demography to phylogenetic trees, utilising techniques such as simulation studies and Markov chain Monte Carlo to estimate the

likelihood of the data given the various models. (Nielsen & Beaumont, 2009). They are very computationally intensive and time consuming and so were not used in this thesis.

4.4.1 Practical applications

These data indicate that COI has the potential to locate the origin of intercepted *C. oculatus*. With one exception, all haplotypes were confined to single archipelagos. The low geographic structuring and the scattering of Eastern *C. o. gilloglyi* amongst each other prevent the provenance of individuals with unsampled haplotypes to be estimated with any certainty. It is also probable that with further sampling, haplotypes that span across different archipelagoes will be found. Before this information can be used in biosecurity operations, it would need to be confirmed that haplotypes are confined to particular island groups. To do this, specimens would need to be looked at from parts of their range that were unable to be sampled over the course of this study, particularly those regions that are important from a trade perspective. Samoa, Niue, and the Cook Islands would be prioritised from a New Zealand point of view. Other island groups required to complete the dataset would be New Caledonia, and Micronesia. Specimens from Hawaii, where the species is believed to have been introduced in the 1960s, would provide an interesting test for this procedure.

Of the subspecies, *C. o. cheesmani* does not appear to present any biosecurity threat, being confined to Vanuatu, and being found only in low numbers there. *Carpophilus o. gilloglyi* potentially presents more of a threat, being widespread throughout the Pacific, and having been recently introduced into Hawaii in the past half century (Dobson, 1993b). In this subspecies, COI has maximum potential to identify the source of intercepted specimens,

as all haplotypes sampled were specific to individual archipelagos. Resolution to the island level is not possible, as there is some overlap between nearby islands, e.g. Moorea and Tahiti; Viti Levu and Vanua Levu. Due to the non-monophyly of populations from the same archipelago, the provenance of intercepted specimens with previously undetected COI haplotypes is not able to be inferred. The subspecies that would be hypothesised from these data to be the greatest risk of being invasive is *C. o. oculatus*. This subspecies has the widest range of the *C. oculatus* subspecies, being found throughout the Pacific, from New Caledonia to French Polynesia. It has a relatively low genetic diversity compared to *C. o. gilloglyi* throughout the range sampled here, which suggests that gene flow may still be taking place. However, in most localities it is relatively rare, being dominated by *C. o. gilloglyi*. Only in Vanuatu and Taveuni (Fiji) was *C. o. oculatus* found in high abundance.

4.4.2 Conclusion

This objective used COI and ITS2 DNA sequences in a phylogeographic analysis to determine the genetic diversity within populations of *C. oculatus* and determine the degree of gene flow. Genetic diversity differed between *C. oculatus* subspecies with *C. o. gilloglyi* having the greatest COI diversity, *C. o. oculatus* having the highest ITS2 diversity *C. o. cheesmani* having the lowest genetic diversity over both markers. Geographic structuring was most obvious in *C. o. gilloglyi* COI data, separating the subspecies into an eastern and a western clade. This split is not reflected as clearly in the ITS2 data, however differing indel positions between these two clades indicate this structure has been established long enough to influence nuclear DNA markers. Genetic diversity measures indicate that the Kermadec Islands

population of *C. o. gilloglyi* is not a recent introduction, but has been there for a significant period of time. Fixation indices indicate that *C. o. oculatus* may have had a relatively recent range expansion, with a potential origin in Fiji, based on nucleotide and haplotype diversity measures. Measures of gene flow were unreliable, but suggested that gene flow is only occurring regularly within *C. o. oculatus* and western *C. o. gilloglyi* populations.

Unfortunately, this study has unbalanced sample sizes that are too small to make definite conclusions. Any future work should aim to both consolidate and add to this body of work, particularly with extensive collecting in Tonga, Samoa, Niue and Micronesia. Coalescent-based analyses should also be used to test these hypotheses of recent population expansion and incipient speciation.

Chapter 5

Carpophilus oculatus: Colour and outline analyses

5.1 Introduction

Carpophilus oculatus is very variable in the colour and shape of the elytral pattern. In some specimens the colour is deep and the pattern forms a clear 'eye' justifying the specific epithet (Figs. 5.1a–c), while in other specimens the colour is less intense and the pattern vague (Fig. 5.1d). This variation presents significant problems for the identification of the species, as this colour pattern provides one of the clearest differences that is supposed to distinguish *C. oculatus* from other *Carpophilus* species, especially *C. maculatus* (Leschen & Marris, 2005).

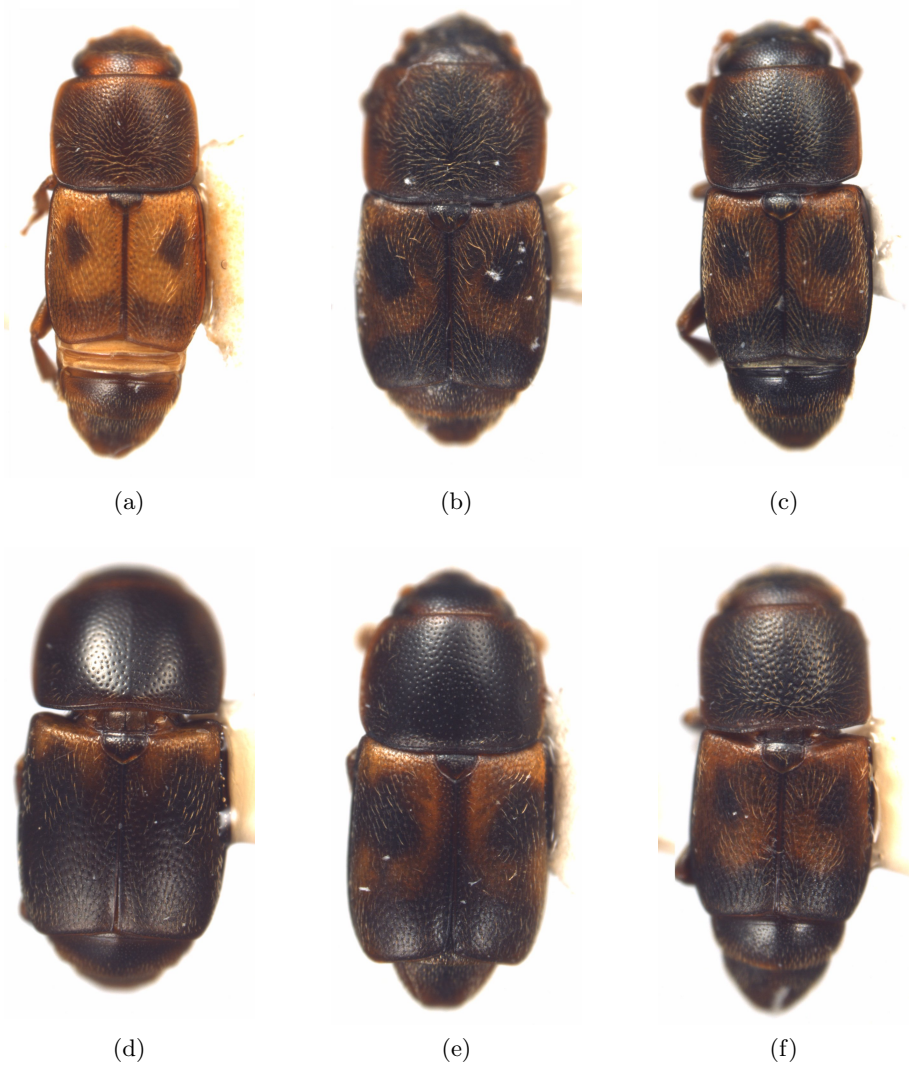
Traditional techniques of investigating these colour patterns involve treating them qualitatively, attempting to organise the variation into discrete groups (Poe & Wiens, 2000). With advances in digital camera technology and morphometrics, it is now possible to bring a greater degree of quantification and objectivity into the process.

The aim of this chapter is to investigate the variability of the shape and colouration of *C. oculatus* elytral patterning and to investigate whether this variation is correlated with subspecific limits, geographic location or host fruit species. These analyses are intended as an exploratory effort to determine the feasibility and usefulness of these techniques in *C. oculatus* identification and systematics.

5.1.1 Colour notation and theory

Colour and the sources and perceptions thereof is a fascinating subject that incorporates fields as diverse as physics, chemistry and philosophy. There remains a lot of debate as to the philosophical and perceptive implications of colour, however the scientific understanding of the phenomenon is well-established and has proved useful in various fields including manufacturing, chemical analysis and technology.

The perceived colour and patterns of insects in general result from the interaction of cuticular pigments and structure of the integument, plus the hairs, scales or waxes on the body of the insect. In some cases the primary source of the colour is structural, being formed by processes such as diffraction and reflection (Seago *et al.*, 2009). Spectacular examples of this include the iridescence displayed by some butterflies (Ghiradella, 1985) and ruteline scarab beetles and entimine weevils (Seago *et al.*, 2009). In most cases however, such as in *C. oculatus*, the colouration is derived primarily from pigments such as melanins, pterines, carotenoids and ommachromes (Fuzeau-Braesch, 1972). These pigments create colour by absorbing certain wavelengths of light, removing them from the light that is being reflected back at the observer. The hue and intensity of the colour depends on distribution of pigments in the integument, how deep it is found, and what



(a) (b) (c)

(d) (e) (f)

Figure 5.1: *Carpophilus oculatus* specimens showing variation in shape and colour of elytral pattern. *C. o. gilloglyi* (a) Taveuni, Fiji; (b) Raoul I., Kermadec Is.; (c) Rapa, Austral Is.; *C. o. oculatus* (d) Espiritu Santo, Vanuatu; (e) Tongatapu, Tonga; *C. o. cheesmani* (f) Efate, Vanuatu.

other pigments are in association with it (Fuzeau-Braesch, 1972). While the exact composition of *C. oculatus* pigments and the influences on their expression have not been determined, blacks and browns are usually formed by melanins and catechols (True, 2003; Roseland *et al.*, 1987).

Colour and colour pattern have been demonstrated to have a genetic component which can be inherited (Favila *et al.*, 2000; Sánchez-Guillén *et al.*, 2005). They can also be influenced by environmental factors such as temperature (Okuda *et al.*, 1997), diet (Grill, 1999) and population density (Barnes & Siva-Jothy, 2000). The conditions in which immature insects develop can also influence the colour of the adult (Grill & Moore, 1998). Colour has correlations with the fitness of the individual (Armitage & Siva-Jothy, 2005; Barnes & Siva-Jothy, 2000), although the effect of this on *C. oculatus* is unknown.

Humans are good at detecting differences in colour, but are unable to quantitatively record them (Kuehni, 2004, pg. 62). Relating colours back to colour chips is a method of standardising colour observations, although differences in colour perception between individuals can cause discrepencies in this method. These differences reveal some of the inadequacies of observer-dependant descriptions of colour. More objective methods of colour determination include spectrophotometry and digital cameras. Spectrophotometry measures the intensity of the wavelengths of light in the UV and visible spectrum that is reflected from a sample object (Kuehni, 2004). Digital cameras focus light onto a semiconductor chip (usually a Charge Coupled Device (CCD)) made up of photodiodes that record the intensity of light that strikes them in a single monochromatic channel. To record colour, a red, green and blue colour filter array is placed over the CCD, such that each photodiode records only the intensity of a single primary colour. These

intensities are then interpolated by the camera's processor to determine the RGB values of each pixel to form a digital image (Brown, 2004). This information is easily extracted from the image using readily available graphics manipulation programs. Both methods have been used for determining the colour of insects (e.g. Akamine *et al.*, 2008; Grill & Moore, 1998; Bezzerides *et al.*, 2007). Disadvantages of spectrophotometry include the cost of the equipment, and measurement areas that are too large for fine-scale work, such as is attempted here. Digital cameras however are unable to record the full UV–visible spectrum, are very sensitive to variation in calibration and lighting, and the method of colour detection may be unsuitable for some applications.

A number of quantitative measures of colour have been developed that seek to describe colours in different ways for different purposes. Systems such as the Munsell Colour Notation and the Optical Society of America Uniform Colour Scale (OSA-UCS) system seek to describe colour in such a way that the colour space is uniform, i.e. the differences between colours will be the same distance whether it's due to changes in hue, chroma or lightness (Kuehni, 2004). The CIELAB system is perception based, and the non-linear relationships between the variables in the system attempt to model human perception of equal differences in colour (Schanda, 2007). Arguably the most common and familiar are the additive systems that have become household names due to their utility in technological systems such as computer monitors, webpages and graphics manipulation programs (Frery *et al.*, 2000). The Red-Green-Blue (RGB) system is based on the concept of primary colours in the form of red, geen and blue phosphors used in televisions and computer monitors. Hue-Saturation-Value (HSV) is related to RGB and provides an interpretation that is related to human colour

perception and thus more intuitive than RGB. The RGB notation consists of three numbers which encode the amount of Red, Green and Blue in the sample on a scale from 0 to 255. An RGB value of 255 255 255 corresponds to pure white, 0 0 0 corresponds to black, 126 126 126 is a mid grey and a value of 255 0 0 corresponds to red. HSV also consists of three numbers, but in this case the first number, Hue, relates to a colour wheel spanning from 0° to 360° (Karcher & Richardson, 2003). The following two numbers relate to the saturation and brightness of the colour, spanning values between 0–100. The theoretical solid formed by RGB calculations is a cube, while that of HSV is a hexagonal cone (Agoston, 2005). This gives the two colour systems different properties and make them incomparable, though they can be converted from one to the other (Agoston, 2005).

Some authors have promoted using RGB as a method for recording and determining the colour of biological organisms (Aguiar, 2005; Berggren & Merilä, 2004; Pullan *et al.*, 2005), however there have been a number of objections to these approaches, due to both to practical difficulties and theoretical principles (Stevens & Cuthill, 2005).

5.1.2 Morphometrics

Elliptic Fourier analysis (EFA) is a method for quantifying shapes that uses the periodic variation in x and y coordinates to calculate a periodic function which is then expanded to provide a number of coefficients that calculate waves (termed harmonics) that describe the shape with increasing accuracy. These coefficients are then used in multivariate analyses to visualise the trends and correlate that with various explanatory variables. EFA has been used to describe the variation in mosquito wings (Rohlf & Archie, 1984) and in analysis of lepidopteran, coleopteran and mantid genitalia (Holwell, 2008;

Kergoard & Alvarez, 2008; Monti *et al.*, 2001). It is useful in situations such as outlines, where the interest lies in the way the shape itself changes, and there are few or no discrete landmarks that can be used. This lack of reliance on landmarks is one of the strengths of EFA, yet it is also one of the problems with the technique. As the orientation of the first harmonic is standardised according to its shape, and not to any specified landmarks, the link between the organism and the mathematical description of shape is severed. This makes it dangerous to compare features, particularly in a phylogenetic framework (Swiderski *et al.*, 2002). Recently however, EFA has been combined successfully with landmark-based Procrustes analyses, minimising this concern (Claude, 2008; Frieß & Baylac, 2003).

Multivariate analysis is a powerful tool for illuminating trends in data which are not immediately obvious. However, it is a hypothesis-generating tool, not a hypothesis-testing one, and its conclusions should refine questions and experimental design for more rigorous testing. It is also tempting to view the results of multivariate analyses as proof of causality, whereas it is more one of correlation. Testing for causality requires carefully designed experiments testing focussed hypotheses. It is also important to remember that colours and patterns obvious to human observers may not necessarily be biologically important (Bennett *et al.*, 1994).

5.2 Methods

5.2.1 Morphometrics

Where available, up to 10 specimens of several *Carpophilus* species were measured using a microscope graticule under 3× magnification. Ten representatives of both the eastern and western clades of *C. o. gilloglyi* were

Figure 5.2: Photograph of *C. o. gilloglyi*, white box showing the location of the 243 pixels × 73 pixels box in which colour histogram was calculated. This specimen was used as a reference for colour and outline analyses.

included. The length and width of both the pronotum and elytra and their combined length was measured. Measurements were taken as recorded in Chapter 2. Pronotal and elytral L/W ratios were calculated from these and analysed using an Analysis of Variance (ANOVA) as implemented in R (R Development Core Team, 2008). *Post hoc* multiple comparisons were calculated using Tukey's honestly significant difference.

5.2.2 Colour and outline analysis

Single images of each *C. oculatus* specimen used in genetic analysis were taken at 3 × magnification under a Nikon SMZ1500 Stereomicroscope fitted with a Qimaging Micropublisher 5.0 RTV digital camera, using the software QCapture pro (Qimaging, Surrey, BC, Canada). The lighting regime of four fibre-optic Zeiss KL1500 LCD spotlights set to 3050 K was set up on an arbitrarily selected reference specimen of *C. o. gilloglyi* before the software was white balanced and a photograph of a grey card was taken at different exposure times to determine the optimum of 83.9 ms. To provide an estimate of

the colour variation between photos, photographs of the grey card and reference specimen were taken before every block of ten specimens, resulting in a total of six photographs of the reference specimen. All photos were taken in one session over a period of three hours. The median, mean and standard deviation of red, green and blue (RGB) values in an area 243 pixels × 73 pixels on the right elytron close to the suture and scutellum (Fig. 5.2) were measured using the 'Histogram' function in the GNU Image Manipulation Program (Kimball *et al.*, 1995-2008). RGB values were converted to HSV values using the conversion equations given in Karcher & Richardson (2003). Images were enhanced with the "Value Invert" function to enable the elytral patterns to be more clearly seen. The shape of the patterns was digitised using ImageJ (Rasband, 1997-2009; Abramoff *et al.*, 2004) as an outline described by 95 to 263 x,y-coordinates. RGB and HSV values were analysed separately using Principal Components Analysis (PCA) in the statistical programming environment R (R Development Core Team, 2008). Previously published functions for elliptic Fourier analysis (Claude, 2008) were used to analyse the outline data and to normalise the Fourier coefficients to standardise for size, rotation and initial starting point of the analysis. Normalised and pre-normalised Fourier coefficients of the first 20 harmonics were analysed separately using PCA. The colour and outline data were analysed both individually and combined and subspecies, host, and distribution data were mapped onto the resulting ordinations.

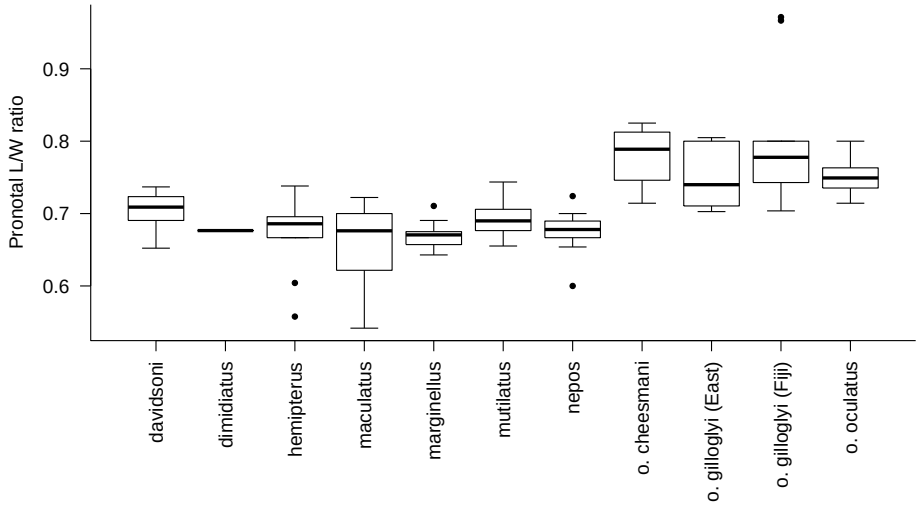


Figure 5.3: Boxplot of median, first and third quartiles and ranges of pronotal L/W ratios of different *Carpophilus* spp.

5.3 Results

5.3.1 Basic morphometrics

Analysis of the pronotal and elytral measurements revealed differences between *Carpophilus* taxa. *Carpophilus oculatus* had a mean pronotal L/W ratio over all subspecies of 0.77. This did not differ significantly within *C. o. oculatus* subspecies, but was significantly different from that of *C. hemipterus*, *C. maculatus*, and *C. nepos* (Figure 5.3).

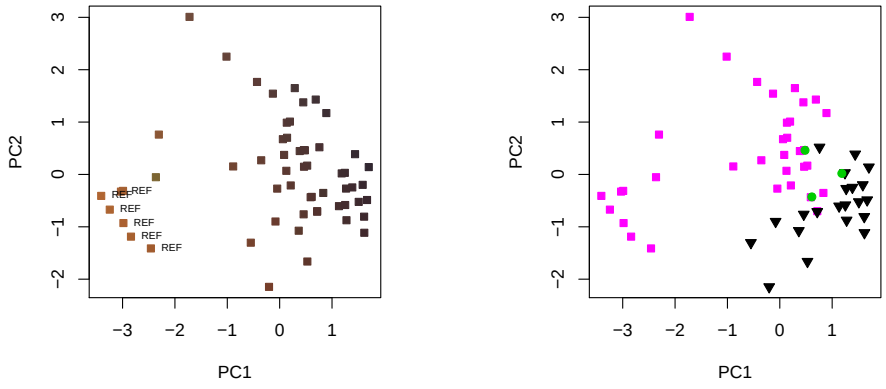
The Eastern and Western clades of *C. o. gilloglyi* were found to have significantly different (p=0.029) pronotal widths having mean widths of 1.26 mm and 1.06 mm respectively. These clades also have significantly different (p=0.025) elytral lengths (1.21 mm vs. 1.04 mm).

5.3.2 Colour analysis

A total of 53 specimens were analysed by colour along with six photos of the reference specimen. PCA showed few differences in the trends revealed by using mean and median RGB values, and in subsequent analyses only the median value was used (Appendix D). HSV scores resulted in a very different result from RGB, and in both cases standardisation made a difference (Appendix D). Superimposition of the recorded colour showed that the first two PCs in all cases were clustering by colour in a logical way (Fig. 5.4a). In the non-standardised analyses, the first PC explained over 95% of the variation, while in standardised analyses this explanatory power of PC1 dropped to 67–82%. In both instances the first two PCs combined explained over 99% of variation.

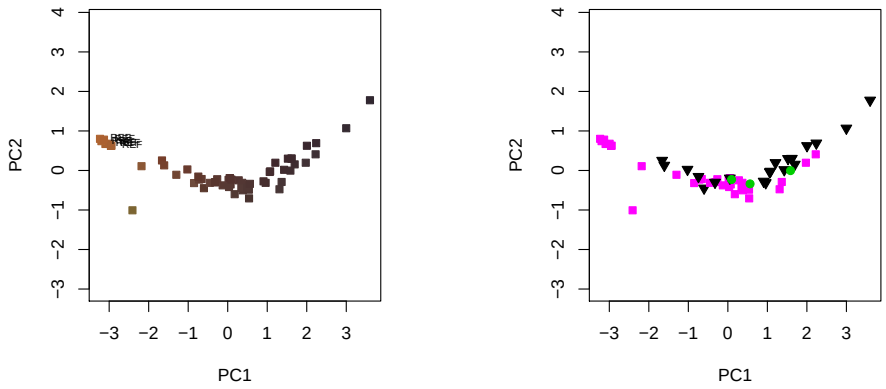
The colour recorded from the reference specimen showed much variability, indicating that the colour measurement technique is not particularly precise. However, the data points from this specimen do form a group, indicating that the method of colour measurement utilised here does give some accuracy. The reference specimen was more closely clustered in the HSV plot. Component correlations of the HSV data showed that the first PC was strongly negatively correlated with saturation and brightness, while hue showed a positive correlation, though much weaker.

Standardised RGB values indicated that there may be some clustering by subspecies (Fig. 5.4b) however, this was not corroborated by the HSV data (Fig. 5.5b). There was broad overlap in the colour of the Eastern and Western populations of *C. o. gilloglyi* (Fig. D.5). The other potential explanatory variables investigated here (distribution and host) did not show any clear groupings, (Figs D.6 & D.7) and seemed to be random across the sampled specimens. This lack of influence of host and distribution was also



(a) (b)

Figure 5.4: Principal Components analysis of median RGB values. Points labeled "REF" indicate colour values derived from the reference specimen. (a) Plot of first two principal components showing gradient according to elytral colour. (b) Same plot, modified to show clustering by subspecies. Pink squares: *C. o. gilloglyi*; green circles: *C. o. cheesmani*; black triangles: *C. o. oculatus*



(a) (b)

Figure 5.5: Principal Components analysis of standardised and corrected HSV values. Points labeled "REF" indicate colour values derived from the reference specimen. (a) Plot of first two principal components showing gradient according to elytral colour. (b) Same plot, modified to show clustering by subspecies. Pink squares: *C. o. gilloglyi*; green circles: *C. o. cheesmani*; black triangles: *C. o. oculatus*

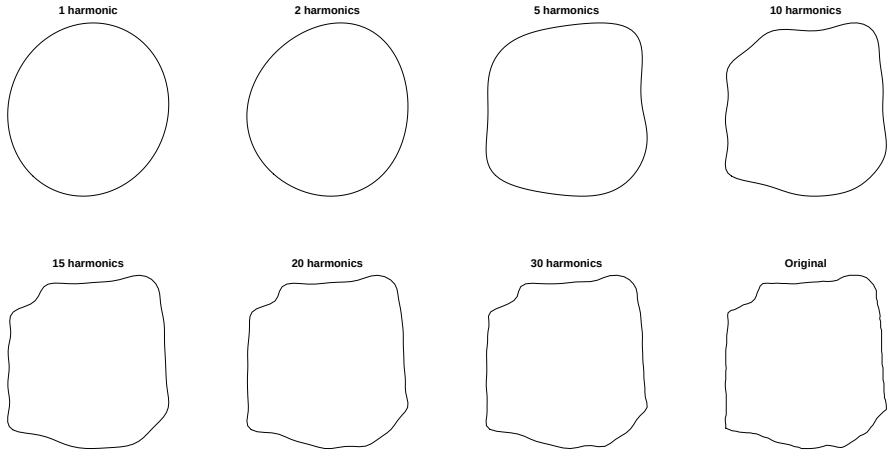


Figure 5.6: Reconstructed outline of reference specimen elytral pattern.

inferred by k-means and UPGMA hierachical clustering methods. However, these methods did not detect the subspecies structure either. Multivariate Analysis of Variance (MANOVA) tests were highly significant, suggesting strong separation between the groups. However, this result can arise when only one group is significantly different from the others; this test is also sensitive to an unbalanced design. This was the case in this situation, therefore the MANOVA results are considered spurious and are not presented here.

5.3.3 Outline analyses

Fifty one specimens had the elytral pattern analysed. Two *C. o. oculatus* specimens (one each from Vanuatu and Tahiti) were excluded from outline analysis because the elytral pattern was not discernable.

The first 20 harmonics were considered to accurately reconstruct the outline (Fig. 5.6), and were used in subsequent ordinations.

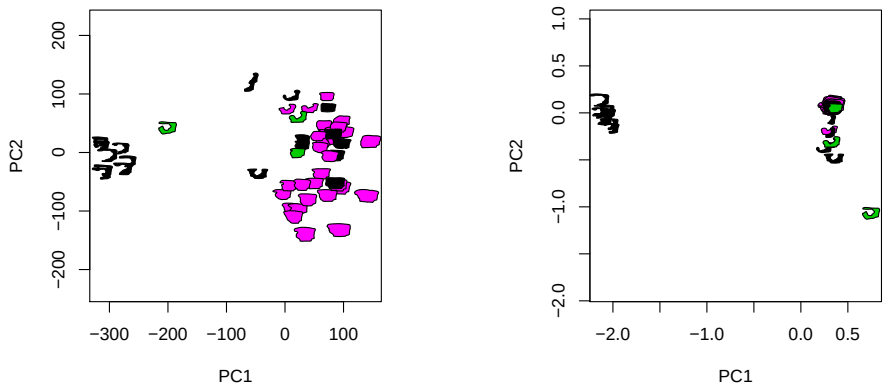
In analyses of general elliptic Fourier harmonics, the first seven PCs explained 99% of the variation (Table 5.1). Normalisation reduced this vari-

Table 5.1: Contributions of Principal Components showing standard deviation (SD) and cumulative proportion (CP) of general and normalised elliptic Fourier analysis.

	General SD	General CP	Normalised SD	Normalised CP
PC1	133.18	0.71	0.86	0.93
PC2	60.66	0.86	0.20	0.97
PC3	36.40	0.91	0.09	0.98
PC4	30.81	0.95	0.06	0.99
PC5	22.48	0.97	0.05	0.99
PC6	14.40	0.98	0.04	0.99
PC7	11.27	0.99	0.03	1.00
PC8	9.09	0.99	0.03	1.00
PC9	7.16	0.99	0.02	1.00
PC10	6.68	0.99	0.02	1.00

ation considerably, requiring only 4 PCs to achieve the same percentage. More surprisingly 93% of variation was explained by the first PC alone. This decrease in variation made a great difference to the ordinations, causing the results of the PCA to be much more tightly clustered (Fig. 5.7b). An interesting difference between the two is the behaviour of one of the *C. o. cheesmani* specimens which overlaps with *C. o. oculatus* in general EFA (Fig. 5.7a), but is very distinct when the data is normalised (Fig. 5.7b).

Elytral outlines did not show any clear clustering according to the predictor variables in this analysis. Results of digitising the pattern of the reference specimen were less variable than the colour quantification (Fig. D.8). *C. o. oculatus* and *C. o. cheesmani* were shown to have much more variable patterning than *C. o. gilloglyi* (Fig. 5.7a), however there was sizable overlap between subspecies. Within *C. o. oculatus*, the groups do not correlate with the clades revealed by either COI or ITS sequences. The Eastern and Western populations of *C. o. gilloglyi* largely overlap and do not show any discrete groups. While analysis of the non-normalised data seem to show



(a) (b)

Figure 5.7: Principal Components analysis of general (a) and normalised (b) elliptic Fourier analysis harmonics of *C. oculatus* elytral pattern, coloured by suspecies. Pink: *C. o. gilloglyi*; green: *C. o. cheesmani*; black: *C. o. oculatus*

some degree of differentiation (Fig. D.8), this structure disappears when size and orientation are factored out (Fig. 5.7b). Geographic distribution did seem to have an effect, with Vanuatu and Rotuman specimens of *C. o. oculatus* having incomplete rings on their elytra when compared with the complete rings of Fijian and French Polynesian *C. o. oculatus* (Fig. D.9). The difference between the two discrete clusters is due to the nature of variation in colour pattern. The shape of the colour pattern of *C. oculatus* is typically a ring that encloses an area darker in colour and nearest the background colour of the elytra (Fig. 5.2). If the edge of this ring is deformed such that the ring is broken, the traced outline is greatly affected—more so than if the pattern was a unicolourous solid.

5.3.4 Combined analyses

Combined analyses looked at the median RGB values and the first 20 harmonics derived from general EFA. The first eight PCs explained 99% of

the variation. Standardising the colour data reduced the number of PCs required for this level of explanation to seven.

Combining the data did not reveal any further clustering that was not already evident in the outline only analyses (Figs. D.11 & D.12).

5.4 Discussion

5.4.1 Basic morphometrics

It is not surprising that species of *Carpophilus* have different pronotal L/W ratios. Indeed, this ratio has been used by many workers as a routine part of describing *Carpophilus* species. The significant difference between *C. oculatus* and *C. maculatus* is important as this is one of the most consistent characters that differentiate the two species.

Also of interest is the difference between of the two clades of *C. o. gilloglyi* in pronotal width and elytral length. These measurments are correlated with body size and indicate that the Western *C. o. gilloglyi* populations are smaller than Eastern populations. This difference is extremely subtle, as evidenced by the fact that it is only statistically significant in two measurements. Therefore it is not useful for distinguishing between the two populations. Measurements for additional specimens may determine whether this significant difference is an artifact of non-random specimen sampling.

5.4.2 Colour and pattern analysis

Discoveries

This analysis provided an initial look into the variability of the colour and patterning of *C. oculatus* elytra. There are discrete clusters in the data, however these are not correlated with any of the variables of collection lo-

cality, host fruit or subspecies identity. There appears to be some degree of differentiation in both shape and colour according to subspecies, although there is a lot of overlap between these groups. The only other trend of potential interest is a possible difference in shape between Vanuatu and Rotuman *C. o. oculatus* and populations of this subspecies from other archipelagoes.

In some individuals, the elytral pattern of *C. o. oculatus* is very similar to that of *C. maculatus*, with lighter patches at the base of the elytra and running down the suture in a way that is characteristic of *C. maculatus*. Further research on these two species in the context of an overall morphological and molecular systematic study would be required to determine if this similarity is due to convergence, or whether this colour pattern is plesiomorphic.

Modifications and experimental deficiencies

In highly hirsute individuals, hairs in the area where colour was measured skewed the mean RGB value towards white. As this was a regular occurrence, it was considered that the median RGB score would most accurately reconstruct the colour of the integument. There was however little difference between the two measures.

There was a lot of error introduced by the method, as revealed by the distribution of points of the reference specimen (Fig. 5.4b). While magnification, lighting setup and yaw of the specimen were kept constant the microscope focus and specimen pitch and roll were optimised for each photo. These effects, combined with the normal variation of an artificial light source probably are the greatest source of this variation in recorded colour. The extent of this variation prevents us from being at all confident about the apparent separation between subspecies according to colour. When trans-

formed to HSV, this variation is diminished significantly, suggesting that HSV might be an appropriate transformation for the subsequent analysis of colour recorded from digital photographs.

Outline analysis of the elytral pattern appeared to be fairly robust and conservative. The multiply recorded shape of the reference specimen showed little variation, and comparison between the normalised and non-normalised Fourier coefficients showed that most of the variation in *C. o. gilloglyi* patterns—which were primarily the typical 'eye' pattern—were due to subtle differences in the size and orientation of the digitised image.

Digitisation of the outline proved difficult for a number of reasons. Firstly, the process involved projecting a pattern on a 3D surface onto a 2D plane which results in a degree of distortion of shape. Secondly, specimens were mounted by gluing the right side of the specimen to the tip of a card point according to standard entomological practice (Walker & Crosby, 1988). The point at which the specimen is glued, and the extent of the glue differs between specimens, coming up onto the elytra more in some specimens than in others and in some cases distorting the lateral edge of the pattern. A possible approach to account for this would be to record the pattern on the other elytron, as the pattern appears to be symmetrical. However, further research would be required to confirm this observation. Finally, by the very virtue that the patterning of *C. oculatus* is highly variable, the outline can be very ill-defined, leading to subjectivity in tracing the outline of the pattern. However, as mentioned previously, analysis of the outline of the reference specimen show that these factors may not influence the results to any great extent. Up to 80 harmonics were made available from EFA, of which the first 20 were used in this analysis. Use of higher harmonics is not advisable because of the inaccuracy in digitising the pattern.

This study relied on visual inspections of PCA ordinations to identify trends in the data. While the human eye is very good at detecting patterns that computers cannot, it is possible for people to see putative clusters in random distributions behoving us to be wary of overanalysing the data. It is also apparent that results are greatly dependant on the methods of recording and transforming the data (RGB vs HSV; normalised vs general EFA).

There are other multivariate methods for determining clusters and determining the effect of multivariate data on categorical variables. Cluster analysis (including k-means and hierachical clustering algorithms) searches the data to determine clusters deemed significant, while Canonical Discriminant Analysis and MANOVA are used for categorical variables. These approaches were experimented with in this analysis, but not utilised fully because of the inadequacy of the data for these analytical techniques. Cluster analysis is optimised for groups that are roughly equal in size (Gordon, 1981), while MANOVA is sensitive to unbalanced experimental designs, as is the case in this study.

For comparing these results with phylogenetic data, there are methods such as Mantel tests that use distance matrices from morphometric and genetic data and identify the correlations between them.

While combined colour and outline analyses showed the same trends as outline alone, this may be because of the relative amounts of data contributed by each method. The use of 20 EFA harmonics to describe the outline resulted in 80 variables as input to the PCA. Colour contributed only three variables. Weighting methods could possibly adjust for this.

While the failure of these techniques indicate that their utility in *C. oculatus* research is limited, they may be useful in other applications.

Implications and applications

An advantage of the system recorded here for analysing colour is that it is cheap and readily available to most researchers and diagnosticians. More accurate colour measurement may be achieved using a spectrophotometer. However such devices are expensive and may be unsuitable for use in small specimens such as insects. Whilst a lot of research goes into colour theory, measurement and notation, this research tends towards physical science and the design industry. Colour is highly important, relevant and interesting in biology, and much more collaboration between these fields would be beneficial. The quantification of colour in taxonomy is a particularly interesting subject and is worth much more investigation.

The lack of correlation between groups revealed by colour analysis and molecular clades may point to a lack of genetic influence on elytral pattern. This possibility would need to be tested rigorously by breeding experiments, or by identification and analysis of the genes involved before it could be accepted. By the same token however, the host fruit did not reveal any influence either. It is important to note that the fruit species that the adult specimens were collected on may not be the fruit they developed on as larvae. Potentially important environmental factors probably have their greatest effect during the developmental stage of the insects lifecycle. In particular, the pupal and eclosion period could plausibly be the most important time with regard to colouration. Breeding experiments combined with host and temperature manipulations may reveal aspects of the mechanism that determine colour.

Discovering the pigments involved in *C. oculatus* colouration will give an indication of the relative importance of genetics vs environment in determining the pattern. Insects are unable to synthesise carotenoids and obtain

them from their diet, usually from plants. Pterines and melanins however are derived by the insects themselves, and thus are more genetically determined.

Normalisation of outlines makes coefficients invariant to size and rotation (Ferson *et al.*, 1985). This would indicate that the variation in *C. o. gilloglyi* elytral patterns primarily consists of minor differences in the size and rotation of the starting ellipse. This also negates any apparent difference in elytral pattern between East and West *C. o. gilloglyi* populations.

The shape of the colour pattern of *C. oculatus* is one of the species' distinguishing traits, and one of the most useful features in its identification. Its variability poses problems for those identifying it, particularly in biosecurity situations where fast, accurate identification is vital. It was surmised in Chapter 4 that within *C. oculatus*, *C. o. oculatus* may be of most interest for biosecurity operations. These results suggest it is also the most variable in pattern. This is confirms that identification of these creatures should be done using more than one character. In the case of *C. o. oculatus*, the pattern can be very similar to that of *C. maculatus*, from which it can be distinguished by having a pronotal L/W ratio of 0.8 (Fig. 5.3), and having round (*c.f.* kidney-shaped) pronotal punctures.

Alhough there appears to be some tenuous clustering according to colour, this is based on relatively few specimens, particularly of *C. o. cheesmani*. Therefore, by these current methods, colour should not be used as a diagnostic tool to distinguish between *C. oculatus* subspecies. As mentioned previously, the measurement of colour relies heavily on the strength, position and colour of the light source, a correctly calibrated camera, and the magnification and orientaton of the specimen. Any departures from the conditions used in computing the standard RGB values would make the results

incomparable. A dedicated setup that can be accurately calibrated over a long time period, accompanied by reference specimens that were included in each photographic run, would be required before this system could possibly be used for identification purposes.

It must be kept in mind that descriptions of colour based on methods such as the one used here only describe the colour that is perceptable and important to humans. Many animals, including beetles (Lin & Wu, 1992; Mishra & Meyer-Rochow, 2006) are able to perceive UV light also, resulting in a very different view of the world. Any suppositions on the biological significance of colour must take this into account (Bennett *et al.*, 1994).

The RGB/HSV system is a convenient measure of colour that is accessible to most researchers. While some authors have promoted describing colours as RGB values in taxonomic papers (Aguiar, 2005), there are a number of considerations to take into account before doing so. For the purpose of communicating colour to users of the information, colour is best recorded in the more intuitive HSV format. The appearence of colour in digital photos is a composite of a large number of pixels with different RGB values. Colour picker tools select the RGB value of a single pixel in most software packages, possibly leading to unrepresentative samples. Histogram tools give an average of the values of all pixels in the selected region, and so give a much better representation of the colour of the area of interest. Unfortunately, the perceived colour of the selection is a composite of that range of pixels, meaning that the single average RGB value may be perceived to be quite different from that calculated. While it may be a more objective measure than verbal descriptions of colour, the limitations of this system also need to be acknowledged. In statistical and multivariate analyses, how best to use the information derived from the process used here requires further thought and

discussion. The three RGB channels should not be separately analysed, as this distinction is neither biologically nor physically meaningful. Depending on the application, the two systems may be considered to be interchangable, however, as converting between the two measures does affect the distribution of the data because of the shape of the theoretical colour solid; the implications of this transformation need to be explored in further detail.

5.5 Conclusion

The aim of this objective was to develop a method to enable quantitative analysis of the variation in elytral colour and pattern in *C. oculatus*. The methods successfully developed here involved taking digital photographs of the specimen and extracting the RGB colour information for colour analysis. The outline of the pattern was digitised and analysed using elliptic Fourier analysis. Both datasets were analysed using principal components analysis. Although the data did not cluster according to any of the predictor variables measured in this study, the method shows potential for further development.

Chapter 6

Conclusion

This thesis set out to investigate the systematics of the *Carpophilus* species found in the South Pacific and focussed primarily on the genetic variation and distinctiveness of the three subspecies of *C. oculatus* and determining the sister taxon of this group. In the process, the following objectives were achieved:

1. A summary of the *Carpophilus* species known from the Pacific and checklists of the species known from each archipelago were produced.

2. Estimates of *Carpophilus* phylogeny using molecular systematics to test the monophyly of *C. oculatus* were produced.

3. A phylogeographic analysis of *C. oculatus* data was able to determine the degree of gene flow and geographic partitioning within the species and subspecies.

4. A colour and outline analysis on *C. oculatus* elytral patterns produced the first quantifiable assessment of the variation within the species.

6.1 Checklist of Pacific *Carpophilus* species

In total, 32 species of *Carpophilus* are known from the Pacific (Chapter 2). The majority of these species have been only collected rarely and appear to be relatively localised in distribution. This number is likely to go up with further collecting in the region, particularly in the Melanesian islands and especially new Guinea. A number of cosmopolitan species such as *C. hemipterus*, *C. dimidiatus*, *C. nepos*, *C. marginellus* and *C. maculatus* are all widespread throughout the islands. Prior to this study, *C. nepos* had not been recorded from the region. *Carpophilus oculatus* is found widely across the Pacific, with specimens confirmed from the area between New Caledonia to Easter Island. Unconfirmed records are also known from the Solomon Islands and Papua New Guinea. As far as is currently known, *C. oculatus* has not been found outside the Pacific.

To assist in the identification of this difficult genus of insects, lists are provided for the species recorded from each Pacific country within the region (Appendix B). A LUCID key is also in preparation by the author to assist with the identification of *Carpophilus*.

Knowledge of the true extent and diversity of *Carpophilus* in the Pacific is hindered by the extremely subtle interspecific variation within the genus, combined with the lack of a comprehensive modern taxonomic review of the genus and biased sampling. A number of species with ranges outside the Pacific are known within the region only from second-hand reports and checklists, the identification accuracy of which are suspect. A number of other *Carpophilus* species are known from old descriptions, which have been mentioned only rarely since their description and then in the form of checklists and other publications that add little to our knowledge of their biology. It is highly recommended that the taxonomy of *Carpophilus* be investigated

in much greater detail, including the inspection of type specimens for these older descriptions, and the evaluation of new characters for the identification of members of the genus.

While a comprehensive biosecurity risk analysis is beyond the scope of this research, field observations and knowledge of *Carpophilus* biology would suggest that *C. maculatus* may be the species in the Pacific of most concern for biosecurity efforts. It is highly abundant, found on a wide range of fruit and vegetables, and is extremely variable and difficult to accurately identify with certainty. The genetic diversity of *C. maculatus* was not sampled comprehensively, but it shows a degree of genetic structure and a reasonably high diversity within the Pacific (Fig. 3.6). *Carpophilus mutilatus* and *C. nepos* are also species of interest due to their presence throughout the region, and their status as agricultural pests in Australia and North America (Arbogast & Throne, 1997; Hossain *et al.*, 2008).

6.2 Molecular systematics

A molecular systematic study of one mitochondrial gene and two nuclear markers (Chapter 3) showed that the three subspecies of *C. oculatus* are genetically distinct, and worthy of consideration for full species status. As subspecies they do form a monophyletic group according to morphological similarity and nuclear markers. However this monophyly is not reflected in the mitochondrial gene, with *C. o. cheesmani* being extremely different from the other two *C. oculatus* subspecies, in a poorly supported relationship with *C. dimidiatus*. The mitochondrial gene also reveals a deep split in *C. o. gilloglyi* between populations in Fiji and Rotuma, and populations in the eastern Pacific from the Kermadec Islands to French Polynesia. This East/West split is not present in the 28S data, but ITS2 reveals a lesser

degree of separation between these two clades. The sister taxon of *C. o. oculatus* and *C. o. gilloglyi* was not resolved with any certainty, appears to be a clade including *C. davidsoni*, *C. maculatus* and *Carpophilus* sp. 2. In no analyses was *C. maculatus* inferred as being the sole sister species of the *C. o. oculatus*–*C. o. gilloglyi* clade.

The inference of higher relationships is made difficult by the incomplete taxon sampling within *Carpophilus*. A member of the genus *Urophorus*, which has traditionally been considered to be a subgenus of *Carpophilus*, came out within the *Carpophilus* clade. However this placement does not fit with morphological evidence and the taxon sampling in that part of the tree is not comprehensive enough to bring its status into question. While COI data was unable to resolve the relationships within the *Myothorax* subgenus with appreciable measures of support, the topology inferred by the ML tree was more congruent with morphological evidence from genitalia than the Bayesian topology. Mapping this morphology onto the trees suggest that the similarity in genitalia between *C. o. gilloglyi* and *C. maculatus* is due to symplesiomorphy rather than convergence.

This research showed that the use of COI as an identification tool is appropriate for *Carpophilus* species, given the availability of a database of correctly identified voucher specimens. However, it is not appropriate for purely DNA-based taxonomy, as it would incorrectly identify two *C. o. maculatus* clades as separate species. It would also identify as separate species the two clades of *C. o. gilloglyi*, which appear to present a case of incipient speciation. The 28S D1-D2 region has been promoted as being a nuclear gene suitable for species identification (Sonnenberg *et al.*, 2007). Within *Carpophilus* however, it performed poorly with essentially identical sequences between species that were distinctly different morphologically and

according to COI.

6.3 Phylogeography

Genetic data was also used in a phylogeographic context to infer the genetic diversity, gene flow and geographic structuring present within *C. oculatus* (Chapter 4). In both COI and ITS2 regions, the major structuring that occurred within the species was at the subspecies level. Geography had no effect other than the major split in *C. o. gilloglyi* described above. Within each subspecies, *C. o. oculatus* had its greatest diversity in Fiji and Rotuma, while *C. o. gilloglyi* had its greatest diversity in the Austral Islands. Little genetic diversity was found in *C. o. cheesmani*. No gene flow was inferred as occurring between the subspecies, nor did there appear to be any between the eastern and western *C. o. gilloglyi* clades. Fixation indices indicated that both *C. o. gilloglyi* and *C. o. oculatus* are expanding, and furthermore that *C. o. gilloglyi* is experiencing secondary contact.

With one exception, no COI haplotypes were found to be shared between archipelagoes. This may provide a method for the determination of the origin of intercepted specimens or introduced populations. However, haplotypes were not monophyletic according to archipelago, so no assumptions can be made regarding the origin of individuals possessing haplotypes that have not been sampled to date. This observation may be an artifact of the sampling in this study, and so many more specimens from across the range of *C. oculatus* would be required before this method could be recommended for such applications.

The ITS2 region in *C. o. oculatus* was shown to have multiple copies within individuals, that usually differed from each other by 2 bp indels in small microsatellite regions. This phenomenon was not present in either of

the other two *C. oculatus* subspecies. These multiple copies do not necessarily come out together in analyses, which may have implications for the phylogeographic utility and analysis of this gene region. The difference between the subspecies is also very interesting and may provide opportunities for the further understanding of the evolution of this marker.

6.4 Colour and outline analyses

Finally, the variation in colour and elytral pattern was investigated using multivariate morphological techniques (Chapter 5). The change in colour quantification between photos was quantified by the use of digital photographs taken through a microscope under standardised conditions. The RGB colour information was then used in a PCA to determine differences in colour. For the elytral pattern, the outline was described using elliptic Fourier harmonic coefficients and analysed in the same way. Results indicated that there may be some separation according to subspecies, however there was large overlap between groups. There were no other groupings according to the other predictor variables of host and locality. The geographic split in *C. o. gilloglyi* also did not appear to have a major effect, though there were significant differences in the pronotal width of the two groups. Differences in the recorded colour from photographs of the reference specimen showed that variation caused by the method was significant. However, variation in the outline within the reference specimen was not as great, suggesting the outline method is more robust than the colour quantification.

Colour has been used extensively in taxonomy in general, and is a key feature of the historical determination of *Carpophilus* (Murray, 1864, e.g.). However, it is a variable character that is subjective and difficult to quantify. While the method presented here may not be perfect, it demonstrates that a

degree of quantification is possible. The use of recording colour in the RGB colour space has been advocated in some papers (Aguiar, 2005). This colour space is extremely useful in contexts such as this one where further analysis is conducted on the data. However, the RGB colour space is unintuitive for the communication of colour to others and the related colour space HSV may be more appropriate for use in species descriptions. For analytical purposes however, the conical shape of the HSV colour space can cause problems. There has been and continues to be extensive research on the properties and the recording of colour. Unfortunately, little of this research filters into the taxonomic community, and the opportunities for further investigation in this area have not yet been capitalised upon.

The use of elliptic Fourier methods for the analysis of outlines was reasonably straightforward and robust. Despite there being no discrete groupings according to these data, the use of elliptic Fourier methods may be a powerful method for the analysis of outlines in other cases. However, the validity of incorporating the data derived from this process into a phylogenetic analysis is still under debate. Some suggest that because the analysis does not rely on landmarks, the assumption of homology between sequences is destroyed (Swiderski *et al.*, 2002). However, more recent research into the method have revealed some methods where this assumption of homology may be preserved (Claude, 2008; Frieß & Baylac, 2003). Even with this advance however, the technicalities of designing models to account for the evolution of these patterns is still an area where much research is needed, and may prove to be different between organisms (Felsenstein, 2002).

Neither of these methods of colour quantification or outline analysis would be suitable in a biosecurity context, as the overlap between subspecies was too great. Moreover, the challenge of standardising conditions to con-

sistently record colour is great and would require much more research to determine the optimum setup to minimise the variation introduced by the method itself.

6.5 Final conclusions and future research

This research offers some insights into the genetic and morphological diversity within *Carpophilus* as a whole and *C. oculatus* in particular, as well as giving some tantalising hints of interesting biological processes. Unfortunately, the scope of this study limited specimen numbers and sampling ranges, resulting in inconclusive results. The exception is the clear differentiation between *C. oculatus* subspecies, leading to a recommendation for the elevation of these subspecies to species status.

The following questions arise from the present study and offer opportunities for further research:

- Are there more, undescribed species of *Carpophilus* in the Pacific islands? The large islands of Melanesia are particularly likely to be inhabited by as yet unknown species of *Carpophilus*.
- To what degree are the two *C. o. gilloglyi* clades geographically separated? Is there overlap in their geographic ranges? If there is, does gene flow occur between the two clades? Are there any morphological or ecological differences between the two?
- Are there any other shared COI haplotypes? Could COI be used to determine the origin of intercepted specimens? Does the inclusion of other island groups such as the Marquesas, Cook Islands, Samoa, Niue and Micronesia change the patterns of diversity?

Finally, a thorough taxonomic revision of *Carpophilus* is desperately needed. The taxonomy and nomenclature of the genus is confused and a comprehensive morphological, molecular and nomenclatural survey of the genus is required to bring some much-needed clarity.

The four objectives of this thesis reveal a wide range of variation over a number of measures and scales, looking at morphological, genetic and species diversity data and investigating the variety of *Carpophilus* in the South Pacific from the generic to the intra-specific level. These analyses show that this region and this genus has great potential to provide further insight into speciation, biogeographic and biological invasion processes in this highly dynamic and diverse region. There remains a lot of scope to flesh out the details and investigate in greater depth many of the phenomena touched on in this thesis. There is a lot more these interesting beetles have to teach us.

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Appendix A

Original description of *Carpophilus littoralis* (Eschscholtz, 1822)

During the course of this research, I was able to obtain a scanned copy of Eschscholtz's original 1822 description of *Carpophilus littoralis*. In the interests of making this description more readily available, the scanned copy is reproduced here (Fig. A.1), and my transcription and translation of the document is presented. The document was transcribed and described using Cunningham (1958) and Scholze-Stubenrecht & Sykes (1994).

A.1 Transcription

24. Nitidula littoralis

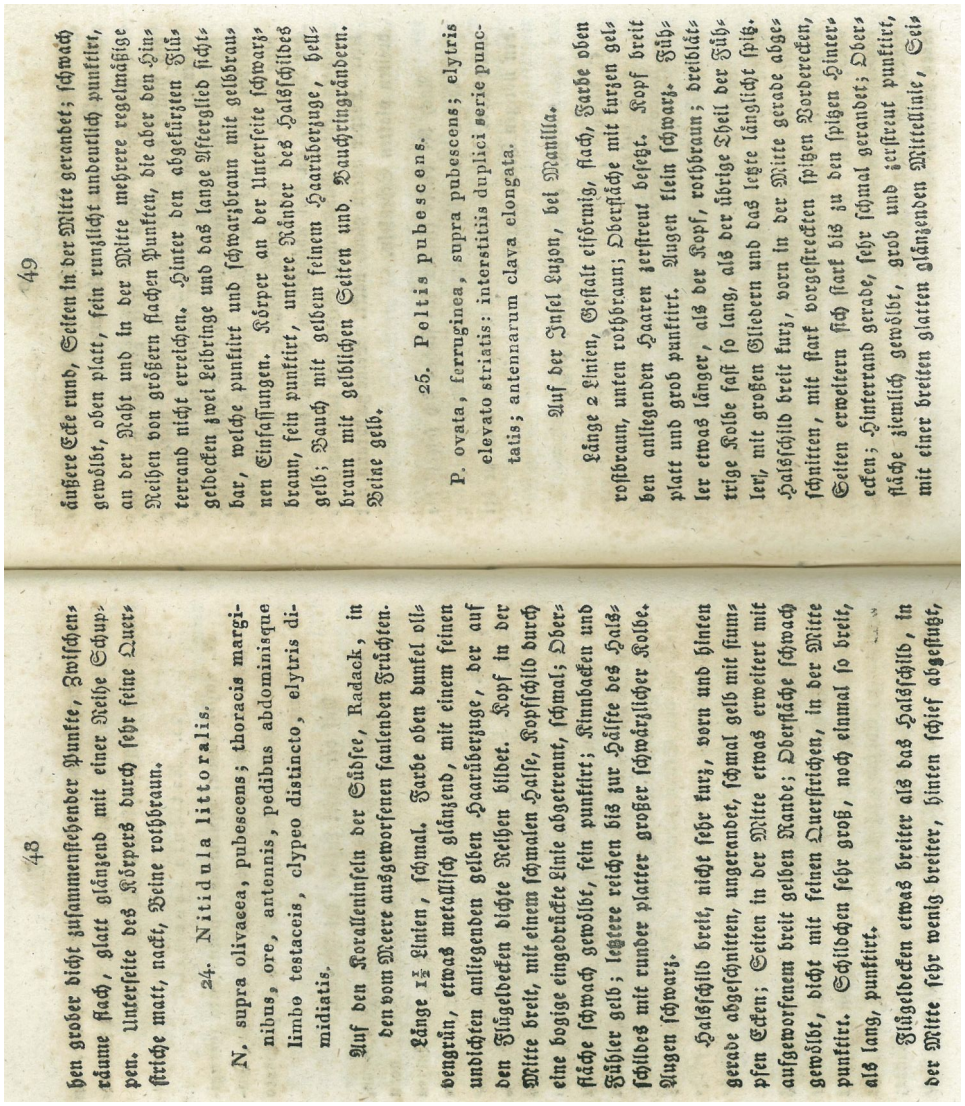
> N. supra olivacea, pubescens; thoracis marginibus, ore, antennis, pedibus abdominisque limbo testacies, clypeo distincto, elytris dimidiatis

Auf den Koralleninseln de Südsee, Radack, in den vom Meere ausgeworfenen faulenden Früchten.

Länge 1($\frac{1}{2}$) linien, schmal. Farbe oben dunkel olivengrün, etwas metallisch glänzend, mit einem feinen undichten anliegenden gelben Haarüberzuge, der auf den Flügeldecken dichte Reihen bildet. Kopf in der Mitte breit, mit einem schmalen Halse, Kopfschild durch eine bogige eingedrückte Linie abgetrennt, schmal; Oberfläche schwach gewölbt, fein punktirt; Kinnbacken und Fühler gelb; letztere reichen bis zur Hälfte des Halsschildes mit runder platter großer schwärzlicher Kolbe. Augen schwarz.

Halsschild breit nicht sehr kurz, vorn und hinten gerade abgeschnitten, ungerandet, schmal gelb mit stumpfen Ecken; Seiten in der Mitte etwas erweitert mit aufgeworfenum breit gelben Rande; Oberfläche schwach gewölbt,

Figure A.1: Facsimile of the original description of *Carpophilus oculatus* (Eschscholtz, 1822)



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hen grober dicht zusammenstehender Punkte, Zwischenräume flach, glatt glänzend mit einer Reihe Schuppen. Unterseite des Körpers durch sehr feine Querstriche matt, nackt, Beine rothbraun.

24. Nitidula littoralis.

N. supra olivacea, pubescens; thoracis marginibus, ore, antennis, pedibus abdominisque limbo testaceis, clypeo distincto, elytris dimidiatis.

Auf den Koralleninseln der Südsee, Radack, in den vom Meere ausgeworfenen faulenden Früchten.

Länge 1½ Linien, schmal. Farbe oben dunkel olivengrün, etwas metallisch glänzend, mit einem feinen undichten anliegenden gelben Haarüberzuge, der auf den Flügeldecken dichte Reihen bildet. Kopf in der Mitte breit, mit einem schmalen Halse, Kopfschild durch eine bogige eingedrückte Linie abgetrennt, schmal; Oberfläche schwach gewölbt, fein punktirt; Kinnbacken und Fühler gelb; letztere reichen bis zur Hälfte des Halsschildes mit runder platter großer schwärzlicher Kolbe. Augen schwarz.

Halsschild breit, nicht sehr kurz, vorn und hinten gerade abgeschnitten, ungerandet, schmal gelb mit stumpfen Ecken; Seiten in der Mitte etwas erweitert mit aufgeworfenem breit gelben Rande; Oberfläche schwach gewölbt, dicht mit feinen Querstrichen, in der Mitte punktirt. Schildchen sehr groß, noch einmal so breit, als lang, punktirt.

Flügeldecken etwas breiter als das Halsschild, in der Mitte sehr wenig breiter, hinten schief abgestutzt,

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äußere Ecke rund, Seiten in der Mitte gerandet; schwach gewölbt, oben platt, fein runzlicht undeutlich punktirt, an der Naht und in der Mitte mehrere regelmäßige Reihen von größern flachen Punkten, die aber den hinterrand nicht erreichen. Hinter den abgekürzten Flügeldecken zwei Leibringe und das lange Afterglied sichtbar, welche punktirt und schwarzbraun mit gelbbraunen Einfassungen. Körper an der Unterseite schwarzbraun, fein punktirt, untere Ränder des Halsschildes gelb; Bauch mit gelbem feinem Haarüberzuge, hellbraun mit gelblichen Seiten und Bauchringrändern. Beine gelb.

25. Poltis pubescens.

P. ovata, ferruginea, supra pubescens; elytris elevato striatis: interstitiis duplici serie punctatis; antennarum clava elongata.

Auf der Insel Luzon, bei Manilla.

Länge 2 Linien, Gestalt eiförmig, flach, Farbe oben rothbraun, unten rothbraun; Oberfläche mit kurzen gelben anliegenden Haaren zerstreut besetzt. Kopf breit platt und grob punktirt. Augen klein schwarz. Fühler etwas länger, als der Kopf, rothbraun; dreiblätrige Kolbe fast so lang, als der übrige Theil der Fühler, mit großen Gliedern und das letzte länglicht spitz. Halsschild breit kurz, vorn in der Mitte gerade abgeschnitten, mit stark vorgestreckten spitzen Vorderecken, Seiten erweitern sich stark bis zu den spitzen Hinterecken; Hinterrand gerade, sehr schmal gerandet; Oberfläche ziemlich gewölbt, grob und zerstreut punktirt, mit einer breiten glatten glänzenden Mittellinie, Sei

dicht mit feinen Querstrichen, in der Mitte punktirt. Schildchen sehr groß, noch einmal so breit, als lang, punktirt.

Flügeldecken etwas breiter als das halsschild, in der mitte sehr wenig breiter, hinten schief abgestutzt, äußere Ecke rund, Seiten in der Mitte gerandet; schwach gewölbt, oben platt, fein runzlicht undeutlich punktirt, an der Naht und in der Mitte mehrere regelmäßige Reihen von größern flachen Punkten, die aber den Hinterrand nicht erreichen. Hinter den abgekürzten Flügeldecken zwei Leibringe und das lange Afterglied sichtbar, welche punktirt und schwarzbraun mit gelbbraunen Einfassungen. Körper an der Unterseite schwarzbraun, fein punktirt, untere Ränder des halsschildes gelb; Bauch mit gelbem feinem haarüberzuge, hellbraun mit gelblichen Seiten und Bauchringrändern. Beine gelb.

A.2 Translation

24. Nitidula littoralis

On the Radack atolls in the South Seas, in rotting fruit washed up from the ocean.

Length 1.5 lines, slender. Colour above dark olive-green, somewhat metallic shining, with a fine closefitting yellow vestiture, which form close rows on the elytra. Head wide in the middle with a slender neck. Clypeus narrowly separated by a bow-shaped depressed line. Surface weakly curved, finely punctured; Gena and antennae yellow; the latter reaching to the midpoint of the pronotum and with large, flat, blackish, round club. Eyes black.

Pronotum wide not very short, anterior and posterior margins obsolete, straight, with blunt yellow angles. Widest medially, lateral margins with projecting wide yellow borders. Surface weakly curved, densely marked with fine diagonal lines, disc punctured. Scutellum very large, twice as wide as long, punctured.

Elytra somewhat wider than pronotum, in the middle very little wider, hind margins obliquely truncate, outer angles round, lateral margins present; weakly curved, disc flat, finely wrinkled and indistinctly punctured at the suture and in the middle many regular rows formed from large, broad punctures, does not reach hind-margin of elytra. Elytra short with two two tergites visible. Genitalia visible, black-brown with yellow-brown border, punctured. Body and underside black-brown, finely punctured, hypomeron yellow; Abodomen with yellow fine setae, sternites light-brown with light yellow sides and margins. Legs yellow.

Appendix B

Regional lists of *Carpophilus* species

Bismarck Archipelago

Carpophilus inconspicuus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus nepos

Caroline Islands

Carpophilus davidsoni
Carpophilus hemipterus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus mutilatus
Carpophilus oculatus indet subsp.
Carpophilus oculatus gilloglyi
Carpophilus truncatus
Urophorus humeralis

Cook Islands

Carpophilus dimidiatus
Carpophilus hemipterus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus mutilatus
Carpophilus nepos
Carpophilus obsoletus
Carpophilus oculatus indet subsp.
Carpophilus oculatus gilloglyi
Carpophilus oculatus oculatus
Carpophilus truncatus

Easter Island

Carpophilus maculatus
Carpophilus oculatus gilloglyi

Fiji

Carpophilus dimidiatus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus mutilatus
Carpophilus nepos
Carpophilus obsoletus
Carpophilus oculatus indet subsp.
Carpophilus oculatus gilloglyi
Carpophilus oculatus oculatus
Carpophilus truncatus
Urophorus humeralis

Gilbert Islands

Carpophilus davidsoni
Carpophilus dimidiatus
Carpophilus hemipterus

Carpophilus maculatus
Carpophilus mutilatus
Carpophilus truncatus
Urophorus humeralis

Guam

Carpophilus davidsoni
Carpophilus dimidiatus
Carpophilus maculatus
Carpophilus mutilatus
Carpophilus truncatus
Urophorus humeralis

Hawaii

Carpophilus dimidiatus
Carpophilus hemipterus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus mutilatus
Carpophilus nepos
Carpophilus oculatus indet subsp.
Carpophilus oculatus oculatus
Urophorus humeralis

West Papua

Carpophilus maculatus
Carpophilus obesus
Carpophilus obsoletus
Carpophilus pallescens
Carpophilus truncatus

Kermadec Islands

Carpophilus oculatus gilloglyi

Kiribati

Carpophilus dimidiatus
Carpophilus maculatus

Marquesas Islands

Carpophilus maculatus
Carpophilus oculatus indet subsp.
Carpophilus oculatus oculatus

Mariana Islands

Carpophilus davidsoni
Carpophilus dimidiatus
Carpophilus hemipterus
Carpophilus maculatus
Carpophilus mutilatus
Carpophilus oculatus indet subsp.
Carpophilus truncatus
Urophorus humeralis

Marshall Islands

Carpophilus davidsoni
Carpophilus dimidiatus
Carpophilus hemipterus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus mutilatus
Carpophilus oculatus indet subsp.
Carpophilus truncatus
Urophorus humeralis

Nauru

Carpophilus maculatus
Carpophilus mutilatus
Carpophilus oculatus oculatus

New Caledonia

Carpophilus dimidiatus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus mutilatus
Carpophilus nepos
Carpophilus obsoletus
Carpophilus oculatus oculatus

Niue

Carpophilus dimidiatus
Carpophilus maculatus
Carpophilus oculatus indet subsp.
Carpophilus oculatus gilloglyi
Carpophilus oculatus oculatus
Carpophilus truncatus

Palau

Carpophilus davidsoni
Carpophilus dimidiatus
Carpophilus frivolus
Carpophilus maculatus
Carpophilus obsoletus
Carpophilus truncatus
Urophorus humeralis

Papua New Guinea

Carpophilus araucariae
Carpophilus bacchusi bacchusi
Carpophilus bacchusi madangensis
Carpophilus bakewelli
Carpophilus dimidiatus
Carpophilus fusus
Carpophilus hemipterus
Carpophilus maculatus
Carpophilus mcnamarai
Carpophilus nepos
Carpophilus obesus
Carpophilus obsoletus
Carpophilus oculatus indet subsp.
Carpophilus pallescens
Carpophilus truncatus
Carpophilus waterhousei

Samoa

Carpophilus maculatus
Carpophilus mutilatus
Carpophilus nepos
Carpophilus obsoletus
Carpophilus oculatus indet subsp.
Carpophilus oculatus gilloglyi
Carpophilus oculatus oculatus

Society Islands

Carpophilus dimidiatus
Carpophilus hemipterus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus mutilatus
Carpophilus nepos
Carpophilus oculatus indet subsp.
Carpophilus oculatus gilloglyi
Carpophilus oculatus oculatus

Solomon Islands

Carpophilus dimidiatus
Carpophilus leveri
Carpophilus maculatus
Carpophilus mutilatus
Carpophilus obsoletus
Carpophilus oculatus indet subsp.
Carpophilus pallescens
Carpophilus truncatus
Carpophilus waterhousei
Urophorus humeralis

Tokelau

Carpophilus dimidiatus
Carpophilus maculatus
Carpophilus oculatus indet subsp.
Carpophilus oculatus oculatus

Tonga

Carpophilus dimidiatus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus obsoletus
Carpophilus oculatus indet subsp.
Carpophilus oculatus gilloglyi
Carpophilus oculatus oculatus

Tuamotu Archipelago

Carpophilus maculatus

Austral Islands

Carpophilus maculatus
Carpophilus oculatus indet subsp.
Carpophilus oculatus gilloglyi

Tuvalu

Carpophilus dimidiatus
Carpophilus maculatus
Carpophilus marginellus
Carpophilus nepos
Carpophilus oculatus gilloglyi
Carpophilus oculatus oculatus
Carpophilus truncatus

Vanuatu

Carpophilus dimidiatus
Carpophilus maculatus
Carpophilus mutilatus
Carpophilus nepos
Carpophilus obsoletus
Carpophilus oculatus cheesmani
Carpophilus oculatus oculatus
Carpophilus pallescens
Carpophilus truncatus
Urophorus humeralis

Appendix C

Details of specimens used for molecular analysis

Taxon	Source/Locality	NCBI Accession numbers COI	28S	ITS2
Omosita discoidea	Genbank	FM877918		
Meligethes gracilis	Genbank	AJ536174		
Meligethes aeneus	Genbank	AJ536173		
Meligethes coracinus	Genbank	AJ536175		
C. maculatus	Hawaii, unpublished COI sequence (C. Ewing pers. comm.)			
C. mutilatus	Hawaii, unpublished COI sequence (C. Ewing pers. comm.)			
Carpophilus sp. JAR-2003	Genbank		AY310664	
Epuraea signata	NZL, New Zealand MC, Rolleston	GU217505	GU217428	–
Epuraea signata	NZL, New Zealand MC, Rolleston	GU217506	–	–
Epuraea ocularis	SOC, French Polynesia, Tahiti, Mahina	GU217504	–	–
Conotelus sp.	USA, United States of America, Idaho, Bonner County	GU217508	GU217431	–
Stelidota sp.	VAN, Vanuatu, Efate, Tagabe Agricultural Research Station	GU217503	GU217432	–
Brachypeplus sp.	AUS, Australia, Queensland, West End	GU217507	–	–
Phenolia sp.	VAN, Vanuatu, Espiritu Santo, Luganville	GU217530	GU217429	–
Aethina concolor	VAN, Vanuatu, Efate, Port Vila	GU217509	GU217430	–
Aethina concolor	VAN, Vanuatu, Efate, Port Vila	GU217510	–	–
Urophorus humeralis	FIJ, Fiji, Vanua Levu, Bua	–	GU217425	–
Urophorus humeralis	AUS, Australia, Victoria, Swan Hill	GU217502	–	–
Carpophilus davidsoni	AUS, Australia, New South Wales, Wandin Valley	GU217435	GU217392	GU217390
Carpophilus hemipterus	AUS, Australia, South Australia, Loxton	GU217437	GU217423	–
Carpophilus hemipterus	NZL, New Zealand MC, Weedons	GU217453	–	–
Carpophilus marginellus	TUV, Fiji, Rotuma, Saolei	GU217446	GU217427	–
Carpophilus marginellus	FIJ, Fiji, Viti Levu, Sigatoka	GU217445	GU217426	–
Carpophilus nepos	NCL, New Caledonia, La Fou	GU217449	GU217413	GU217388
Carpophilus nepos	USA, United States of America, Illinois, NCAUR culture	GU217481	–	–
Carpophilus nepos	AUS, Australia, Queensland, West End	GU217448	GU217407	GU217383
Carpophilus nepos	SOC, French Polynesia, Tahiti, Punaauia	GU217469	–	–
Carpophilus nepos	VAN, Vanuatu, Efate, Tagabe Agricultural Research Station	–	GU217402	–
Carpophilus maculatus	SAM, Western Samoa, Upolu, Lalomanu Village	GU217436	–	GU217387
Carpophilus maculatus	NCL, New Caledonia, La Fou	–	GU217393	–

Carpophilus maculatus	NCL, New Caledonia, La Fou	–	GU217394	–
Carpophilus maculatus	NCL, New Caledonia, La Fou	–	GU217395	–
Carpophilus maculatus	TUV, Wallis and Futuna, Wallis, Wharf	GU217438	GU217396	–
Carpophilus maculatus	TUV, Wallis and Futuna, Wallis, Wharf	GU217439	GU217397	–
Carpophilus maculatus	TUV, Wallis and Futuna, Wallis, Wharf	GU217440	GU217398	GU217384
Carpophilus maculatus	TUV, Wallis and Futuna, Wallis, Mata Utu	GU217443	GU217399	–
Carpophilus maculatus	TUV, Wallis and Futuna, Wallis, Mata Utu	GU217444	GU217400	–
Carpophilus maculatus	NAU, Nauru	GU217441	–	GU217385
Carpophilus maculatus	AUS, Australia, Queensland, West End	GU217447	GU217405	–
Carpophilus maculatus	AUS, Australia, Queensland, West End	–	GU217406	–
Carpophilus maculatus	SOC, French Polynesia, Moorea, Central	GU217433	–	GU217386
Carpophilus maculatus	BIS, Papua New Guinea, New Britain, Kerewat	GU217516	–	–
Carpophilus sp. 1	BIS, Papua New Guinea, New Britain, Kerewat	GU217468	GU217420	–
Carpophilus mutilatus	NAU, Nauru	GU217442	GU217401	–
Carpophilus mutilatus	SOC, French Polynesia, Tahiti, Punaauia	GU217456	GU217411	–
Carpophilus dimidiatus	SOC, French Polynesia, Tahiti, Punaauia	GU217517	GU217418	–
Carpophilus sp. 2	BIS, Papua New Guinea, New Britain, Kerewat	GU217455	–	–
Carpophilus sp. 2	BIS, Papua New Guinea, New Britain, Kerewat	GU217457	GU217412	GU217376
Carpophilus sp. 2	BIS, Papua New Guinea, New Britain, Kerewat	–	–	GU217378
Carpophilus sp. 2	BIS, Papua New Guinea, New Britain, Kerewat	–	–	GU217377
Carpophilus obsoletus	BIS, Papua New Guinea, New Britain, Kerewat	GU217467	–	–
Carpophilus corticinus	USA, United States of America, Illinois, Tazewell County	GU217492	GU217419	–
Carpophilus antiquus	USA, United States of America, Illinois, Tazewell County	GU217497	GU217416	–
Carpophilus lugubris	USA, United States of America, Illinois, Tazewell County	GU217483	–	–
Carpophilus lugubris	USA, United States of America, Illinois, Tazewell County	GU217484	GU217424	–
Carpophilus bakewelli	AUS, Australia, New South Wales, Wandin Valley	GU217498	–	–
Carpophilus davidsoni	AUS, Australia, New South Wales, Wandin Valley	GU217499	–	–
Carpophilus gaveni	NZL, New Zealand AK, Auckland	GU217500	GU217414	GU217389
Carpophilus discoideus	USA, United States of America, Idaho, Bonner County	GU217501	GU217417	–
Carpophilus o. oculatus	NAU, Nauru	GU217485	–	GU217362
Carpophilus o. gilloglyi	FIJ, Fiji, Viti Levu, Vuci	–	GU217403	–
Carpophilus o. gilloglyi	FIJ, Fiji, Viti Levu, Koronivia Research Station	–	GU217404	–
Carpophilus o. gilloglyi	FIJ, Fiji, Viti Levu, Koronivia Research Station	GU217464	–	–
Carpophilus o. gilloglyi	FIJ, Fiji, Viti Levu, Koronivia Research Station	GU217477	–	GU217346
Carpophilus o. gilloglyi	FIJ, Fiji, Viti Levu, Suva	GU217474	–	GU217347
Carpophilus o. gilloglyi	FIJ, Fiji, Viti Levu, Suva	GU217494	–	–
Carpophilus o. gilloglyi	FIJ, Fiji, Viti Levu, Suva	GU217475	–	GU217348

Carpophilus o. gilloglyi	FIJ, Fiji, Viti Levu, Suva	GU217495	–	–
Carpophilus o. oculatus	FIJ, Fiji, Taveuni, Matei	GU217493	–	GU217372
Carpophilus o. oculatus	FIJ, Fiji, Taveuni, Matei	–	–	GU217373
Carpophilus o. oculatus	FIJ, Fiji, Taveuni, Matei	GU217496	–	–
Carpophilus o. oculatus	FIJ, Fiji, Taveuni, Matei	GU217490	–	GU217374
Carpophilus o. oculatus	FIJ, Fiji, Taveuni, Matei	–	–	GU217375
Carpophilus o. oculatus	FIJ, Fiji, Taveuni, Matei	–	GU217408	–
Carpophilus o. gilloglyi	FIJ, Fiji, Taveuni, Matei	GU217454	–	GU217349
Carpophilus o. gilloglyi	FIJ, Fiji, Vanua Levu, Bua	GU217487	GU217409	–
Carpophilus o. gilloglyi	FIJ, Fiji, Vanua Levu, Bua	GU217434	GU217410	GU217351
Carpophilus o. gilloglyi	FIJ, Fiji, Vanua Levu, Delaikoro	GU217486	–	GU217352
Carpophilus o. gilloglyi	FIJ, Fiji, Vanua Levu, Savusavu	GU217488	–	GU217353
Carpophilus o. gillogyli	TUV, Fiji, Rotuma, Saolei	GU217463	–	GU217350
Carpophilus o. oculatus	TUV, Fiji, Rotuma, Saolei	GU217476	GU217391	GU217361
Carpophilus o. gilloglyi	TUB, French Polynesia, Rapa, Tavaitau	GU217450	–	GU217343
Carpophilus o. gilloglyi	TUB, French Polynesia, Rapa, Tavaitau	GU217482	–	GU217344
Carpophilus o. gilloglyi	TUB, French Polynesia, Rapa, Tavaitau	GU217451	–	–
Carpophilus o. gilloglyi	TUB, French Polynesia, Rapa, Tavaitau	GU217479	–	–
Carpophilus o. gilloglyi	TUB, French Polynesia, Rapa, Tavaitau	GU217480	–	–
Carpophilus o. gilloglyi	TUB, French Polynesia, Rapa, Tavaitau	GU217452	–	GU217339
Carpophilus o. gilloglyi	TUB, French Polynesia, Rapa, Tavaitau	–	–	GU217338
Carpophilus o. gilloglyi	TUB, French Polynesia, Rimatara	GU217513	–	GU217354
Carpophilus o. oculatus	SOC, French Polynesia, Tahiti, Pueu	GU217459	–	GU217359
Carpophilus o. gilloglyi	SOC, French Polynesia, Tahiti, Toahotu	GU217465	–	–
Carpophilus o. gilloglyi	SOC, French Polynesia, Tahiti, Vairao	GU217460	–	–
Carpophilus o. gilloglyi	SOC, French Polynesia, Tahiti, PK46	GU217461	–	–
Carpophilus o. gilloglyi	SOC, French Polynesia, Moorea, Baie de Cook	GU217458	–	GU217340
Carpophilus o. oculatus	TON, Tonga, Tongatapu	GU217489	–	GU217369
Carpophilus o. oculatus	TON, Tonga, Tongatapu	–	–	GU217370
Carpophilus o. oculatus	TON, Tonga, Tongatapu	GU217462	GU217415	GU217358
Carpophilus o. gilloglyi	TON, Tonga, Tongatapu	GU217466	–	–
Carpophilus o. gilloglyi	NZL, New Zealand KE, Kermadec Islands, Raoul I.	GU217470	–	–
Carpophilus o. gilloglyi	NZL, New Zealand KE, Kermadec Islands, Raoul I.	GU217471	–	–
Carpophilus o. gilloglyi	NZL, New Zealand KE, Kermadec Islands, Raoul I.	GU217472	GU217421	GU217341
Carpophilus o. gilloglyi	NZL, New Zealand KE, Kermadec Islands, Raoul I.	GU217478	–	GU217345
Carpophilus o. gilloglyi	NZL, New Zealand KE, Kermadec Islands, Raoul I.	GU217491	–	–
Carpophilus o. gilloglyi	NZL, New Zealand KE, Kermadec Islands, Raoul I.	GU217473	–	GU217342

Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Naonepan Landing	GU217512	–	–
Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Luganville	GU217514	–	GU217356
Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Luganville	–	–	GU217357
Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Luganville	–	–	GU217363
Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Luganville	–	–	GU217364
Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Luganville	GU217523	–	GU217360
Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Luganville	GU217524	–	–
Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Luganville	GU217511	–	–
Carpophilus o. oculatus	VAN, Vanuatu, Espiritu Santo, Ipayato	GU217519	–	GU217365
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Port Vila	GU217526	–	–
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Port Vila	GU217515	–	–
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Port Vila	GU217522	–	GU217366
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Port Vila	–	–	GU217367
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Port Vila	–	–	GU217368
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Port Vila	GU217518	–	–
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Tagabe Agricultural Research Station	GU217520	–	GU217371
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Tagabe Agricultural Research Station	–	–	GU217355
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Tagabe Agricultural Research Station	GU217521	–	–
Carpophilus o. cheesmani	VAN, Vanuatu, Efate, Tagabe Agricultural Research Station	GU217527	–	GU217380
Carpophilus o. oculatus	VAN, Vanuatu, Efate, Tagabe Agricultural Research Station	GU217525	–	–
Carpophilus o. cheesmani	VAN, Vanuatu, Efate, Rainbow Garden	GU217528	–	GU217379
Carpophilus o. cheesmani	VAN, Vanuatu, Efate, Rainbow Garden	GU217529	GU217422	GU217381
Carpophilus o. cheesmani	VAN, Vanuatu, Efate, Rainbow Garden	–	–	GU217382

Appendix D

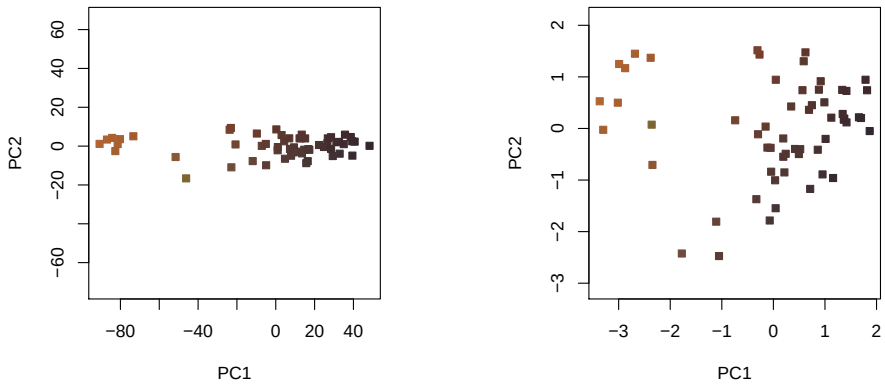
Colour analysis

Mean (Figure D.1) and median (Figure D.2) RGB values seemed to differ in PCA plots primarily by being reflected along the x-axis. There is a little bit of other movement, but essentially, there are no other features that differentiate the two. As the mean values may differ by being skewed to towards white, it was decided to use the median RGB values in the calculation of HSV values, and in combined analyses with outline data.

The raw HSV plots (Figure D.3) show two distinct groups. This however is an artifact of the HSV system, where colour is described as being a continuum on a disc. The hue is described as a value between 0° and 360°, with the red area of the spectrum crossing the 0° mark. Thus, the difference between 359° and 1° is exactly the same as between 8° and 10°. When uncorrected, this system of colour description produced a PCA plot showing two discrete groups. When corrected by centering the values around 0 (Figure B.4), PCA correctly identifies the continuum. This analysis only works in this instance when the colours are confined to one part of the spectrum. Were colours distributed around the whole HSV sphere, the discontinuities of the system of notation would create distortions in a similar way to map projection.

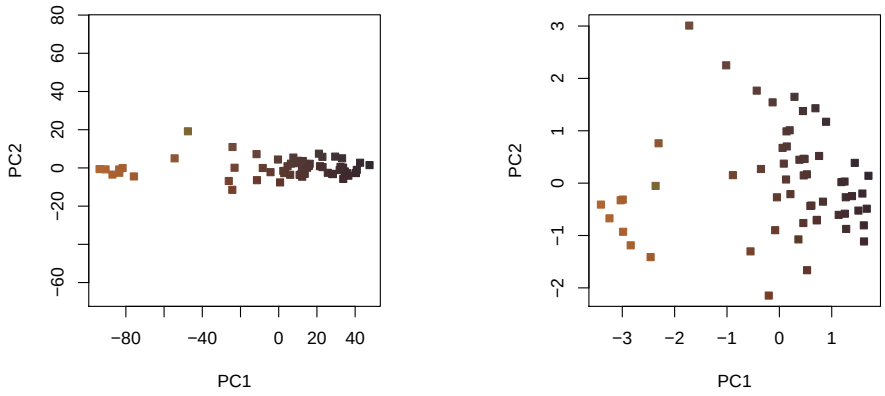
Outline analysis showed great differences between the results of general and normalised EFA. When size, rotation and starting point are removed from the data, variation decreases significantly.

Both of these plots indicate the importance of standardisation and transformation of data on the results of subsequent analyses.



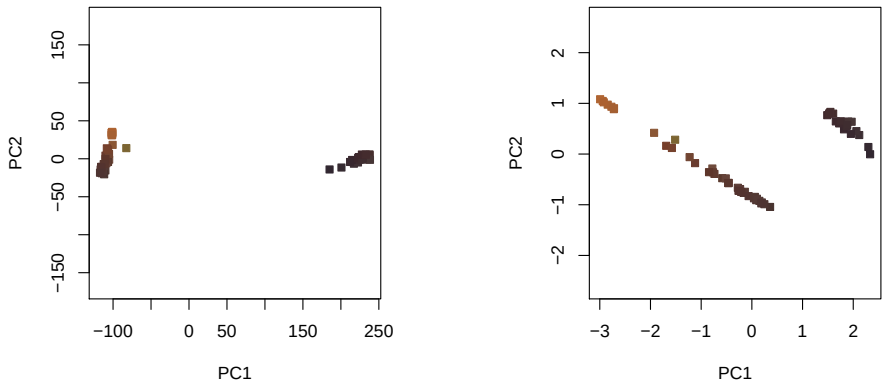
(a) (b)

Figure D.1: Principal Components analysis of mean RGB values showing elytral colour gradient. (a) Raw values. (b) Standardised values.



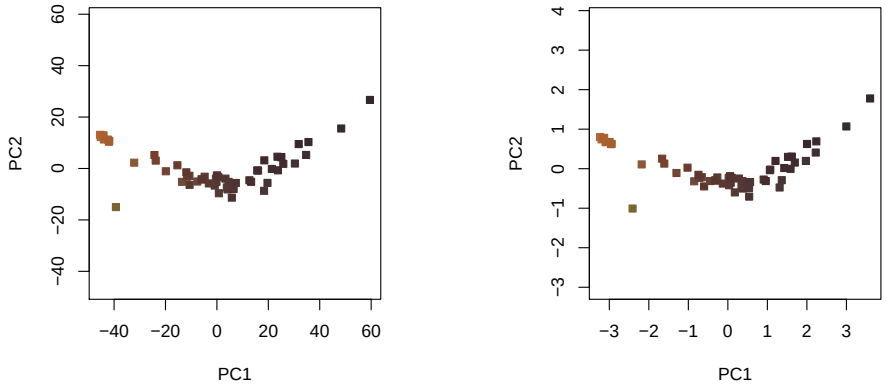
(a) (b)

Figure D.2: Principal Components analysis of median RGB values showing elytral colour gradient. (a) Raw values. (b) Standardised values.



(a) (b)

Figure D.3: Principal Components analysis of uncorrected calculated HSV values showing elytral colour gradient. (a) Raw values. (b) Standardised values.



(a) (b)

Figure D.4: Principal Components analysis of corrected calculated HSV values showing elytral colour gradient. (a) Raw values. (b) Standardised values.

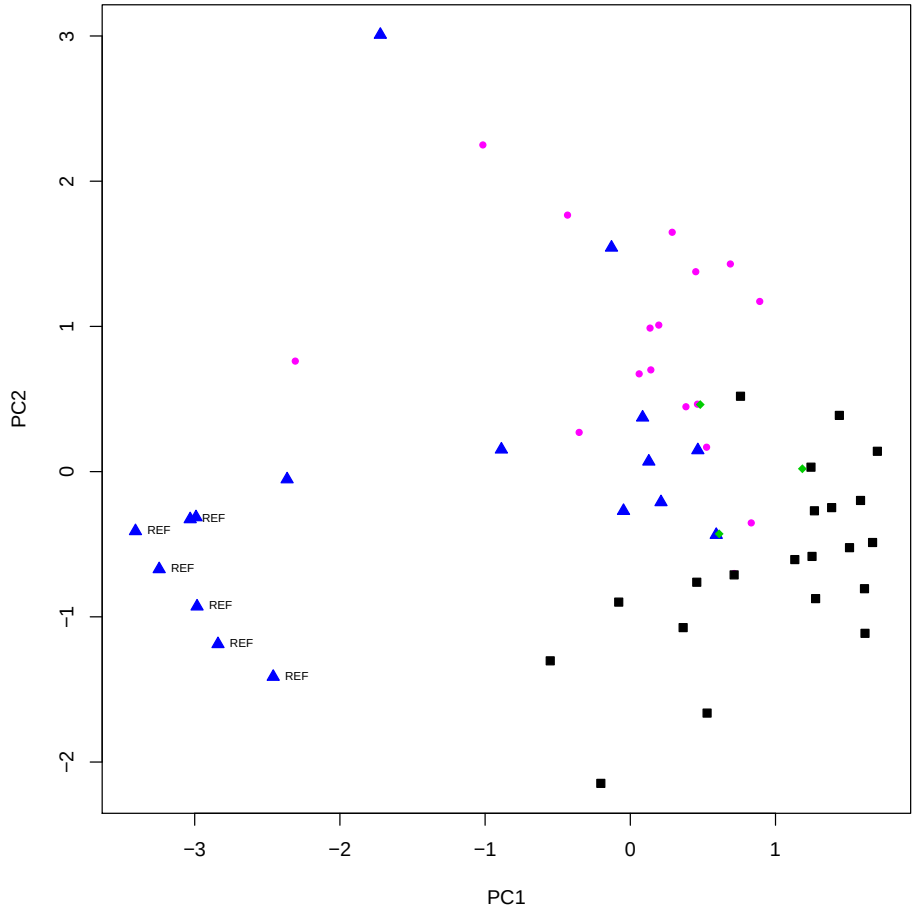


Figure D.5: Principal Components analysis of standardised median RGB values, coloured according to mtDNA phylogeny. Points labeled "REF" indicate colour values derived from the reference specimen. Blue triangles - Western *C. o. gilloglyi* populations; pink circles - Eastern *C. o. gilloglyi* populations; black squares - *C. o. oculatus*; green diamonds - *C. o. cheesmani*.

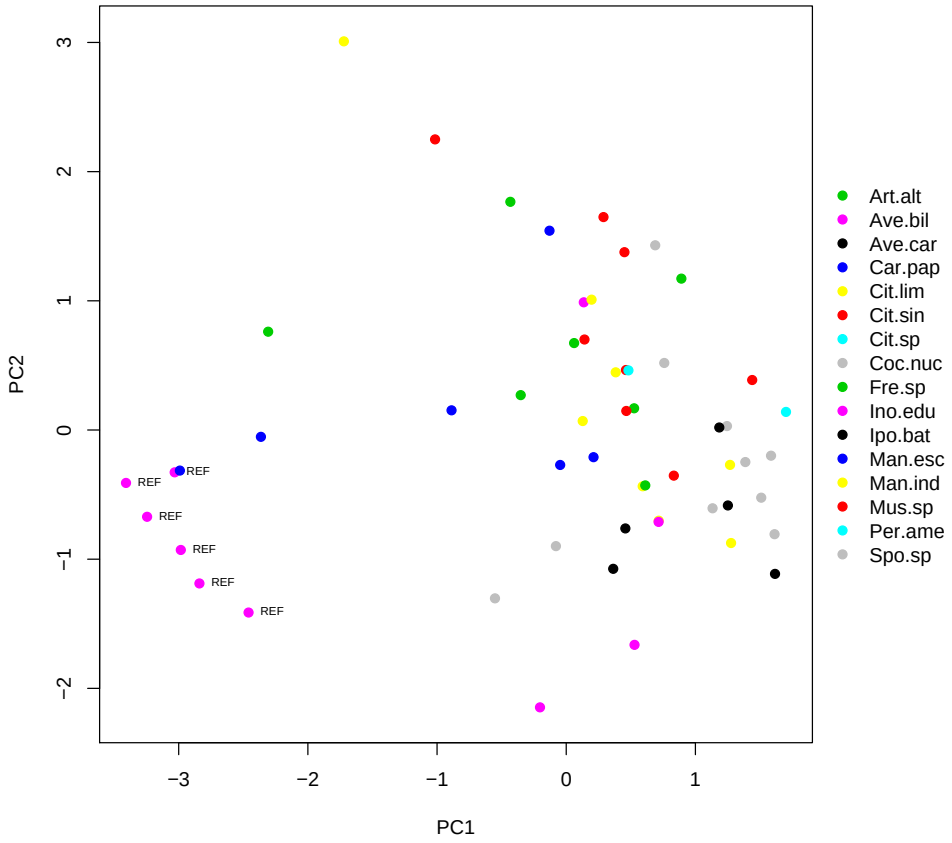


Figure D.6: Principal Components analysis of standardised median RGB values, coloured according to host. Points labeled "REF" indicate colour values derived from the reference specimen. Host abbreviations: Art.alt - *Artocarpus altitus* (Breadfruit) (Moraceae); Ave.bil - *Averrhoa bilimbi* (Bilimbi) (Oxalidaceae); Ave.car - *Averrhoa carambola* (Starfruit); Car.pap - *Carica papaya* (Pawpaw) (Caricaceae); Cit.lim - *Citrus limon* (Lemon) (Rutaceae); Cit.sin - *Citrus sinensis* (Orange) (Rutaceae); Cit.sp - *Citrus* spp. (Citrus) (Rutaceae); Coc.nuc - *Cocos nucifera* (Coconut) (Arecaceae); Fre.sp - *Freycinetia* sp. (Kiekie) (Pandanaceae); Ino.edu - *Inocarpus edulis* (Tahitian Chestnut) (Fabaceae); Ipo.bat - *Ipomoea batatas* (Sweet Potato) (Convolvulaceae); Man.esc - *Manihot esculenta* (Cassava) (Euphorbiaceae); Man.ind - *Mangifera indica* (Mango) (Anacardiaceae); Mus.sp - *Musa* spp. (Banana) (Musaceae); Per.ame - *Persea americana* (Avocado) (Lauraceae); Spo.sp - *Spondias* spp. (Golden apple) (Anacardiaceae).

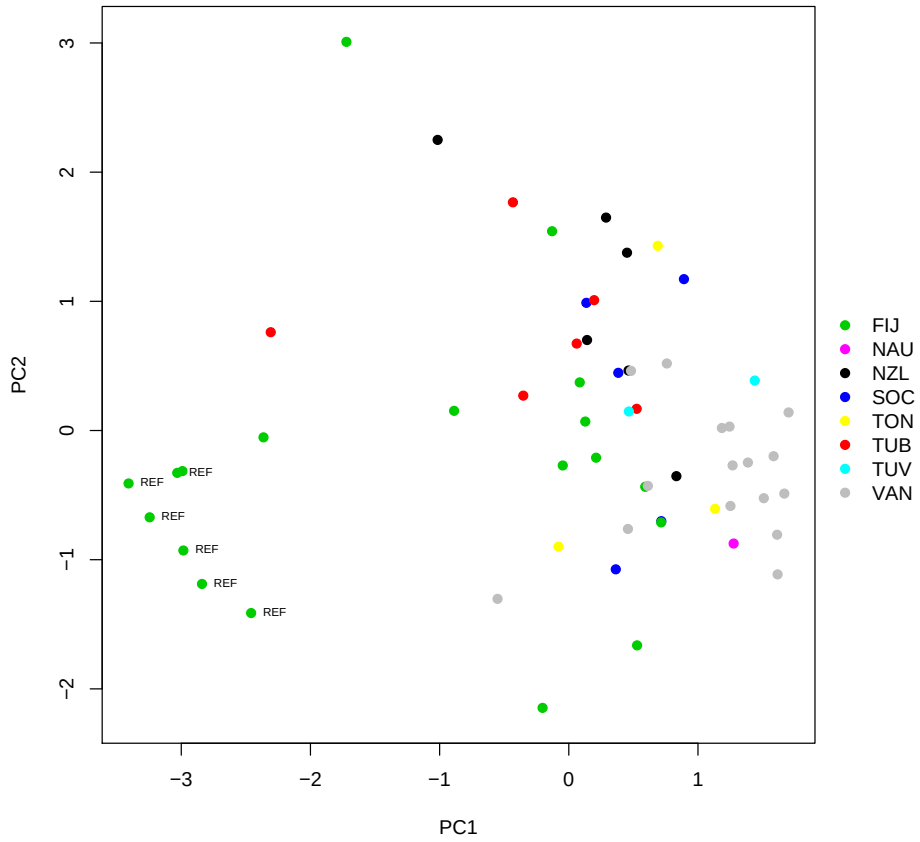


Figure D.7: Principal Components analysis of standardised median RGB values, coloured according to archipelago. Points labeled "REF" indicate colour values derived from the reference specimen. Legend abbreviations: FIJ - Fiji; NAU - Nauru; NZL - Kermadec Islands (New Zealand); SOC - Society Islands (French Polynesia); TON - Tonga; TUB - Tubuai Islands (a.k.a. Austral Is., French Polynesia); TUV - Rotuma (close to Tuvalu); VAN - Vanuatu.

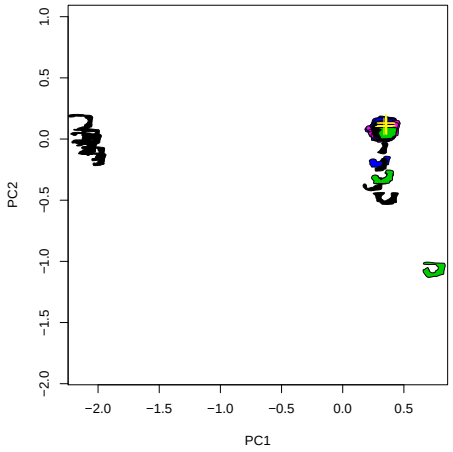


Figure D.8: Principal Components analysis of normalised elliptic Fourier analysis on elytral patterns. Black - *C. o. oculatus*; green - *C. o. cheesmani*; pink - Eastern *C. o. gilloglyi*; blue - Western *C. o. gilloglyi*. Yellow crosses indicate position of reference specimen replicates.

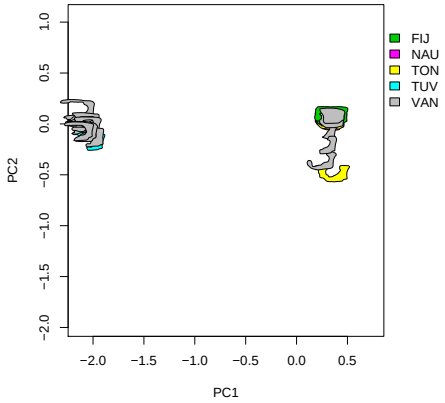


Figure D.9: Principal Components analysis of general elliptic Fourier analysis on *C. o. oculatus* elytral patterns. Archipelago codes as for Fig D.7

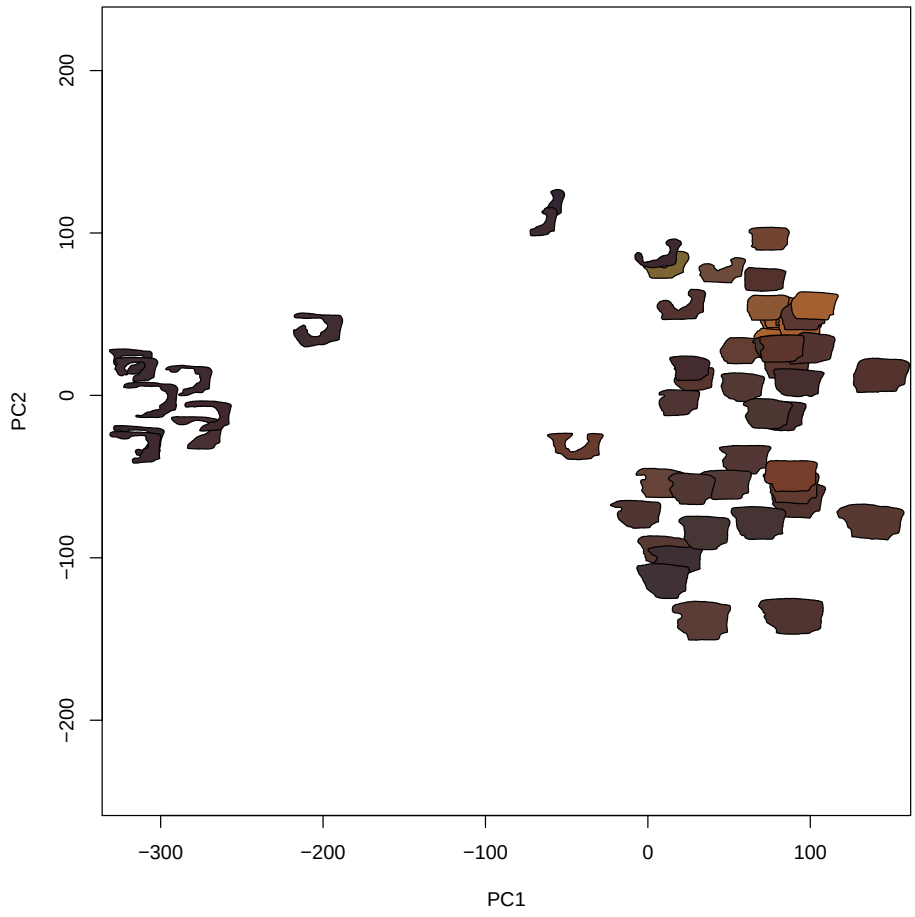


Figure D.10: First two Principal Components of combined general elliptic Fourier analysis harmonics and colour of *C. oculatus* elytral patterns. Colours correspond to the recorded RGB values of the elytral pattern.

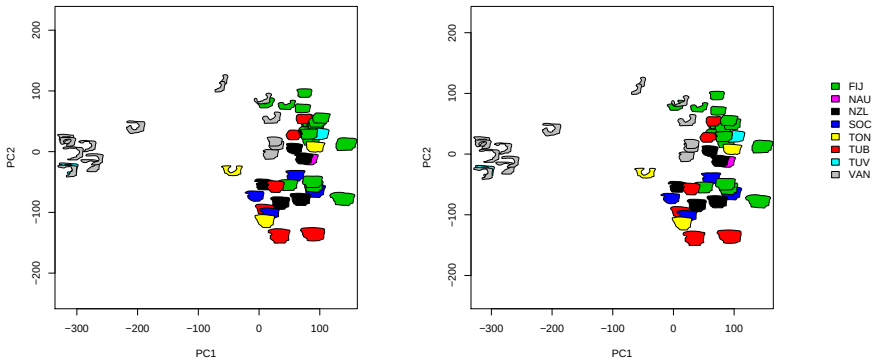


Figure D.11: First two Principal Components coloured according to archipelago. Left: Combined shape and colour data. Right: Shape data only.

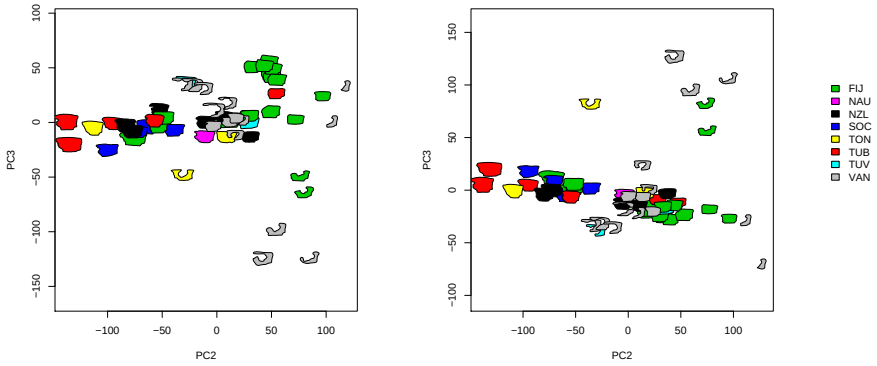


Figure D.12: Second and third Principal Components coloured according to archipelago. Left: Combined shape and colour data. Right: Shape data only.