

Phylogeny of the *Heterodoxus octoseriatus* group (Phthiraptera: Boopiidae) from rock-wallabies (Marsupialia: *Petrogale*)

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Accepted for publication 18th June, 1990.

Abstract

The phylogenetic position of the *Heterodoxus octoseriatus* group is inferred from morphological characters. Two character states support monophyly of this group. Another indicates that its sister-group is a group of 11 other *Heterodoxus* species (here called the *H. calabyi* group), that infest at least seven other genera of macropodid marsupials. Fourteen potential apomorphies, associated with the male and female genitalia, are identified. Evident rapid and divergent evolution of the genitalia, however, precludes determination of their polarity by comparison with an out-group (the sister-group). Consequently, phylogeny in the *H. octoseriatus* group is inferred from the close similarity of morphological characters. In light of the phylogenetic analysis and a phenogram, speciation and the evolution of morphological characters in the *H. octoseriatus* group is discussed.

Introduction

Rock-wallabies (*Petrogale* spp.) are infested by two groups of amblyceran lice from the family Boopiidae, the *Heterodoxus octoseriatus* and *H. ampullatus* groups (Clay, 1981; Barker, 1991). Eleven species are presently recognised in the *H. octoseriatus* group (Barker, 1991): all occur in eastern Australia (Barker & Close, 1990).

Inference of phylogeny in the *H. octoseriatus* group is important. First, it will allow the evolution of associations among lice and rock-wallabies to be examined and the co-evolution model to be tested. Of all the macroparasites, lice are most likely to co-evolve with their hosts because they lack intermediate hosts, are totally dependent on the micro-environment of their hosts and are wing-less (Price, 1980). Second, it may provide insight into speciation in the *H. octoseriatus* group and in the genus *Petrogale*, and third, it should provide insight into the evolution of morphological characters in the genus *Heterodoxus*.

This study was conducted simultaneously with a taxonomic review (Barker, 1991) and a zoogeographical analysis (Barker & Close, 1990) of the *H. octoseriatus* group. Within the *H. octoseriatus* group general morphology and chaetotaxy is conservative (Barker, 1991), thus precluding phylogenetic inference based on those character. In contrast, the morphology of the genitalia, particularly the male genitalia, differs widely among species. In this study 17 characters were identified, their suitability to cladistic analyses determined and the phylogeny of the *H. octoseriatus* group inferred, though not by the cladistic method.

Materials and methods

To find a suitable out-group to the *H. octoseriatus* group for cladistic analyses, I searched the literature and examined specimens for apomorphies that might reveal the sister-group of *H. octoseriatus* group. Morphological data for the out-group

were obtained from specimens mounted on glass slides, specimens viewed with a scanning electron microscope and from the published work of Clay (1969, 1981) and von Kéler (1971). Morphological data for the *H. octoseriatus* group were obtained from specimens mounted on glass slides (listed in Barker, 1991). Louse eggs were examined with scanning electron and light microscopes. Eggs of the following species were examined (locality and host data in tables I-III of Barker & Close, 1991): *H. lesouefi* (host number – S726), *H. briscoei* (S733, S736), *H. insulatus* (S879, B149), *H. octoseriatus* (S885), *H. maynesi* (S713), *H. closei* (S625), *H. insularis* (S462 a captive *P. assimilis* Mareeba) and *H. orarius* (S621). Phylogenies were constructed by hand.

Results

Monophyly of the H. octoseriatus group and identification of its sister-group

The Amblycera, which is supported by at least two apomorphies (Lyal, 1985), comprises the Menoponidae and Boopiidae, together with four other families (Clay, 1970). A formal cladistic analysis of the Amblycera is wanting. Clay (1970), however, derived the Boopiidae directly from bird infesting menoponid stock, implying a sister-group relationship between the Boopiidae and Menoponidae. Based on this, the Menoponidae was used as an out-group to the Boopiidae.

With *Heterodoxus* three binary characters, which shed light on the relationships of the *H. octoseriatus* groups to other boopiid species, were identified.

1. Position of third temporal seta.

Plesiomorphic – third temporal seta nearer the alveolus of seta 2 than the posterior margin of the antennal groove. This character state is characteristic of the Menoponidae (see Clay, 1969). It occurs also in *H. ampullatus* and the undescribed taxa (specimens in the Australian National Insect Collection) examined by Clay (1981) which were placed by her in the *H. ampullatus* group (per-

sonal observations), and in *Therodoxus oweni* (Clay, 1971). [Note that seta 2 is seta 27 of Clay (1969, fig. 2) and seta 3 is most probably seta 30 of Clay (1969, figs 2, 3) according to Clay (1981)].

Apomorphic – third temporal seta nearer to the posterior margin of the antennal groove than to the alveolus of seta 2. Present in the 11 species of the *H. octoseriatus* group (Clay, 1981; Barker, 1991) and in *H. calabyi*, *H. ancoratus*, *H. longitarsus*, *H. maai*, *H. macropus*, *H. quadriseriatus*, *H. spiniger*, *H. mitratus*, *H. ualabati*, *H. alatus* and *H. pygidialis* (see von Kéler, 1971). To aid discussion the last 11 species are hereafter referred to as the *H. calabyi* group.

2. Lateral structure on egg.

Plesiomorphic – egg without lateral structure. Apparently present throughout the Menoponidae (M.D. Murray, unpublished data; examples in Balter, 1968a, b) and in all boopiid species examined (Table I), except those of the *H. octoseriatus* group.

Apomorphic – egg with lateral structure derived from the surface scales (Fig. 1). Present in the 8 species of the *H. octoseriatus* group examined (eggs of *H. hughendensis*, *H. harrisoni* and *H. murrayi* were not available).

3. Vesica.

Plesiomorphic – male genitalia with vesica not divided into two parts (the vesica and Clay's (1981) sac-like part of the copulatory apparatus are synonymous). Present in all of the described boopiid species, except those of the *H. octoseriatus* group (Clay, 1981; Barker, 1991).

Apomorphic – vesica divided into two areas (Barker, 1991, figs 1,6,7). Present in the 11 species of the *H. octoseriatus* group (Clay, 1981; Barker, 1991).

Genitalia of the type seen in the Boopiidae have apparently not been recorded in the Menoponidae. Thus, at this level out-group comparison using the Menoponidae is uninformative. However, the position of the third temporal seta (character 1) unites into a monophyletic group, the 11 species of the *H. octoseriatus* group and

Table 1. Boopiid species known to have eggs lacking lateral structure. Abbreviations: *H.*, *Heterodoxus*; *P.*, *Petrogale*; UQ-Ento, Museum of the University of Queensland Department of Entomology; ANIC, Australian National Insect Collection; AC, authors collection.

Species	Host	Coll. No.	Collection
<i>H. calabyi</i>	<i>Macropus eugenii</i>	B192	UQ-Ento
<i>H. longitarsus</i>	<i>Macropus rufus</i>	1/C81	UQ-Ento
<i>Boopia</i> sp.	bandicoot	6/12/40	UQ-Ento
<i>Boopia</i> sp.	<i>Isoodon macrourus</i>	****	UQ-Ento
<i>Boopia</i> sp.	<i>Vombatus ursinus</i>	****	UQ-Ento
<i>H. spiniger</i>	dog	McCulloch	(1933)
<i>H.</i> sp. 14	<i>P. persephone</i>	B155	AC
<i>H.</i> sp. 15	<i>P. lat. purpureicollis</i>	S887	AC
<i>H. ampullatus</i>	<i>P. p. penicillata</i>	82-130	AC
<i>H.</i> sp.	<i>P. xanthopus celeris</i>	B169	ANIC
<i>H.</i> sp.	<i>P. lateralis pearsoni</i>	84-1339	ANIC
<i>H.</i> sp.	<i>P. lateralis hacketti</i>	RW224	AC

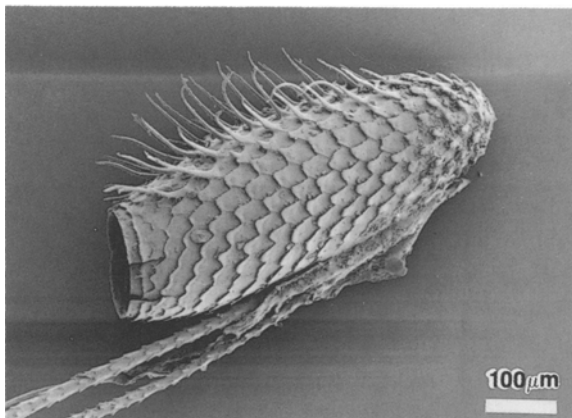
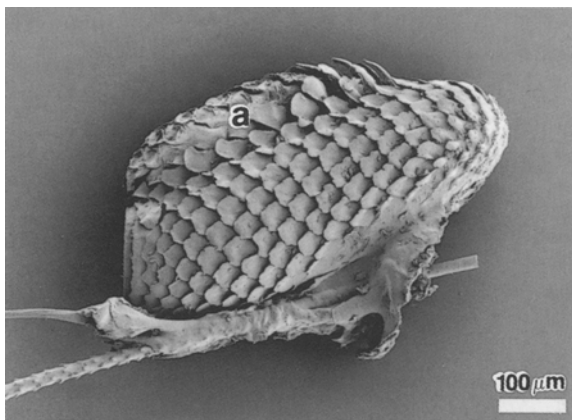


Fig. 1. Eggs of *Heterodoxus insularis* (top), and an undescribed species from the *H. ampullatus* group (ex *Petrogale persephone* from Kelsey Creek, via Proserpine, Queensland). Abbreviation: a, lateral structure.

the 11 species of *H. calabyi* group. Thus, a suitable out-group for the *H. octoseriatus* group and *H. calabyi* group is the remaining 30 described boopiid species. At this level, the division of the vesica into two parts is apomorphic.

Phylogeny within the *H. octoseriatus* group

From the preceding analysis the *H. calabyi* group was identified as the sister-group, and thus, a suitable out-group for a cladistic analysis of the *H. octoseriatus* group. Fourteen potentially apomorphic characters (listed below) were identified in the *H. octoseriatus* group. Ten of these occurred in the dorsal plate, the ventro-lateral sclerite and the vesica of the male genitalia: four were associated with the internal genital sclerite of the female. Comparison with the out-group revealed that these characters have diverged to such an extent that it was impossible to identify with confidence homologous structures in the in- and out-groups. Thus, I was unable, by out-group comparison, to determine whether the 14 character states were plesiomorphic or apomorphic. Consequently the phenetic method was used: a phylogeny was inferred from the close similarity of morphological characters. Where both the Hennigian and phenetic methods are applied to a group of characters, similar estimates of phylogeny may result (e.g. Colless, 1970), though this is certainly not always the case (e.g. Mickevich & Johnson, 1976).

4. Dorsal plate of male with sub-median processes posteriorly (synonymous with sclerite c of Clay, 1981, figs. 5–7) with mammiliform projections on the processes. Present in *H. octoseriatus* and *H. maynesi* (Barker, 1991, fig. 3D–E).
5. Dorsal plate of male with stout sclerotised point (*sensu* Clay, 1981, p. 74, figs 22–24) without mammiliform projections. Present in *H. insularis*, *H. murrayi* and *H. orarius* (Barker, 1991, fig. 3I–J).
6. Dorsal plate of male with rounded globose lobes posteriorly. Present in *H. briscoei* and *H. insulatus* (Barker, 1991, fig. 3B–C).
7. Vento-lateral sclerite of male with broad posterior surface. Present in *H. octoseriatus* and *H. maynesi* (Barker, 1991, fig. 4D–E).
8. Vento-lateral sclerite of male with rounded posterior arms and the outermost (primary) median indentation on the posterior surface shallow [cf. *H. closei* (Barker, 1991, fig. 4F)]. Present in *H. hughendensis* and *H. harrisoni* (Barker, 1991, fig. 4G–H).
9. Vento-lateral sclerite of male with two distinct, broad plates connected by a thickened central plate (synonymous with sclerite f of Clay) (Clay, 1981, figs 25–27). Present in *H. insularis*, *H. orarius* and *H. murrayi* (Barker, 1991, fig. 4I–K).
10. Posterior part of vesica of male covered only in scales which are more or less uniform in size and shape (Clay, 1981, figs 4, 5, 13). Present in *H. maynesi* and *H. octoseriatus* (Barker, 1991, fig. 6D–E).
11. Posterior part of vesica of male with long colourless spines, tending to radiate from the mid-point of that part of the vesica (Clay, 1981, figs 3, 23–24). Present in *H. insularis* and *H. orarius* (Barker, 1991, fig. 7B–C). Unlike the long spines sometimes seen in *H. harrisoni* (e.g. Clay, fig. 17; Barker, fig. 7F) the spines in *H. orarius* and *H. insularis* are covered in small mammiliform projections which are clearly discernible when Differential Interference Contrast Microscopy is used.
12. Posterior part of vesica of male covered in an orderly array of broad-based colourless spines, smaller and more numerous than those in character 11, with the anterior and posterior parts of the vesica distinct (Clay, 1981, fig. 21). Present in *H. lesouefi*, *H. briscoei* and *H. insulatus* (Barker, 1991, fig. 6A–C).
13. Posterior part of vesica of male folded on itself and thus, appearing thickened. Present in *H. closei*, *H. hughendensis* and *H. harrisoni* (Barker, 1991, fig. 7D–F).
14. Internal genital sclerite of female without indentations on the inner surfaces of the arms (Clay, 1981, figs 8–11). Present in *H. lesouefi*, *H. briscoei*, *H. insulatus*, *H. octoseriatus*, *H. maynesi*, *H. closei*, *H. hughendensis* and *H. harrisoni* (Barker, 1991, fig. 5A–H).
15. Internal genital sclerite of female with short, stout arms, with indentations on the inner surfaces of the arms (synonymous with genital sclerite Type II, Clay, 1981, figs 19, 20). Present in *H. insularis*, *H. orarius* and *H. murrayi* (Barker, 1991, fig. 5I–K).
16. Anterior arms of the internal genital sclerite of female broad. Present in *H. closei*, *H. hughendensis* and *H. harrisoni* (Barker, 1991, fig. 5F–H).
17. Anterior arms of the internal genital sclerite of female slender (cf. character 16) (Clay, 1981, figs 8–11). Present in *H. lesouefi*, *H. briscoei*, *H. insulatus*, *H. octoseriatus* and *H. maynesi* (Barker, 1991, fig. 5A–E).

Phylogeny and area phenogram of the H. octoseriatus group

A phylogeny is inferred in Fig. 2. Species groups were named, to aid discussion, after the first species in each group (Fig. 2). Characters derived

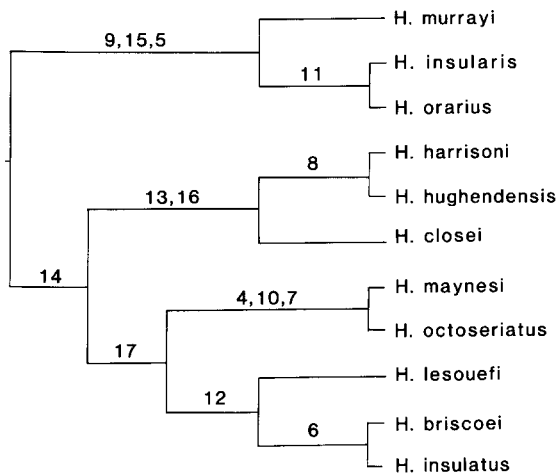


Fig. 2. Phylogeny of the *Heterodoxus octoseriatus* group inferred from a phenetic analysis of morphological characters. See text for key to characters 4–17.

from the morphology and armature of the dorsal plate, ventro-lateral sclerite, the vesica and the internal genital sclerite, were absolutely correlated (Fig. 2).

An area phenogram of the *H. octoseriatus* group, shows that except for the *H. lesouefi* group, the species in each group are in close proximity to one another (Fig. 3). Further, the *H. murrayi* and *H. harrisoni*-*H. maynesi*-*H. lesouefi* species groups are also in geographical proximity to one another.

Discussion

Species in the sister-group of the *Heterodoxus octoseriatus* group, the *H. calabyi* group, are known to infest hosts from seven genera of macropodoid marsupials: *Thylogale*, *Macropus*, *Dorcopsis*, *Setonix*, *Dorcopsulus*, *Wallabia* and *Dendrolagus* (Murray & Calaby, 1971).

The phenetic analysis of homologous morphological characters indicates that the *H. octoseriatus* group comprises four groups of species which are more closely related to one another other than any is to other species in the *H. octoseriatus* group: these have been termed species groups. All but the *H. lesouefi* species group are more strongly supported than are the clusters of species

groups (i.e. the *H. maynesi*-*H. lesouefi* and the *H. harrisoni*-*H. maynesi*-*H. lesouefi* clusters). One morphological feature, the sclerotised ridge on the dorsal plate of *H. insulatus* (Barker, 1991) indicates that *H. insulatus* and thus perhaps the other species in the *H. lesouefi* species group, may be more closely related to the *H. murrayi* species group than to the *H. harrisoni* and *H. maynesi* species groups. This ridge resembles the form of the stout sclerotised point of the dorsal plate present in the *H. murrayi* species group. An alternative view, favoured here, is that the similarity of the ridge and the stout sclerotised point is due to homoplasy.

Where allopatric speciation events predominate, area phenograms may provide independent tests of inferred phylogenies. With one exception, in the *H. lesouefi* species group, the geographical arrangement of the species and species groups is consistent with the phylogeny predicted from morphological characters. The three species of the *H. lesouefi* species group are, geographically, widely separated and *H. insularis* lies between *H. insulatus* and *H. briscoei*. The ancestor of the *H. lesouefi* species group may have had a continuous geographical range encompassing the present geographical ranges of *H. lesouefi*, *H. insulatus* and *H. briscoei*. The geographical range apparently became fragmented, leading to three allopatric speciation events. Range expansion by *H. insularis*, either associated with range expansion by its host or through the colonisation of new hosts, may have caused fragmentation of ancestral taxa also. Unless these hosts were uninfested the colonisation of new hosts apparently led to displacement of the louse species they were previously infested with.

Speciation in the *H. octoseriatus* group

Three types of allopatric speciation which may have occurred in the *Heterodoxus octoseriatus* groups are:

1. Speciation caused by the disruption of the geographical ranges of hosts

The geographical ranges of louse species may be

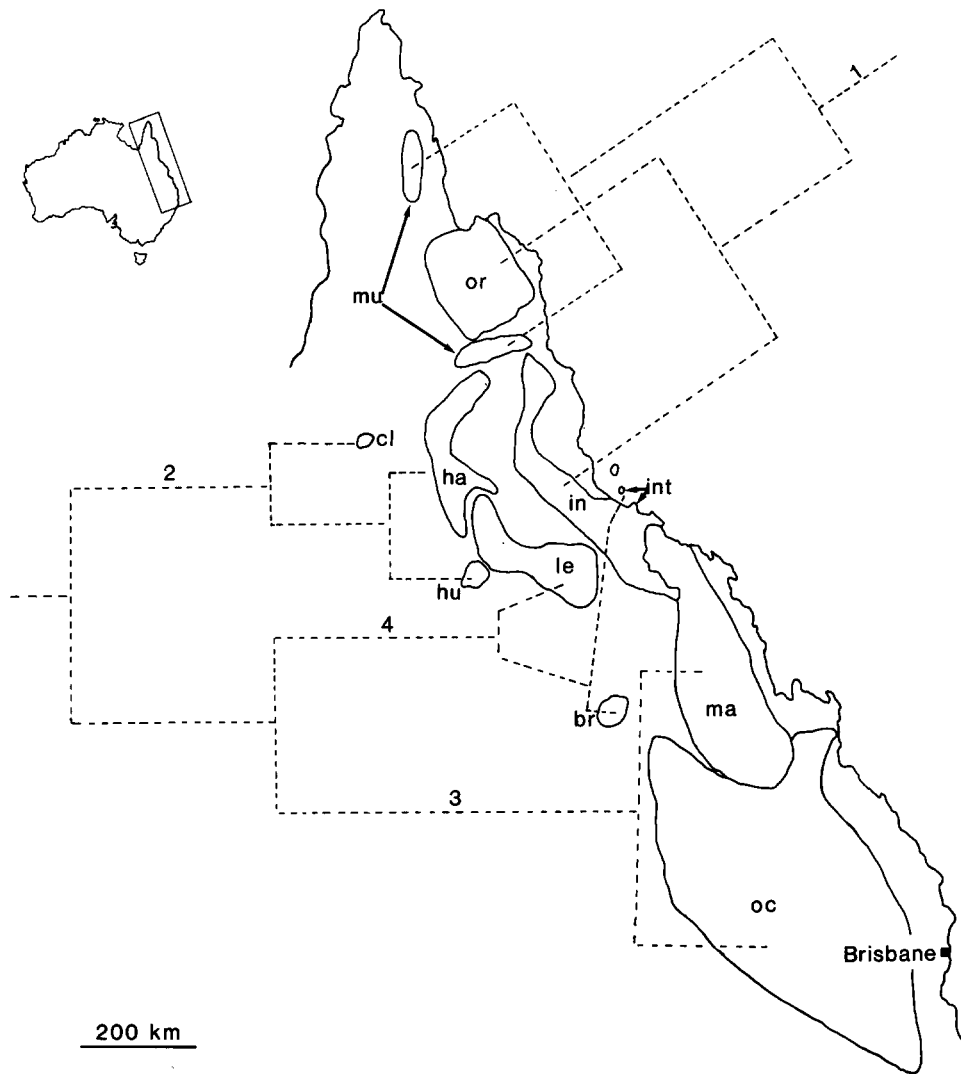


Fig. 3. Area phenogram of the *Heterodoxus octoseriatus* group 1. *H. murrayi* species group; 2. *H. harrisoni* species group; 3. *H. maynesi* species group; 4. *H. lesouefi* species group. Abbreviations: mu, *H. murrayi*; or, *H. orarius*; cl, *H. closei*; ha, *H. harrisoni*; hu, *H. hughendensis*; le, *H. lesouefi*; in, *H. insularis*; int, *H. insulatus*; br, *H. briscoei*; ma, *H. maynesi*; oc, *H. octoseriatus*. Rock-wallabies are not known from the region marked by the western margins of the geographic ranges of *H. murrayi* and *H. orarius*, and the western coastline of Queensland.

come fragmented through the fragmentation of their host's range: divergence of the isolated populations may ensue. Species infesting peripheral host populations that occupy habitats which become uninhabitable with small climatic changes may be especially susceptible. *H. closei* and *H. hughendensis* may have arisen in this way as they infest some of the western-most populations of *P. a. assimilis* in the semi-arid part of Queensland (Barker & Close, 1990).

2. Speciation arising from temporary parapatry and host switching

The geographical ranges of *Petrogale* taxa, carrying different species of lice, may have alternated between allopatry and parapatry. Populations of lice that colonised atypical hosts (host switching) during parapatry, may have diverged from the parent stock when the host taxa became allopatric again. Populations of *H. octoseriatus* apparently diverged in this way. Electrophoretic analysis re-

vealed that *H. octoseriatus* comprises a northern and a southern form (Barker, 1988). The ancestors of the southern form apparently colonised *P. p. penicillata*, during a period of parapatry between *P. p. herberti* and *P. p. penicillata*, and then diverged from the northern populations of *H. octoseriatus* when *P. p. penicillata* and *P. p. herberti* became allopatric again.

The two types of allopatric speciation discussed rely on changes in the geographical ranges of hosts. The next type, relies entirely on changes in the geographical ranges of parasites that are independent of changes in the ranges of their hosts.

3. Speciation arising from the dissection of louse distributions by range expansion of other louse species

Barker & Close (1990) have shown that some species of the *H. octoseriatus* group have colonised new hosts at zones of parapatry, and then expanded their geographical ranges into the former ranges of neighbouring species. In this way, some may have dissected the geographical ranges of others, leading to allopatric speciation of the isolated populations. That the speciation of *H. insulatus* and *H. briscoei* may have been caused by the range expansion of *H. insularis* has been discussed. In a similar way range expansion by *H. murrayi*, may have caused the fragmentation of the geographical range of the *H. insularis*-*H. orarius* ancestor (Fig. 3; Barker & Close, 1990, fig. 5). The presence of two disjunct populations of *H. murrayi*, separated by the geographical distribution of *H. orarius*, suggests that a once continuous distribution of *H. murrayi* has been dissected by a westward extension of the geographical range of *H. orarius* (see Fig. 5; Barker & Close, 1991).

In these examples, however, it is unclear whether the changes in the geographical ranges of louse species were completely independent of changes in the geographical ranges of their hosts.

Evolution of morphological characters in the H. octoseriatus group

Electrophoretic analysis (Barker, 1988) revealed

that in general there is much inter-specific genetic variation in the *H. octoseriatus* group: a number of species were fixed for different alleles at more than 50% of loci examined. Yet, except for differences in the genitalia, they are in general morphologically indistinguishable (Clay, 1981; Barker, 1991). Conservative morphological evolution in parasites, formalised in Manter's Rule, is a widely held view (e.g. Jordan, 1942, for fleas; Hopkins, 1949, for lice). In contrast, genitalia in the *H. octoseriatus* group, like many other animal groups with internal fertilization (Eberhard, 1985), have diverged strongly, to the point where homologous structures in other congeners cannot be identified. This is in accord with the female choice hypothesis (Eberhard, 1985). From this hypothesis Eberhard predicted "runaway evolution" driven by sexual selection. This selection is generated by female choice on apparently arbitrary aspects of male genitalia. Similar sexual selection in the *H. octoseriatus* group may have led to the seemingly rapid and divergent evolution of male genitalia reported here.

Acknowledgements

I thank: M. D. Murray, R. L. Close, D. A. Briscoe and T. H. Cribb for valuable discussion and for criticising earlier drafts; K. Aplin for stimulating discussions on the Hennigian method; my three doctoral referees for helpful comments; R. Oldfield for the photographs; D. Scott for help with the figures; and S. Doyle for assistance with electron microscopy. This study was completed with the assistance of an Australian Commonwealth Postgraduate Research Award and Australian Biological Resources Study grants.

References

- Barker, S.C. (1991) Taxonomic review of the *Heterodoxus octoseriatus* group (Phthiraptera: Boopidae) from rock-wallabies with the description of three new species. *Systematic Parasitology*.
- Barker, S.C. (1988) *Evolution in the Heterodoxus octoseriatus group (Boopidae: Phthiraptera) and the development of host*

- associations with *Petrogale* (Marsupialia). Doctoral Thesis: Macquarie University, Sydney, NSW.
- Barker, S.C. & Close, R.L. (1990) Zoogeography and host associations of the *Heterodoxus octoseriatus* group and *H. ampullatus* (Phthiraptera: Boopidae) from rock-wallabies (Marsupialia). *International Journal for Parasitology*, **20**, 1081–1087.
- Balter, R.S. (1968a) Lice egg morphology as a guide to taxonomy. *Medical and Biological Illustration*, **18**, 94–95.
- Balter, R.S. (1968b) The microtopography of avian lice eggs. *Medical and Biological Illustration*, **18**, 166–179.
- Clay, T. (1969) A key to the genera of the Menoponidae (Amblycera: Mallophaga: Insecta). *Bulletin of the British Museum (Natural History)* [Entomology], **24**, 1–26.
- Clay, T. (1970) The Amblycera (Phthiraptera: Insecta). *Bulletin of the British Museum (Natural History)* (Entomology), **25**, 75–98.
- Clay, T. (1971) A new genus and two new species of Boopidae (Phthiraptera: Amblycera). *Pacific Insects*, **13**, 519–529.
- Clay, T. (1981) A report on a collection of lice (Boopidae: Phthiraptera) on *Petrogale* (rock wallabies). *Proceedings of the Linnean Society of New South Wales*, **105**, 65–78.
- Colless, D.H. (1970) The phenogram as an estimate of phylogeny. *Systematic Zoology*, **19**, 352–362.
- Eberhard, W.G. (1985) *Sexual selection and animal genitalia*. London: Harvard University Press, 244 pp.
- Hopkins, G.H.E. (1949) The host-associations of the lice of mammals. *Proceedings of the Zoological Society of London*, **119**, 387–604.
- Jordan, K. (1942) On *Parapsyllus* and some closely related genera of Siphonaptera. *Revista Espanola de Entomologia Madrid*, **18**, 7–29.
- Kéler, S. von (1971) A revision of the Australasian Boopidae – (Insecta: Phthiraptera) with notes on the Trimenoponidae. *Australian Journal of Zoology*, Supplementary Series, **6**, 1–126.
- Lyal, C.H.C. (1985) Phylogeny and classification of the Psocodea, with particular reference to the lice (Psocodea: Phthiraptera). *Systematic Entomology*, **10**, 145–146.
- McCulloch, R.N. (1933) A Kangaroo louse infesting dogs. *Agricultural Gazette of New South Wales*, **44**, 617–9.
- Mickevich, M.F. & Johnson, M.S. (1976) Congruence between morphological and allozyme data in evolutionary inference and character evolution. *Systematic Zoology*, **25**, 260–270.
- Murray, M.D. & Calaby, J.H. (1971) The host relations of the Boopidae. *Australian Journal of Zoology*, Supplementary Series, **6**, 81–84.
- Price, P.W. (1980) *Evolutionary biology of parasites*. San Francisco: Princeton University Press, 237 pp.