

***Attenborougharion* gen. nov.**
(Mollusca: Pulmonata: Helicarionidae):
A Likely Case of Convergent Evolution in
Southeastern Tasmania

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ABSTRACT. *Helicarion* Férussac, 1821 from southeastern Australia currently comprises five species of endemic semislugs. Analyses of comparative morphological data and partial sequences of the mitochondrial genes cytochrome c oxidase subunit I (COI) and 16S rRNA (16S) reveal that one of these species, *Helicarion rubicundus* Darnall & Kershaw, 1978, which is restricted to southeastern Tasmania, is not closely related to the other known species of this genus. This species is distinguished from *Helicarion* in several key morphological characters, such as the bright two-toned red and green colouration of its larger body with a flattened tail that is keeled only at the tip, the triangular shape of the pneumostome, the degree and type of folding present in the spermooviduct and free oviduct, the presence of a longer, more slender bursa copulatrix, the presence of a small epiphallic caecum and a hooked flagellum, and the presence of irregular longitudinal pilasters in the penial interior in contrast to the v-shaped rows of papillose lamellae seen in *Helicarion*. Moreover, the mitochondrial phylogeny provides evidence that this species is phylogenetically distinct from *Helicarion* as well as any other currently described genus from southeastern Australia. Based on these findings, we here describe a new genus, *Attenborougharion*, for this species.

KEYWORDS. Helicarionoidea; morphology; mitochondrial DNA; land snail; taxonomy.

HYMAN, ISABEL T., AND FRANK KÖHLER. 2017. *Attenborougharion* gen. nov. (Mollusca: Pulmonata: Helicarionidae): a likely case of convergent evolution in southeastern Tasmania. *Records of the Australian Museum* 69(2): 65–72. <https://doi.org/10.3853/j.2201-4349.69.2017.1676>

The Helicarionidae is a family of land snails in which about half of all species have evolved a reduced, ear-shaped shell into which they cannot fully retract (i.e., they have evolved into so-called semislugs). The type genus of the family, *Helicarion* Férussac, 1821, is part of a monophyletic radiation that is restricted to southeastern Australia and includes six additional genera (*Mysticarion* Iredale, 1941, *Parmavitrina* Iredale, 1937, *Cucullarion* Stanistic, 1998, *Peloparion* Iredale,

1937, *Ubiquitarion* Hyman, Lamborena & Köhler, 2017 and *Brevisentis* Hyman, 2007), all of which have recently been revised comprehensively (Hyman & Ponder, 2010; Hyman, Lamborena & Köhler, 2017). This clade is supported by several morphological synapomorphies, including the presence of a spiralling, spinose spermatophore, a flagellum with internal cryptae, at most a very short vagina, and the absence of an epiphallic caecum. The seven genera included

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Figure 1. Living animal of *Attenborougharion rubicundus* from Forestier Peninsula (QVM 9:15514). Courtesy of Simon Grove, TMAG.

in this radiation demonstrate a wide variation in body types, including fully shelled snails (*Brevisentis*) as well as semislugs exhibiting varying degrees of shell reduction, from *Mysticarion* with its slightly reduced, globose shell, to the highly reduced shell seen in *Cucullarion*. While the phylogenetic relationships within this clade have not been fully and consistently resolved yet, it is clear that shell reduction has either repeatedly evolved or has undergone reversals in this subclade of Helicarionidae (Hyman, Ho & Jermiin, 2007; Hyman, submitted).

Helicarion contains five currently recognised species of small to moderately large semislugs: *H. cuvieri* Férussac, 1821 and *H. rubicundus* Darnall & Kershaw, 1978 from Tasmania, *H. niger* (Quoy & Gaimard, 1832) from Victoria, and *H. mastersi* (Cox, 1868) and *H. leopardinus* Iredale, 1941 from New South Wales. More detailed anatomical descriptions are available for the first four of these species (Darnall & Kershaw, 1978; Kershaw, 1979, 1980, 1981; Hyman & Ponder, 2010). A comparison of the available anatomical data highlights some differences between *H. rubicundus* and the other taxa, particularly in the presence of a small epiphallial caecum (although this character was questioned by Kershaw, 1980), the presence of a penial papilla complex, a folded free oviduct, and spirally beaded protoconch microsculpture. Herein we revise the taxonomy of *H. rubicundus* based on comparative examination of the anatomy and morphology as well as analyses of partial sequences of the mitochondrial genes cytochrome *c* oxidase subunit I (COI) and 16S rRNA (16S).

Material and methods

This study is based on the examination of ethanol-preserved specimens and supplementary dry material from the Australian Museum, Sydney (AM), the Tasmanian Museum and Art Gallery, Hobart (TMAG) and the Queen Victoria Museum and Art Gallery, Launceston (QVMAG), including freshly collected material from southeastern Tasmania. Adult specimens of *Helicarion rubicundus*, *H. cuvieri*, *H. mastersi*, *H. niger* and *H. leopardinus* were selected for anatomical examination (see Table 1). At least four specimens were dissected for each species. Genital anatomy was examined through dissection of ethanol-preserved specimens using a Leica MZ8 stereo microscope with a drawing apparatus. In species descriptions, proximal and distal are used with reference to the centre of the body (i.e. the gonad).

DNA extraction, PCR and sequencing were undertaken as described by Hyman *et al.* (2017). Electropherograms were corrected for misreads and forward and reverse DNA strands were merged into one sequence file using CodonCode Aligner v. 3.6.1 (CodonCode Corp., Dedham, MA). Sequences of previous helicarionid studies (Hyman *et al.*, 2007; Hyman *et al.*, 2017) were retrieved from GenBank and included in our dataset while all newly produced sequences have been deposited in GenBank under the accession numbers KY769386–KY769398.

The 16S sequences were aligned using the online version of MAFFT (version 7) (see Reference section) by employing the iterative refinement method E-INS-i suitable for sequences with multiple conserved domains and long gaps

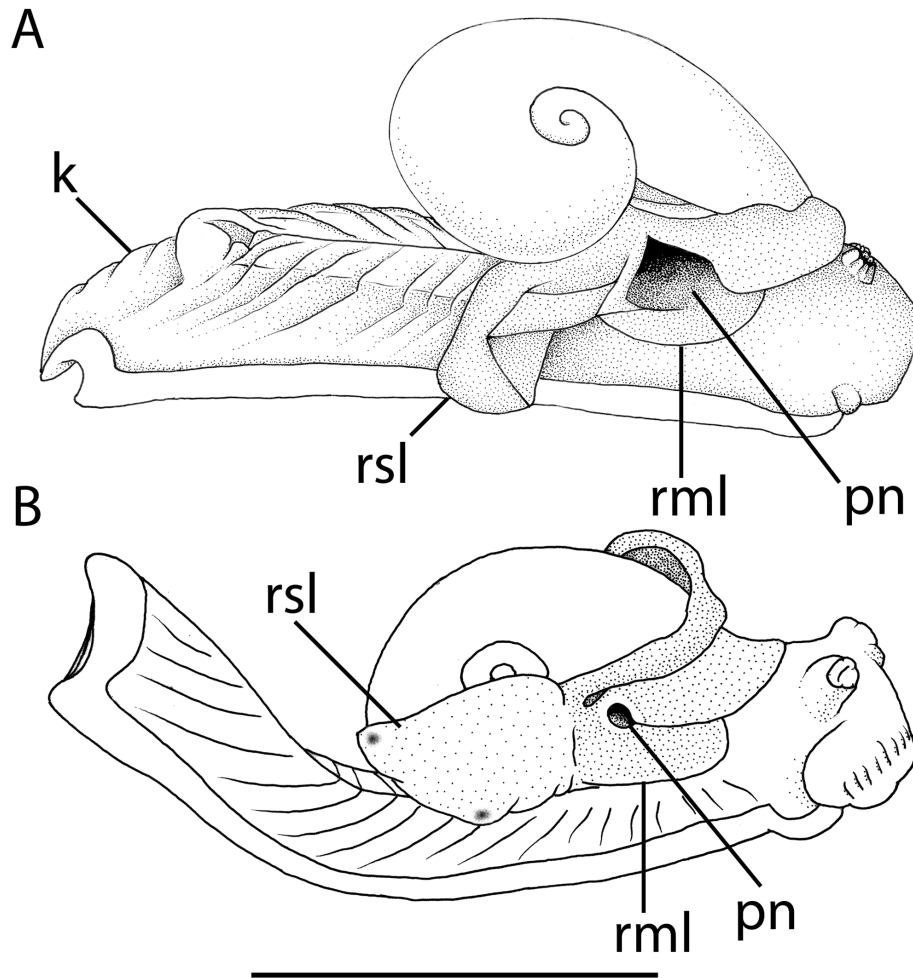


Figure 2. Comparative external morphology. (A) *Attenborougharion rubicundus* from Eaglehawk Neck (QVM 9:9665). (B) *Helicarion cuvieri* from Forestier Peninsula (ex-QVM:9:9671). Scale bar = 5 mm. Abbreviations: *k*, keel; *pn*, pneumostome; *rml*, right mantle lobe; *rsl*, right shell lobe.

(Katoh *et al.*, 2002). The aligned 16S and COI sequences were then concatenated into one partitioned data set. Four partitions have been defined (16S, each of three codon positions of COI) and their parameters have been allowed to be modelled independently. A maximum likelihood-based method of tree reconstruction was employed to estimate phylogenetic relationships. We analysed the concatenated and partitioned sequence dataset using the program RAxML version 8.2.9 available on the Cipres Science Gateway (Stamatakis, 2014). Nodal support of the tree topology was assessed by performing 500 rapid bootstrap replicates.

Results

Comparative morphology

Helicarion rubicundus has a very short vagina and a flagellum with internal cryptae (visible as small bumps on the outside of the flagellum), indicating the presence of a spinose spermatophore. However, unlike other members of the southeastern Australian helicarionid clade, all of which have no epiphallic retractor caecum, *H. rubicundus* has a small bulge in the epiphallus at the point where it attaches

to the penial retractor muscle. This may constitute a remnant of an epiphallic caecum.

A comparison of *H. rubicundus* with other members of *Helicarion* reveals a number of morphological differences. Its body length (type specimens 27.5–45 mm in alcohol; Dartnall and Kershaw, 1978) is greater than other *Helicarion* species (*H. cuvieri*: 18–28 mm; *H. leopardinus*: 21–25 mm; *H. mastersi*: 19–24 mm; *H. niger*: 32 mm). Its tail is flattened dorsally for most of its length, with only a short keel at the tip (Figs 1, 2A), and it has a distinctly triangular pneumostome. In contrast, in other members of *Helicarion* the tail has a longer keel and the pneumostome is rounded (Fig. 2B). The shell of *H. rubicundus* is flatter and the protoconch not raised, and both protoconch and teleconch have indistinct to absent spiral grooves but robust radial growth lines and wrinkles (Fig. 3), in contrast to the smooth glossy shell with incised spiral grooves seen in other members of *Helicarion*. We did not observe the spirally beaded protoconch microsculpture recorded by Dartnall and Kershaw (1978), although very faint beading is present on the protoconch.

There are also several differences in their genitalia. All semislugs have some degree of folding in the spermoviduct as an adaptation to the shortened pallial space caused by

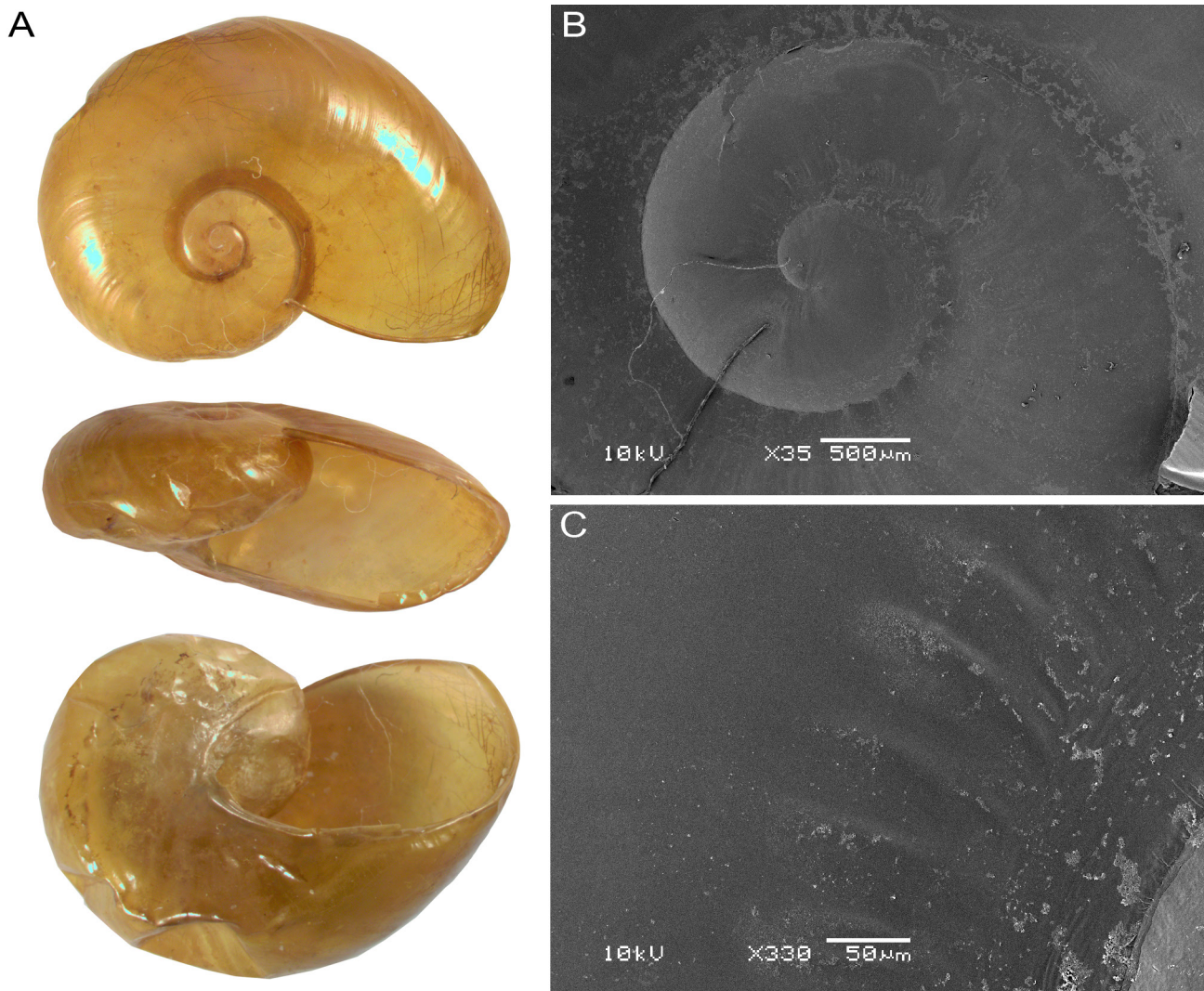


Figure 3. Shell of *Attenborougharion rubicundus* from Forestier Peninsula QVM 9:15514. (A) Photograph of shell viewed from top, front, and below. Scale bar = 5 mm. (B) Scanning electron micrograph showing protoconch. (C) Scanning electron micrograph showing micro-sculpture of fine radial wrinkles near suture on protoconch.

shell reduction (Hyman, submitted). In *Helicarion cuvieri*, *H. leopardinus*, *H. mastersi* and *H. niger* the type and degree of folding is highly consistent and characteristic: from the albumen gland, the spermoviduct is directed into the foot and is folded once to return out of the foot cavity towards the head. The free oviduct is straight and unfolded. In *H. rubicundus*, the spermoviduct is not directed into the foot cavity but instead is slightly folded alongside itself, as is the free oviduct (Fig. 4A). In addition, when the spermoviduct is in its folded position, the long, slender bursa copulatrix of *H. rubicundus* reaches nearly to the albumen gland, in contrast to the more rounded bursa of other *Helicarion* species, which reaches only halfway along the spermoviduct. This is not apparent in the drawing that accompanies the original description (Dartnall & Kershaw, 1978: fig. 1a), where the bursa copulatrix appears to be only about one third of the length of the spermoviduct. However, in their dissection Dartnall & Kershaw (1978) did not preserve the folding of the spermoviduct and in its straightened form it appears longer relative to the bursa copulatrix.

The penis interior is sculptured with irregular longitudinal pilasters in *H. rubicundus* (Fig. 4B) in contrast to all other

members of *Helicarion*, which have v-shaped rows of papillose lamellae instead. The epiphallic flagellum is large and strongly hooked in *H. rubicundus*, with cryptae present proximal to the epiphallus but absent from the slender tip. In other members of *Helicarion* the flagellum is smaller and not hooked, and cryptae extend further along it. This indicates that the gross spermatophore shape may differ in *H. rubicundus*. This could not be confirmed through direct comparison as no spermatophores were observed in the current study, and the illustration shown by Dartnall & Kershaw (1978: fig. 3) may not represent a complete spermatophore. Finally, the small remnant of an epiphallic caecum in *H. rubicundus* is completely absent in the rest of *Helicarion*, and indeed all other members of the southeastern Australian helicarionid radiation. In contrast to the findings of Kershaw (1980), who described this structure as a bulging of the penis retractor muscle rather than a true caecum, we observed a small outpocketing of the epiphallus and therefore consider the term “caecum” to be appropriate. No penial verge or papilla is present in any *Helicarion* species examined herein, including *H. rubicundus*.

Molecular analyses

The molecular phylogenetic data set contained 57 concatenated mitochondrial DNA sequences representing nearly all species and all genera of the southeastern Australian helicarionid clade as well as several species that do not belong to this clade, which were used as outgroup to root the tree. The sequence data set had a total length of 1,588 base pairs (16S: 933 bp, COI: 655 bp).

In the best Maximum Likelihood phylogram (Fig. 5), the southeastern Australian clade as recognized previously as well as all genera as presently circumscribed were found to be monophyletic. Specimens belonging to *Helicarion rubicundus* formed a well-supported and well-individualized clade that did not group with other species of *Helicarion*. Instead these sequences were found to represent the most basally diverging clade within the southeastern Australian helicarionid radiation. Bootstrapping provided strong evidence for the monophyly of the *Helicarion rubicundus* clade as well as for its membership in the southeastern Australian helicarionid clade.

Systematics

Attenborougharion gen. nov.

Type species. *Helicarion rubicundus* Darnall & Kershaw, 1978.

Etymology. Named for Sir David Attenborough, Lifetime Patron of the Australian Museum, in recognition of his lifetime's contribution to the fields of natural science and conservation. The Latin noun *arion* refers to a "kind of snail or slug"; masculine.

Diagnosis

External appearance. Large, shell ear-shaped, flattened, thin, golden, glossy, whorls rounded, base membranous. Protoconch with radial wrinkles near suture; otherwise sculptured with very faint beading and indistinct to absent spiral grooves; teleoconch with very fine, indistinct spiral grooves and more prominent radial growth lines. Body colour green and burgundy. Mantle lobes and shell lappets of moderate size, none fused; shell lappets elongate, lacking pigmented warts; slime network prominent; caudal horn well-developed. Keel confined to very tip of tail; most of tail dorsally flattened. Pneumostome impressed, distinctly triangular.

Genitalia. Spermoviduct embedded in digestive gland. Talon and carrefour embedded in albumen gland. Spermoviduct slightly folded into a small curve but not folded downwards into foot (as in *Helicarion*). Free oviduct folded alongside spermoviduct. Bursa copulatrix inserted on free oviduct; very long, nearly the length of the spermoviduct, bursa very slender, pointed. Vagina very short. Penis long, broad, with internal sculpture of longitudinal pilasters connected by irregular wavy transverse ridges. Penial tunica attached by muscle fibres to middle of epiphallus; epiphallus enters penis through simple pore; very small epiphallic caecum present; hook-shaped epiphallic flagellum with axial filament present, containing spiralling rows of internal cryptae adjacent to epiphallus; flagellum tip slender with no cryptae. Spermatophore a soft-walled capsule with hard tail-pipe; branching spines present in spiralling pattern along tail-pipe.

Attenborougharion rubicundus

(Darnall & Kershaw, 1978) comb. nov.

Figs 1–4

Helicarion rubicundus Darnall & Kershaw, 1978: 2;
Kershaw, 1980: 213.

Material examined. Holotype: TMH E9028 (northeast flank of Hawkes Hill, near Eagle Hawk Neck, Tasmania); paratypes (2 specimens): QVM 9:184, previously labelled 1975/9/1, Eagle Hawk Neck, Tasmania, leg. Darnall, 1974; and non-type material (see Table 1).

Description

External appearance (Figs 1–3): Large semislug, body 27.5–45 mm in alcohol, shell golden, 3.0–3.3 whorls. Head, eyestalks, neck and middle of tail burgundy; shell lappets, mantle lobes, sole and top of tail green; mantle lobes edged with burgundy. Border between burgundy and green colouration along tail visible even in spirit-preserved material (although colours cannot be identified).

Genitalia (Fig. 4): Penis long, tubular, distal half enclosed in penial tunica. Penis interior with 4–5 longitudinal pilasters, smooth basally, pustulose proximally, connected by irregular wavy transverse ridges. Epiphallus slightly greater than penis length, entering penis apically, with a very short epiphallic caecum at point of attachment of penial retractor muscle, about one third along distance between penis and vas deferens. Epiphallic flagellum present, large, curved back alongside penis, with numerous internal cryptae adjacent to the epiphallus, flagellum tip slender with no cryptae. Spermatophore (from Darnall & Kershaw, 1978: fig. 2) with one large, multiply branched spine at base of capsule, followed by spines of decreasing complexity.

Distribution and conservation status

Attenborougharion rubicundus is found only on the Tasman and Forestier Peninsulas in Tasmania (Taylor, 1991; Otley *et al.*, 1999). The total known extent of occurrence of this species is 85 km², leading to its listing as Vulnerable on the IUCN Red List of Threatened Species. In addition to its restricted range, within this area *Attenborougharion rubicundus* inhabits only closed wet forests and is not found in dry forests or damp sclerophyll forests (Otley *et al.*, 1999), making it vulnerable to habitat loss through the effects of climate change as well as habitat destruction through changed land use.

Discussion

Attenborougharion gen. nov. is described herein as a new genus for the type species *Helicarion rubicundus*. This is based on evidence for its evolutionary distinctness from *Helicarion*, underpinned by the morphological characters outlined above, and its isolated position in the mitochondrial phylogeny. The morphological differences, while consistent with differences between other helicarionid genera (e.g., Hyman *et al.*, 2017), are subtle, and juvenile specimens of *A. rubicundus* have frequently been misidentified as the sympatric *H. cuvieri*. The only juvenile specimens available

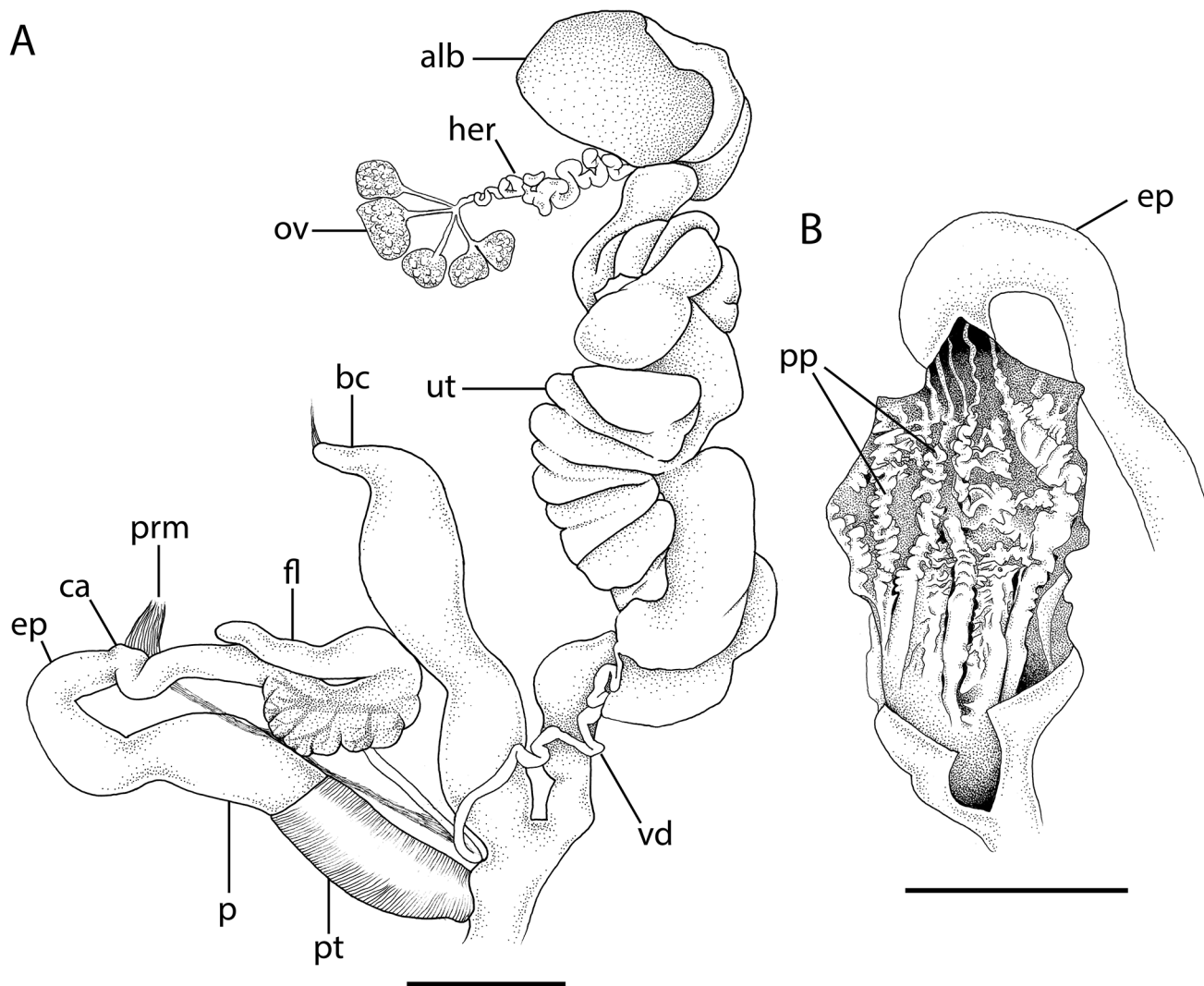


Figure 4. Reproductive anatomy of *Attenborougharion rubicundus* from Forestier Peninsula (QVM 9:15514). (A) Reproductive system. (B) Interior of penis. Scale bars = 5 mm. Abbreviations: *alb*, albumen gland; *bc*, bursa copulatrix; *ca*, caecum; *ep*, epiphallus; *fl*, flagellum; *her*, hermaphroditic duct; *p*, penis; *pp*, penial pilasters; *prm*, penial retractor muscle; *ut*, uterus; *vd*, vas deferens.

for examination were spirit-preserved, so no comments can be made on their living body colour; however, they can be distinguished from *H. cuvieri* by their triangular pneumostome and flattened tail with only a short keel.

Despite the similarity between *A. rubicundus* and *H. cuvieri*, the two species are not closely related. *Attenborougharion* is the most basally diverging member of the southeastern Australian helicarionid radiation. This basal position is consistent with the presence of an epiphallic caecum in *A. rubicundus* and all outgroup taxa (except *Malandena suturalis* (Odhner, 1917) and *Echonitor cyrtochila* Gude, 1905; Hyman & Ponder, 2010) and absence in the rest of the southeastern Australian radiation.

Semislugs such as *Attenborougharion* have evolved from snails in a process known as limacisation (Tillier, 1984). During limacisation, the pallial cavity is shortened, leading to changes in organs contained in the pallial cavity such as the kidney, ureter and gas exchange surface, the oesophagus and the spermoviduct (Solem, 1974; Tillier, 1984; Hyman, submitted). However, it is unclear whether this complex

process could be reversed. If reversal is impossible, then the phylogenetic tree indicates that shell reduction must have evolved independently in every semislug genus in the southeastern Australian radiation. This premise is supported, in some cases, by variation in the folding pattern of the spermoviduct. Folding of the spermoviduct occurs due to the shortening of the pallial cavity during limacisation. The pattern of folding seen in *Helicarion* differs from that seen in *Attenborougharion*, and similar genus-specific patterns are present in *Mysticarion* and *Cucullarion* (Hyman *et al.*, 2017), suggesting that in each case shell reduction has indeed evolved independently. These differences can most easily be observed if the genital system is dissected without being removed from the body and with minimal disturbance, so that any folding pattern of the spermoviduct is maintained during dissection.

Previous studies of the anatomy of *Helicarion* noted differences in the entry of the epiphallus into the penis, described as simple in *H. cuvieri*, via a small fleshy ridge in *H. niger*, and through a small rounded verge (later described

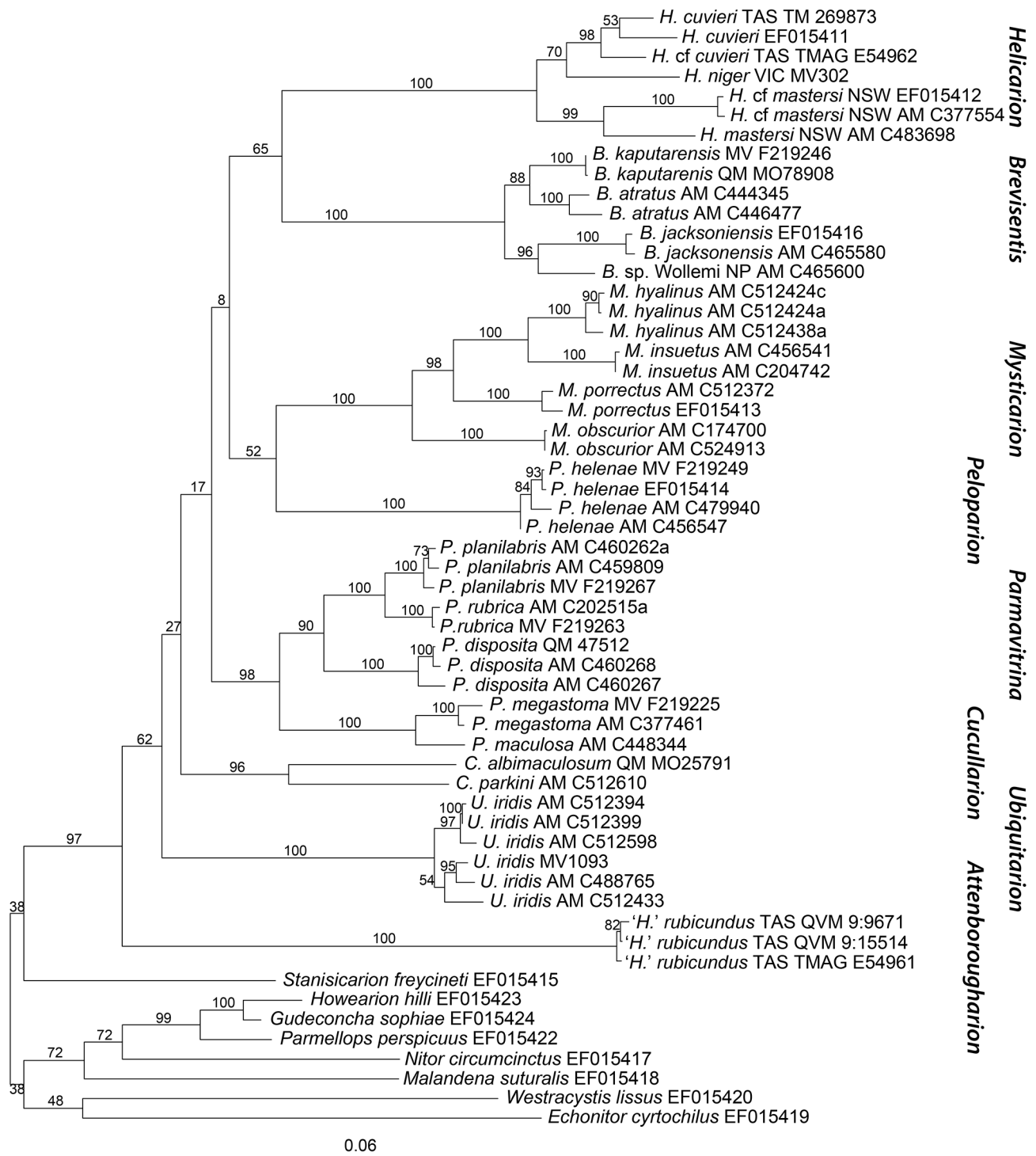


Figure 5. Best Maximum Likelihood phylogram based on analysis of the concatenated data set of 16S and COI in four partitions. Sequences downloaded from GenBank are identified by their GenBank number; new sequences are identified by their museum and collection number. Scale bar = 6% of modelled genetic distance. Numbers on branches indicate nodal support by bootstrapping.

as a complex compound papilla; Kershaw, 1980) in *H. rubicundus* (Dartnall & Kershaw, 1978). No such structures were observed in the current study; all species exhibited entry of the epiphallus into the penis through a simple pore. However, in the penis interior of *Helicariion* species, the opening to the epiphallus is surrounded by a series of small, elongate papillae. During dissection, particularly if

any damage was sustained to this area, one of these papillae could easily be mistaken for a small penial verge or papilla, as could one of the irregularly folded pilasters present in *Attenborougharion*. However, despite this similarity in penis-epiphallus boundary, sufficient clear and consistent differences exist to confirm that *Attenborougharion* and *Helicariion* can be considered as separate entities.

ACKNOWLEDGMENTS. This work has been made possible through financial support from the Australian Government (ABRS grant RF215-49), which is thankfully acknowledged. We also extend our thanks to David Manyard, Kevin Bonham, Mandy Reid and Simon Grove for assistance with collecting and the loan of museum specimens. We are also grateful to Michael Shea for carrying out the anatomical drawings, to Simon Grove (Tasmanian Museum & Art Gallery) for the live photographs and to Sue Lindsay for the SEM images. Finally, we would like to thank two reviewers, Heike Reise and John Stanisc, for their detailed comments on an earlier version of this manuscript. Their reviews helped to improve the quality of this work.

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Supplementary data (published 17 Oct 2017)
(this link inserted in PDF 17 Oct 2017 by the Editor)
Table 1. *Attenborougharion* Hyman & Köhler
<https://doi.org/10.6084/m9.figshare.5504608>

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