



Systematics of the subfamily Aclyvolvinae (Caenogastropoda: Ovulidae) based on molecular and morphometric analyses

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ABSTRACT

Molecular phylogenetic research on the octocoral-associated gastropod family Ovulidae is still in its infancy and, as a consequence, the relationships between subfamilies and genera are not well defined. Previous research on various ovulid genera has shown that their conchological characters are often too fluid when dealing with species delimitations. For this study, Ovulidae were collected in Indonesia and Malaysia, with some additional specimens obtained from Thailand and the Red Sea. Relationships between the Aclyvolvinae and other ovulid subfamilies were assessed using sequence data from two mitochondrial genes (cytochrome *c* oxidase subunit I (COI) and 16S rRNA); the dataset contained ovulid species (including type species) from the subfamilies Eocypraeinae, Ovulinae, Pediculariinae and Simniinae. The type species of the subfamilies Eocypraeinae and Sulcocypraeinae are fossils, and hence could not be included in the analyses. The phylogeny and systematics of the subfamily Aclyvolvinae were assessed based on four DNA gene regions (mitochondrial COI and 16S rRNA, and nuclear 28S rRNA and histone H3) and morphometric analyses. Shell morphological characters were analysed to help clarify species delimitations within the Aclyvolvinae. The results from the molecular analyses showed that the subfamilies Aclyvolvinae, Eocypraeinae and Simniinae are polyphyletic, whereas the Ovulinae and Pediculariinae appear to be monophyletic. Within the subfamily Aclyvolvinae, the type species of *Hiatavolva*, *H. depressa*, did not form a clade with the other species of *Hiatavolva*. Instead, *H. rugosa* and *H. coarctata* formed a clade that is sister to the clade comprising *Aclyvolva lamyi*, *A. lanceolata* and *A. nicolamassierae*, and are therefore now considered as belonging to the genus *Aclyvolva*. *Aclyvolva lamyi* and *A. nicolamassierae* were shown to be synonyms of *A. lanceolata*, and *A. rugosa* (n. comb.) is a synonym of *A. coarctata* (n. comb.). The genus *Kuroshiovolva* could not be retrieved in a fixed phylogenetic position within the Aclyvolvinae, nor did it cluster with *H. depressa* or *Aclyvolva* spp. Our morphometric analyses are in agreement with the results of the molecular analyses, and furthermore show that juvenile shells are morphologically significantly different from their adult conspecifics. Photographs of the type material of *Ovulum lanceolatum*, *O. coarctatum*, *Neosimnia lamyi*, *Hiata rugosa* and *A. nicolamassierae* are provided, and new information is given on the geographical distribution and host species of Aclyvolvinae. The subfamily Aclyvolvinae is redefined and now includes only *A. lanceolata* and *A. coarctata*. The genus *Hiatavolva* is now monotypic, containing only *H. depressa*, but the subfamily to which this genus belongs remains unclear. *Kuroshiovolva* is not part of the Aclyvolvinae, but its subfamily level placement is unclear.

INTRODUCTION

Species of the family Ovulidae Fleming, 1882, occur in tropical, subtropical and temperate waters, but their diversity is highest in the tropical waters of the Indo-Pacific. Most species in this family are obligate associates of octocoral species. To provide camouflage

against visual predation, the mantle colour and pattern of ovulids is usually similar to that of their octocoral hosts (Cate, 1973; Rosenberg, 1992; Lorenz & Fehse, 2009, but see Reijnen & van der Meij, 2017). Some ovulid species can mimic typical morphological octocoral host structures such as polyps (Fig. 1).

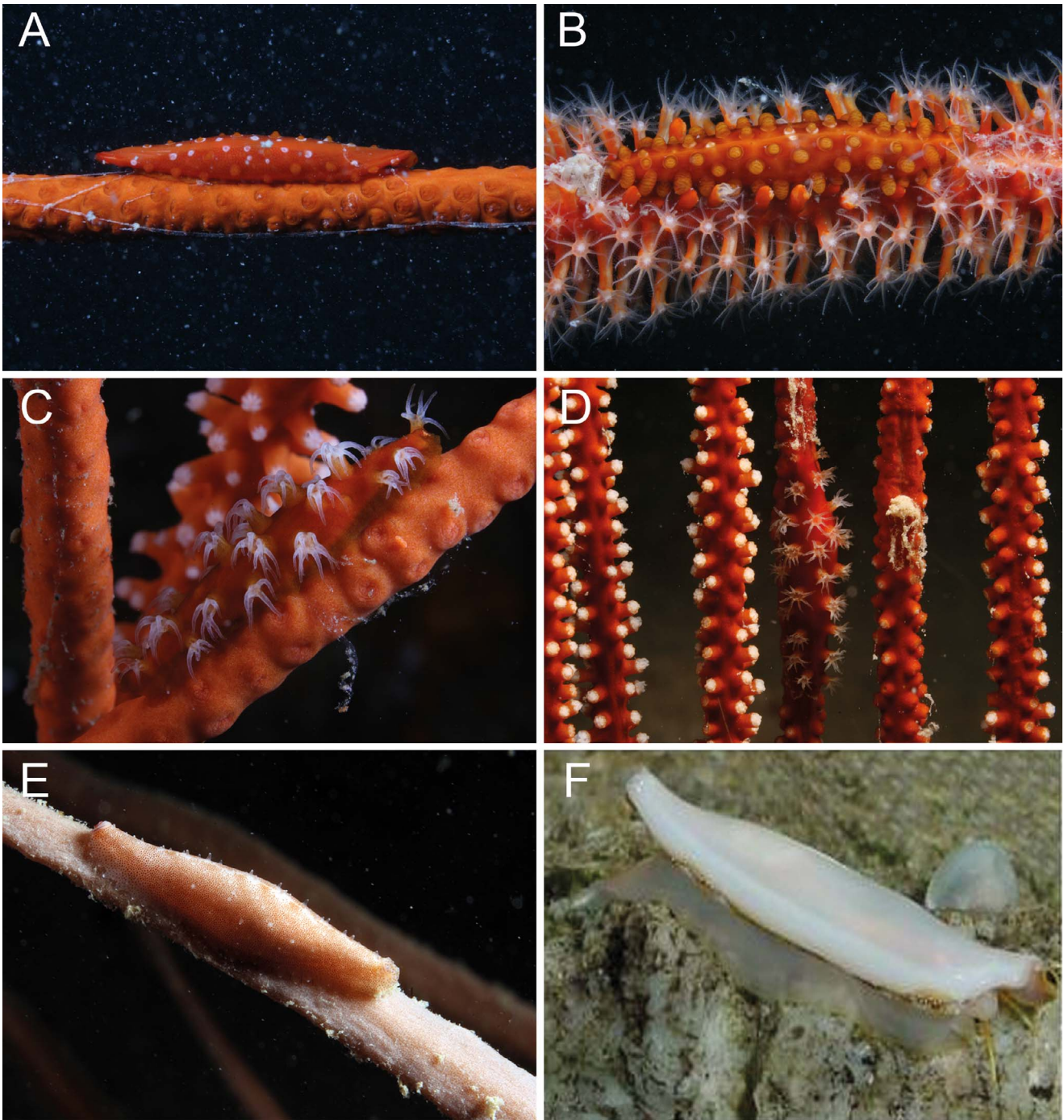


Figure 1. *In situ* images of Aclyvolvinae and their coral hosts. **A.** *Aclyvolva lanceolata* (RMNH.MOL.164192) on *Viminella* sp. at Kudat, Malaysia. **B.** *Aclyvolva lanceolata* (RMNH.MOL.164181) on *Junceella* sp. at Kudat, Malaysia. **C.** *Aclyvolva coarctata* (RMNH.MOL.164234) on *Ellisella* sp. at Lembah Strait, Indonesia. **D.** *Aclyvolva coarctata* (RMNH.MOL.164197) on *Ctenocella* sp. at Pulau Banggi, Malaysia. **E.** *Hiatovolva depressa* (RMNH.MOL.164147) on *Alertigorgia orientalis* (Ridley, 1884) at Pulau Banggi, Malaysia. **F.** *Kuroshiovolve shingoi* at Bohol, Philippines. Photographs: **A–E**, B.T. Reijnen; **F, E**, Guillot de Suduiraut.

The family Ovulidae was subdivided by Fehse (2007) into four subfamilies, namely Ovulinae Fleming, 1822, Simniinae Schilder, 1927, Aclyvolvinae Fehse, 2007 and Prionovolviniae Fehse, 2007. The division into subfamilies by Fehse (2007) was based on the study by Schiaparelli *et al.* (2005), which was the first molecular phylogenetic reconstruction of the Ovulidae and was based on DNA sequence data for the mitochondrial 16S rRNA gene. This phylogenetic reconstruction showed a polytomy involving five clades (A–E), with strong support for each clade in some or all

analyses. No taxonomic revisions were made by Schiaparelli *et al.* (2005), but Fehse (2007) erected the subfamilies Aclyvolvinae and Prionovolviniae based on their results and provided morphological characters for these two groups on the basis of descriptions by Simone (2004). Bouchet *et al.* (2017) recognized six subfamilies in the Ovulidae (Ovulinae, Aclyvolvinae, Eocypraeinae Schilder, 1924, Pediculariinae Gray, 1853, Simniinae and Sulcocypreaeinae Schilder, 1932) and considered Prionovolviniae a junior synonym of Eocypraeinae.

Schiaparelli *et al.* (2005) included two species of *Aclyvolva* Cate, 1973 in their analyses, *A. lanceolata* (Sowerby, 1848) and *A. cf. lamyi* (Schilder, 1932). These species were found to form a clade (Schiaparelli *et al.*, 2005: fig. 1, see clade C). The relationships between this clade and the four other clades in their study remain unresolved. Despite the lack of supporting molecular data, Fehse (2007) included the genera *Hiatavolva* Cate, 1973, and *Kuroshiovolva* Azuma & Cate, 1971, in the new subfamily Aclyvolvinae (type species *A. lanceolata*). The shells of Aclyvolvinae *sensu* Fehse (2007) can be distinguished from those of other ovulids by their lanceolate form and the absence of a well-developed funiculum. Species-level differences in this subfamily are based on conchological characters, such as the density and coarseness of the striae, the presence or absence of longitudinal growth lines and shell colour (Lorenz & Fehse, 2009). However, when presented with sizeable shell collections, appreciable interspecific overlap in morphology becomes apparent, hampering identification based purely on these morphological characters. To add to the confusion, the conchological characters are lacking or expressed differently in juvenile shells. As a consequence, many names have become available for similar-looking lanceolate shells and there is disagreement among ovulid workers. Cate (1973) described two new genera and two new species in what is currently known as Aclyvolvinae *sensu* Fehse (2007), while resurrecting other species. Lorenz & Fehse (2009) synonymized many species in the genera *Aclyvolva* and *Hiatavolva*, and subsequently *Kuroshiovolva lacanientae* Lorenz, 2009, was described. This currently leaves nine recognized species in the Aclyvolvinae.

All Aclyvolvinae species are restricted to the central Indo-Pacific, except for *A. nicolamassierae* Fehse, 1999, which occurs in the western Indian Ocean and the Red Sea (Fehse, 1999; Lorenz & Fehse, 2009). Most species of *Aclyvolva* and *Hiatavolva* are hosted by gorgonians of the family Ellisellidae (Schiaparelli *et al.*, 2005; Lorenz & Fehse, 2009; Reijnen, 2010), with the exceptions of *H. depressa*, which is associated with the genus *Alertigorgia* (Anthothelidae), and *Kuroshiovolva* species, which are associated with the genus *Plumarella* (Primnoidae) (Lorenz, 2009). Unfortunately, most of the ovulid material that is deposited in museum collections is not accompanied by data on the host species (this should ideally be a piece of the host coral), limiting our ability to identify and check published host records.

The taxonomic uncertainties in the Aclyvolvinae indicate the need for an integrated molecular and morphological study to clarify the interspecific relationships and validity of the nominal taxa. In this study, using DNA sequence data from seven nominal species of Aclyvolvinae and four gene regions, we reconstruct the phylogenetic relationships between the Aclyvolvinae and the ovulid subfamilies Ovulinae, Simniinae and Pediculariinae. Our aim is to test generic assignments and clarify the taxonomic status of available species-level taxa. In addition, we analyse data on shell morphological characters gathered from specimens for which molecular data were available, to help clarify species delimitations made on the basis of DNA sequence data.

MATERIAL AND METHODS

Sampling and identification

A total of 83 specimens of Ovulidae were included in this study; for each ovulid specimen, a tissue sample of its host is available in the collections of Naturalis Biodiversity Center (NBC) in Leiden, The Netherlands. The cypraeid *Ransoniella punctata* was used as an outgroup. Specimens belonging to the Aclyvolvinae represented seven nominal species: *Aclyvolva lamyi* ($n = 3$), *A. lanceolata* ($n = 9$), *A. nicolamassierae* ($n = 1$), *Hiatavolva coarctata* (Sowerby II in Adams & Reeve, 1848) ($n = 13$), *H. depressa* (Sowerby III, 1875) ($n = 2$), *H. rugosa* Cate & Azuma in Cate, 1973 ($n = 17$) and *Kuroshiovolva shingoi* Azuma & Cate, 1971 ($n = 1$). The type species of the subfamilies Aclyvolvinae (*A. lanceolata*), Ovulinae (*Ovula ovum* (Linnaeus, 1758))

and Simniinae (*Simnia nicaensis* Risso, 1826) were also included in the dataset. *Simnia nicaensis* is now considered a synonym of *S. spelta* (Linnaeus, 1758) (Dolin & Ledon, 2002). Several species of the Eocypraeinae and Pediculariinae were included in the phylogenetic reconstruction. The type species of Eocypraeinae (*Cypraea inflata* Lamarck, 1802) and Sulcocypraeinae (*Cypraea lineata* Conrad, 1848) are fossils. We were unable to include *Pedicularia sicula* Swainson, 1840, the type species of Pediculariinae, in our study due to the lack of suitable material or GenBank sequence data. The same was true for *Sphaerocypraea incomparabilis* (Briano, 1993), which is the only non-fossil representative of the Sulcocypraeinae.

Ovulid specimens were collected mainly from Indonesia and Malaysia, with a few specimens being obtained from Saudi Arabia and Thailand (see Supplementary Material Table S1 for more information). Voucher specimens were fixed in 70% ethanol and deposited in the mollusc collection of NBC (registration numbers include the code RMNH.MOL). The voucher specimen of *K. shingoi* is in the National Museum of Natural History, Smithsonian Institution, Washington D.C. (USNM); the cytochrome *c* oxidase subunit I (COI) and 16S rRNA sequences from this voucher were provided by C.P. Meyer. Identifications were based on comparisons with photographs of the type specimens of *A. nicolamassierae*, *H. rugosa*, *Neosimnia lamyi*, *A. lanceolata* and *Ovulum coarctatum* (Figs 2, 3) and the relevant literature, of which the major works are the ovulid monographs by Cate (1973) and Lorenz & Fehse (2009). A stereomicroscope (Leica MZ16) was used to examine material. The genera *Hiatavolva* and *Aclyvolva* were separated by their shell outlines; *Aclyvolva* has tapering terminals (Fig. 2), whereas the shell shape is stout in *Hiatavolva* (Fig. 3). The cnidarian hosts were identified based on Grasshoff (1999) and Fabricius & Alderslade (2001).

DNA extraction and sequencing

Sequence data were generated for four gene regions for the 42 specimens belonging to the Aclyvolvinae: the mitochondrial markers 16S rRNA and COI, and the nuclear markers 28S rRNA and histone H3 (Table 1). In addition, sequence data were generated for 16S rRNA and COI for the other 41 specimens of Ovulidae (15 nominal species). Not all markers were successfully amplified for all specimens, and an overview of the sequence and locality data is provided in Supplementary Material Table S1. Sequence data for seven ovulid species (*Crenavolva aureola* (Fehse, 2002) ($I = 4$), *C. striatula* (Sowerby I, 1828) ($n = 1$), *C. trailli* (Adams, 1856) ($n = 2$), *Cyphoma acicularis* (Lamarck, 1811) ($n = 3$), *Cyphoma gibbosum* (Linnaeus, 1758) ($n = 6$), *Primovula rosewateri* (Cate, 1973) ($n = 1$), *Simnia patula* (Pennant, 1777) ($n = 1$) and *S. spelta* ($n = 1$)) were obtained from GenBank (see Supplementary Material Table S1). These sequences were generated in earlier studies by Reijnen *et al.* (2010), Reijnen (2015) and Reijnen & van der Meij (2017).

Tissue for DNA extraction was obtained from the foot and/or mantle of the snails. The DNeasy Kit (QIAGEN) was used according to the corresponding protocol for animal tissue (v. 07/2006). Digestions were performed overnight for approximately 16 h and DNA elution was performed with 100 μ l of buffer AE. DNA extracts were diluted (1:100 or 1:300) before PCR amplification. The PCR mixture contained the following: 2.5 μ l PCR CoralLoad Buffer (containing 15 mM MgCl₂) (QIAGEN); 0.5 μ l dNTPs (2.5 mM); 1.0 μ l each primer (10 μ M); 0.3 μ l Taq polymerase (15 units/ μ l) (QIAGEN); 18.7 μ l extra pure water; and 1.0 μ l (diluted) DNA extract. For amplification of the 28S rRNA marker, 5.0 μ l water was replaced by 5.0 μ l QSolution (QIAGEN).

All PCR cycles consisted of an initial denaturing step of 95 °C for 1 min followed by 39 cycles of 95 °C for 10 s, annealing at the appropriate temperature (see Table 1) for 1 min and extension at 72 °C for 1 min. The final PCR cycle was followed by an elongated extension step of 72 °C for 5 min. Successfully amplified samples were sent to Macrogen Europe for PCR cleaning and sequencing on an ABI Automated Sequencer 3730xl. A total of 237 novel



Figure 2. Dorsal and ventral views of *Aclyvolvula lanceolata*, including type specimens. **A.** Lectotype (left, NHMUK 1969134/1) and paralectotypes of *Ovulum lanceolatum* (NHMUK 1969134/2–3). **B.** *Aclyvolvula lanceolata* (RMNH.MOL.164179). **C.** Holotype of *Neosimnia lanyi* (MNHN-IM-2000-27 664). **D.** *Aclyvolvula lanceolata* (RMNH.MOL.164165). **E.** Holotype of *Aclyvolvula nicolamassierae* (HNC 46684). **F.** *Aclyvolvula lanceolata* (RMNH.MOL.337794). Photographs: **A, A.** Salvador (NHMUK); **B–D, F.** B.T. Reijnen; **E.** V. Wiese (Haus der Natur). Scale bar = 5 mm.

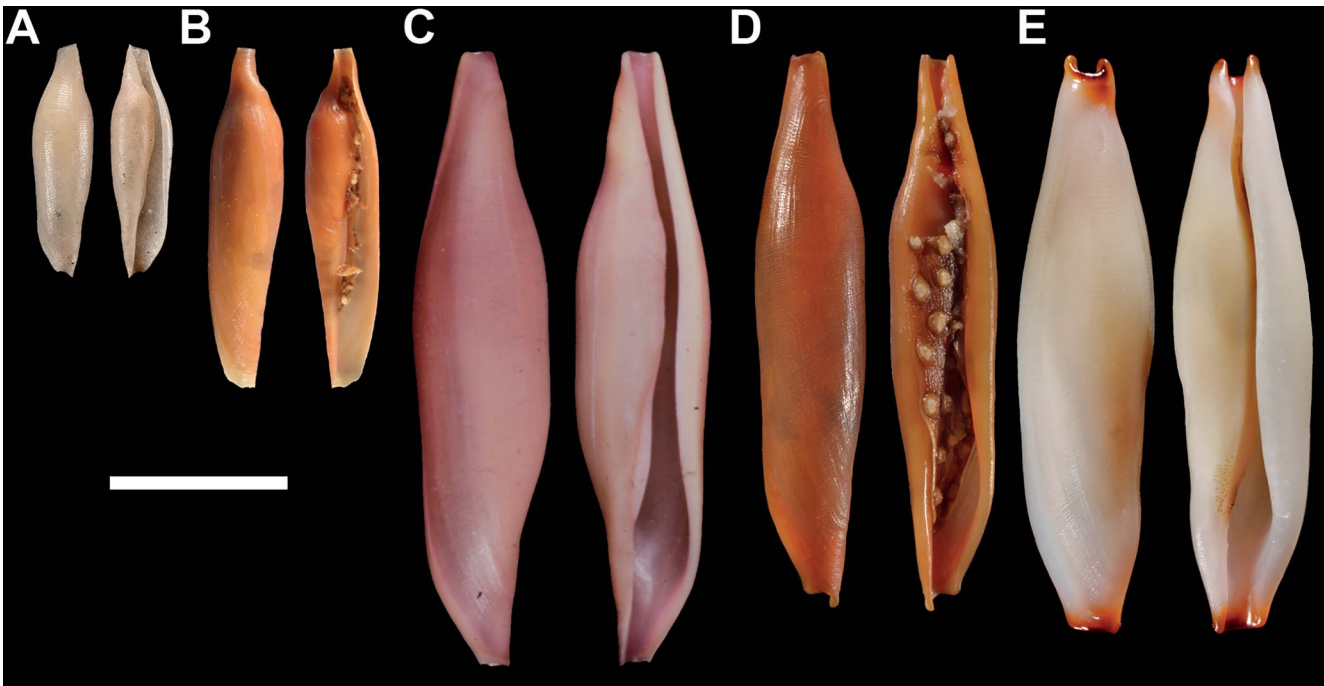


Figure 3. Dorsal and ventral views of *Aclyvolvula coarctata*, including type specimens. **A.** Holotype of *Ovulum coarctatum* (NHMUK 1879.2.26.147). **B.** *Aclyvolvula coarctata* (RMNH.MOL.164185). **C.** Holotype of *Hiata rugosa* (15 603, private collection Masao Azuma). **D.** *A. coarctata* (RMNH.MOL.164234). **E.** *Hiatavolvula depressa* (RMNH.MOL.164182). Photographs: **A.** A. Salvador (NHMUK); **B, D, E.** B.T. Reijnen; **C.** G. Rosenberg (ANSP). Scale bar = 5 mm.

Table 1. Details of gene regions and associated primer pairs (forward primers listed first) used in the study.

Gene region	Fragment size	Primer name	Primer sequence	Annealing temperature	Reference
Histone H3	~380	H3F	ATGGCTCGTACCAAGCAGACVGC	50	Colgan <i>et al.</i> (2000)
		H3R	ATATCCTTRGGCATRATRGTGAC		Colgan <i>et al.</i> (2000)
28S rRNA	~800	LSU5	TAGGTCGACCCGCTGAAYTTAAGCA	50	Littlewood <i>et al.</i> (2000)
		LSU800rc	GACTCCTTGGTCCGTGTTTT		This study
16S rRNA	~540	16Sar	CGCCTGTTTATCAAAAACAT	52	Palumbi (1996)
		16Sbr	CCGGTCTGAACTCAGATCACGT		Palumbi (1996)
COI	~660	LCO-1490	GGTCAACAAATCATAAAGATATTGG	40–44	Folmer <i>et al.</i> (1994)
		HCO-2198	TAAACTTCAGGGTGACCAAAAAATCA		Folmer <i>et al.</i> (1994)

sequences for four molecular markers were generated. These have been uploaded to GenBank under accession numbers KP259314–KP259547 and KP271159–KP271161.

Molecular analyses

Sequences were edited using either Geneious Pro v. 5.6.4 or Sequencher v. 4.10.1 and aligned using MAFFT on the GUIDANCE2 server (Sela *et al.*, 2015), resulting in an alignment score of 0.98. Unreliable columns below 0.93 were removed. All newly acquired sequences were checked against GenBank to check for similarity with sequence data previously submitted by Meyer (2003) and Schiaparelli *et al.* (2005). Sequences were concatenated with the help of SequenceMatrix (Vaidya *et al.*, 2011) to create two concatenated datasets, one containing ovulid species from five subfamilies (based on 16S rRNA and COI genes) and a second dataset consisting solely of the Aclyvolvinae (based on 16S rRNA, COI, histone H3 and 28S rRNA genes). The aligned Ovulidae dataset was 1,105 bp in length, including indels; the aligned Aclyvolvinae dataset was 2,296 bp long, including indels.

Nucleotide substitution models for phylogeny reconstruction were selected for each of the single marker datasets using jModeltest v. 2 (Durriba *et al.*, 2012). Phylogenies were reconstructed with maximum likelihood (ML), using Phyml v. 3.1 (Guindon *et al.*, 2010) in the Seaview platform (Gouy *et al.*, 2010), and Bayesian inference (BI), using MrBayes v. 3.2.2 (Ronquist *et al.*, 2012). Support values for the ML trees were determined over 1,000 bootstrap iterations. For BI, analyses were run for over 3 million generations using the Dirichlet method (the standard deviation of split frequencies was 0.007); trees were sampled every 100 iterations; and burn-in was set to 7,500. Consensus trees were visualized in FigTree v. 1.4.3 (Rambaut, 2009). To check for non arbitrary species delimitation all COI sequences used in this study were submitted to the online programme ABGD (Automatic Barcode Gap Discovery) (Puillandre *et al.*, 2012).

Morphological measurements and analyses

Shell morphological features were analysed by plotting landmarks on photographs of the dorsal side of the sequenced specimens in standard orientation (Figs 2, 3); a total of 151 landmarks were plotted along the entire shell outline. The Tps software package (tpsUtil, tpsDig2 and tpsRelw) (Rohlf, 2006) was used to create

the morphological dataset and to calculate relative warps. The resulting relative warp data was exported into the programme PAST (Hammer *et al.*, 2001) and a principal component analysis (PCA) was carried out. The length of all Aclyvolvinae specimens was measured with a calibrated digital calliper (Mitutoyo 500) following Rosenberg (2010).

RESULTS

Molecular analyses

The phylogenetic reconstructions of the Ovulidae dataset (five ovulid subfamilies) showed that relationships between the subfamilies Aclyvolvinae, Ovulinae, Eocypraeinae and Simniinae were unresolved (Fig. 4). The ingroup consists of two well-supported deep-level clades: one of these clades comprises *Pedicularia pacifica* Pease, 1865, and *P. vanderlandi* Goud & Hoeksema, 2001, (Pediculariinae) and the other all other Ovulidae (Aclyvolvinae + Eocypraeinae + Ovulinae + Simniinae). The relationships between these two clades are unresolved. Relationships within the Aclyvolvinae *sensu* Fehse (2007) are only partly resolved. While the genus *Aclyvolva* was maximally supported in both ML and BI analyses as sister to the clade comprising *Hiatavolva coarctata* and *H. rugosa* (Fig. 4), *H. depressa*, together with *Naviculavolva deflexa*, forms part of a strongly-supported clade that is dominated by taxa belonging to the Eocypraeinae. The clade of *Aclyvolva* + *H. coarctata* + *H. rugosa* and the clade of Eocypraeinae + *H. depressa* + *Naviculavolva* are nested within a larger and strongly-supported clade (Aclyvolvinae + Eocypraeinae + Ovulinae + Simniinae, bootstrap = 89%, posterior probability = 100%), which contains the genus *Kuroshiovolva*. Not only do these results indicate that the genus *Hiatavolva* is polyphyletic, they suggest that the Aclyvolvinae and Simniinae, as currently conceived, are also polyphyletic.

The cladogram based on the Aclyvolvinae dataset (Fig. 5) showed that the genetic distance between the nominal species *Aclyvolva lanceolata*, *A. nicolamassierae* and *A. lamyi*, and between *H. coarctata* and *H. rugosa*, were small in relation to typical interspecific distances. The non arbitrary approach for species delimitation (based on the COI dataset) in the ABGD analysis supported this finding. The differences in intra *vs* interspecific sequence variation resulted in four groups of species (Fig. 5). These were (1) *A. lanceolata* + *A. nicolamassierae* + *A. lamyi*; (2) *H. coarctata* + *H. rugosa*; (3) *H. depressa* and (4) *Kuroshiovolva shingoi*.

Morphological analyses of Aclyvolvinae

The PCA was based on 44 relative warp coordinates of 151 landmarks. Principal components 1, 2 and 3 accounted for 88% of the variation among samples. *Hiatavolva coarctata* and *H. rugosa* formed two largely distinct clusters, with each species being represented in each cluster (Fig. 6). The *Aclyvolva* species also clustered together without further noticeable separation by species. Apart from an outlier of *H. rugosa*, which was located close to one of the two specimens of *H. depressa*, *H. depressa* occupied a distinct part of the plot. On examining the two clusters of *H. coarctata* and *H. rugosa* more closely, we found that the specimens falling within the oval area shown in the plot were smaller in size (mean length \pm SD = 12 \pm 2.56 mm, n = 11) than specimens outside the oval area (mean \pm SD = 15.81 \pm 2.70 mm, n = 19). Moreover, while shells on the left side of the plot had a less developed and less calloused shell, which is typical of juveniles or subadults, specimens on the right side of the plot generally had the well-developed labrum and adapical and abapical canals typical of adult specimens.

DISCUSSION

Molecular phylogeny and subfamilial classification of the Ovulidae

Our phylogeny of the Ovulidae (Fig. 4) was largely unresolved, but the patterns observed are nonetheless inconsistent with the classifications proposed by Fehse (2007) and Bouchet *et al.* (2017). Although a limited number of representatives from the five subfamilies were included in the present study, our results suggest that the Aclyvolvinae, Eocypraeinae and Simniinae, as currently defined, are not monophyletic groups. Although all the species of Eocypraeinae included in our study formed part of a single, well-supported clade, this clade also included *Hiatavolva depressa* (Aclyvolvinae) and *Naviculavolva deflexa* (Sowerby II, 1848) (Simniinae). The phylogeny therefore suggests that both the Aclyvolvinae and Simniinae are polyphyletic. This leaves the Ovulinae and Pediculariinae as the only monophyletic subfamilies within the Ovulidae; Schiaparelli *et al.* (2005) and Fehse (2007) also found the Ovulinae to be monophyletic. The ingroup in our phylogeny of the Ovulidae comprises two major clades, the Pediculariinae and a clade comprising all other ovulids; the relationships between these two clades were unresolved. Additional research, including molecular data for the type species, is needed to assess whether the Pediculariinae are indeed monophyletic and perhaps deserving of family rank. On the basis of anatomical data, Simone (2004, 2011) gave the Pediculariinae family ranking.

Our phylogenetic reconstructions show that ovulid shell shapes (e.g. rhomboid, lanceolate, globose or pyriform) are not restricted to specific clades; this is in line with the results of Schiaparelli *et al.* (2005). Species having a lanceolate shell shape (Aclyvolvinae *s. l.*) occur in three distinct parts of the phylogeny, and may reflect convergent evolution in shell shape rather than common ancestry. Studies on homoplasy and convergent evolution in marine gastropods (e.g. Marko & Vermeij, 1999; Johannesson, 2003) have shown that ecological factors can influence shell morphological features.

Classification of Aclyvolvinae *s. s.*: molecular and morphological evidence

The species of Aclyvolvinae *sensu* Fehse (2007) included in this study were found in three different positions in the phylogeny (Fig. 4). *H. depressa* (type species of *Hiatavolva*) and *Kuroshiovolva shingoi* (type species of *Kuroshiovolva*) do not form part of the highly supported clade containing *Aclyvolva lanceolata*, the type species of *Aclyvolva* (type genus of Aclyvolvinae), and are therefore no longer considered to part of the subfamily Aclyvolvinae *s. s.* As strong support was recovered for the sister-group relationships of *H. coarctata* and *H. rugosa* to the *Aclyvolva* clade, we suggest transfer of those two species to the genus *Aclyvolva* pending further information (see below); these new combinations will be used from here onwards. *Hiatavolva depressa* has indented terminals such that there are two tooth-like projections at either terminal end of the shell. This character is not shared by any other member of the Aclyvolvinae, and this could explain the distinct position this species occupies in the PCA. The relationships of *K. shingoi* to other ovulid species remain unclear. This requires further molecular studies, which should preferably include data for *K. lacanientae*.

Molecular data can be used for overcoming difficulties in morphological species identifications in the Ovulidae (Reijnen, 2015; Reijnen & Van der Meij, 2017). The sequence data for 16S rRNA generated for our study were checked against the molecular data of Schiaparelli *et al.* (2005) deposited in GenBank. The sequences of specimens here identified as *A. coarctata/rugosa* were strikingly similar to material identified by Schiaparelli *et al.* (2005) as *A. lanceolata*. Similarly, material identified by us as *A. lanceolata* corresponded closely with sequences provided by Schiaparelli *et al.* (2005) for *A. cf. lamyi*. Comparison of photographs of

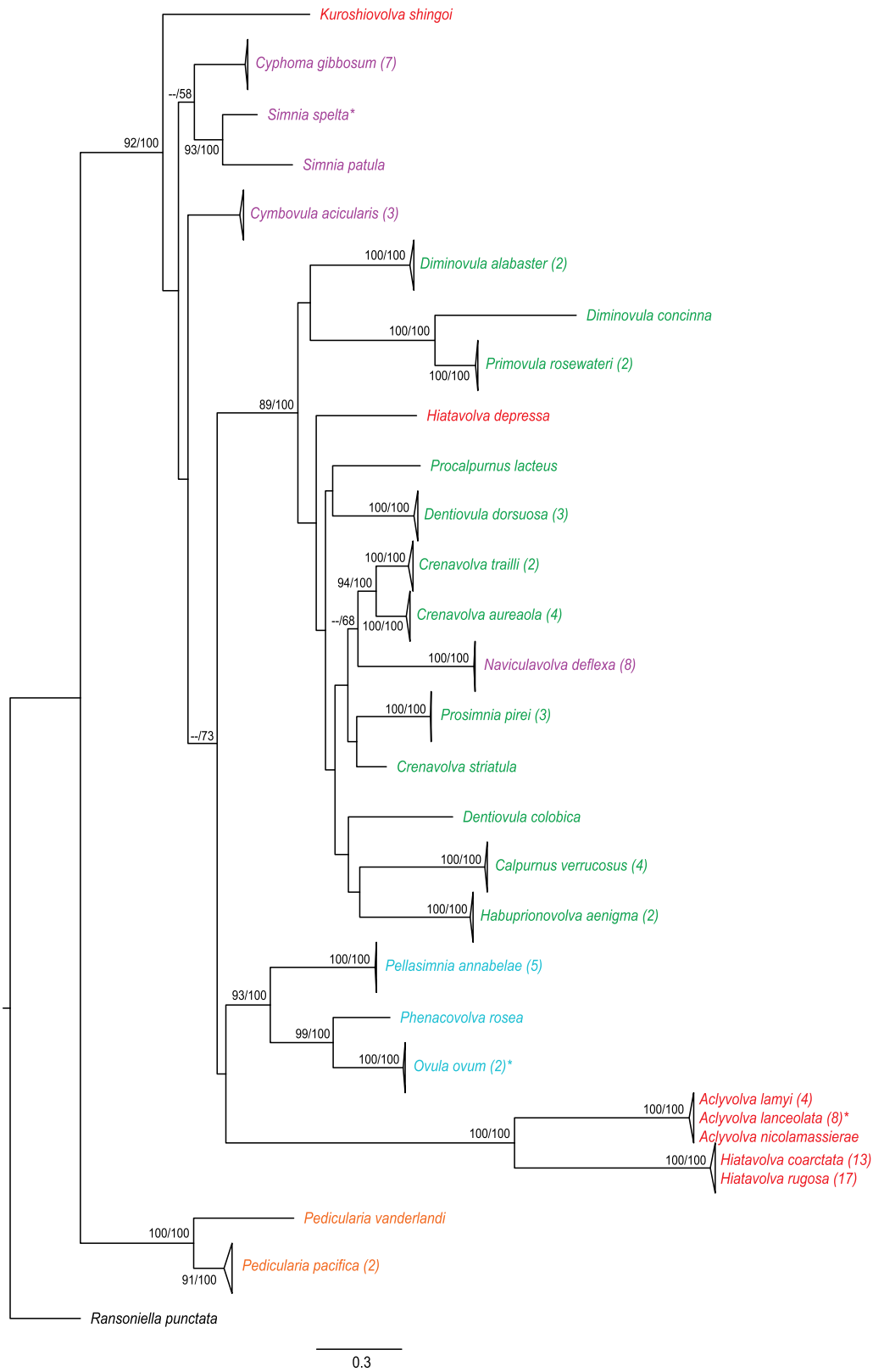


Figure 4. Phylogeny of the Ovulidae based on 16S rRNA and COI. Species belonging to the different ovulid subfamilies are colour-coded as follows: red, Aclyvolvinae; green, Eocypraeinae; blue, Ovulinae; orange, Pediculariinae; and purple, Simniinae. Type species of the subfamilies are indicated with an asterisk and numbers within parentheses indicate the number of specimens sampled for each nominal species. Numbers on branches denote support values with bootstrap and posterior probabilities on the left and right, respectively. Following the taxonomic changes recommended in this paper, *Aclyvolva lamyi* and *A. nicolamassierae* are referred to as *A. lanceolata*, and *H. coarctata* and *H. rugosa* as *A. coarctata*.

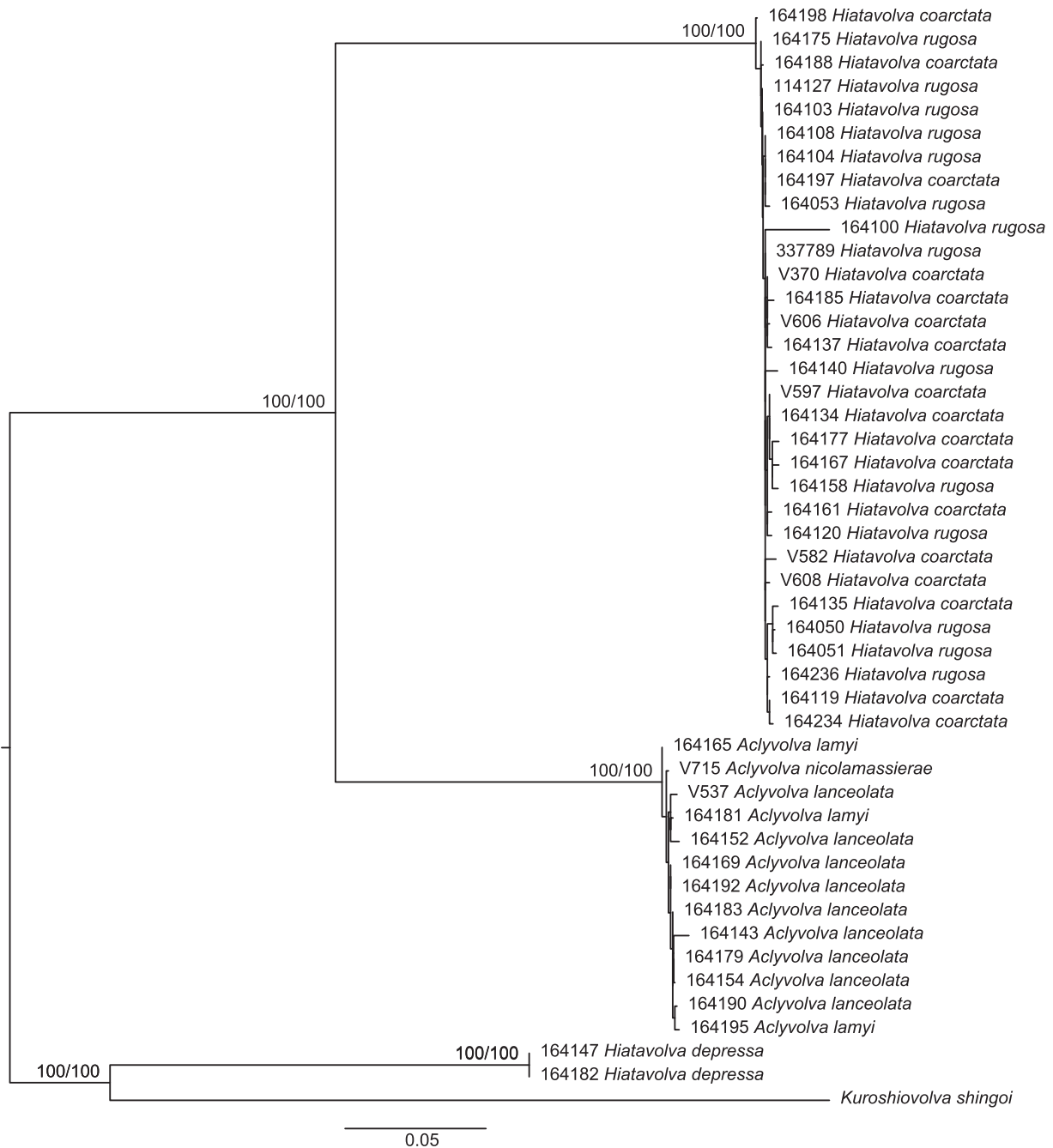


Figure 5. Phylogenetic reconstruction of the Aclyvolvinae based on 16S rRNA, COI, histone H3 and 28S rRNA. Numbers on branches are support values, with bootstrap and posterior probabilities on the left and right, respectively. Following the taxonomic changes recommended here, *Aclyvolva lamyi* and *A. nicolamassierae* are referred to as *A. lanceolata*, and *H. coarctata* and *H. rugosa* as *A. coarctata*.

the living animals and their respective shells, as provided by Schiaparelli *et al.* (2005: figs 3h, i, l, m, 4f–i, l), with specimens figured by Cate (1973) and Lorenz & Fehse (2009), and the images of the holotypes (Fig. 2), indicate that Schiaparelli *et al.* (2005) likely misidentified the *Aclyvolva* species included in their study (see also Fehse, 2006: 19). Schiaparelli *et al.* (2005) did note that the relationship between *A. lanceolata* and *A. rugosa* was unclear and that the morphological characters defining the genera *Aclyvolva* and *Hiatavolva* were rather inconsistent. Furthermore, they suggested that *A. lanceolata* and *A. rugosa* could be conspecific (the authors incorrectly assumed *A. rugosa* to be the type species of *Hiatavolva*).

The type specimen of *A. coarctata* is a subadult shell and lacks most of the adult characters that are used to distinguish species. Indeed, the last sentence of the original description by Sowerby II (1848: 21) states “It may, however, very possibly be a young shell”. Liltved (1989) agreed that the type of *A. coarctata* is probably a subadult shell. Additionally, Liltved (1989: 132) questioned the differences in shell morphology between *A. coarctata* and *A. rugosa*. Fehse (1999) disagreed with the observations by Liltved (1989) and considered *A. coarctata* to have a smaller and slightly more inflated shell, shorter terminals and different colour when compared with *A. rugosa*. Two of these characters reflect the growth stage of the shells: subadult shells tend to be smaller in size than adults and

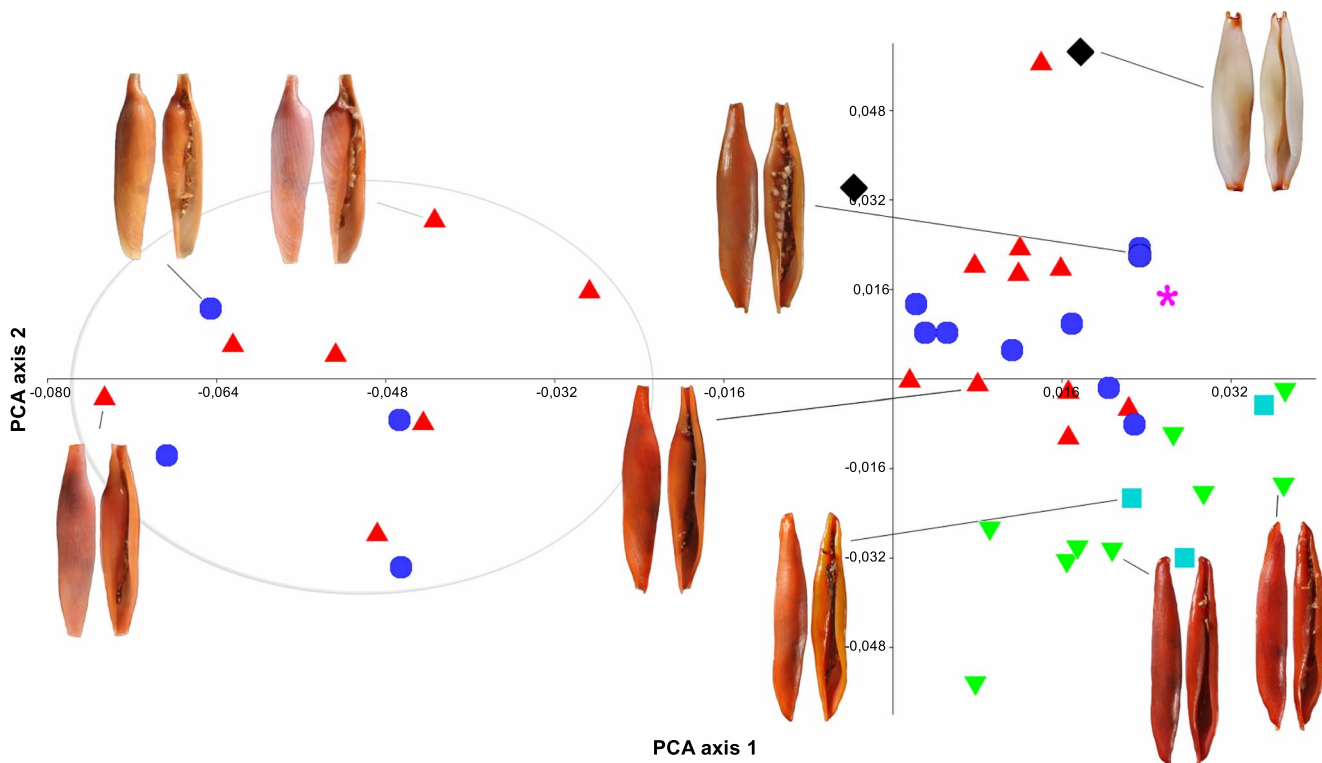


Figure 6. PCA with 44 relative warps and 151 landmarks. Taxa are indicated as follows: square, *Aclyvolvula lamyi*; inverted triangle, *A. lanceolata*; asterisk, *A. nicolamassierae*; circle, *Hiatavolvula coarctata*; diamond, *H. depressa*; triangle, *H. rugosa*. The oval area shown on the left indicates juveniles of *H. coarctata* and *H. rugosa*. Images of the shells are not to scale. Following the taxonomic changes recommended in this paper, *A. lamyi* and *A. nicolamassierae* are referred to as *A. lanceolata*, and *H. coarctata* and *H. rugosa* as *A. coarctata*.

have shorter terminals. Lorenz & Fehse (2009), who considered shell colour not to be a useful diagnostic character, used terminal length and longitudinal sculpture to separate *A. coarctata* from *A. rugosa*. Material collected for this study, which includes both subadult and adult stages, was morphologically assigned to either *A. coarctata* or *A. rugosa* on the basis of these two characters (Fig. 3B, D). While our morphometric analyses showed that juveniles are morphologically distinct from adults and that the differences are not just restricted to size (Fig. 6), our molecular results do not show genetic differences that correspond to the division into two morphologically defined nominal species (Figs 4, 5). These results support the conclusion that *A. coarctata* and *A. rugosa* are conspecific, as suggested by Liltved (1989). Similarly, we did not find any genetic evidence that the nominal species *A. lamyi*, *A. nicolamassierae* and *A. lanceolata* are distinct (Fig. 5). Since juveniles in the family Oculidae can differ substantially from conspecific adults (Reijnen *et al.*, 2010), utmost care has to be taken when describing new species on the basis of adult or juvenile specimens alone (e.g. see Lorenz & Melaun, 2011).

Interspecific differences in shell morphology are often not clear-cut in Aclyvolvinae, but mantle patterns and structures can provide an additional tool for species identification. Images of *A. coarctata* *in situ* in its natural habitat show that this ovulid has compound papillae that mimic the polyps and tentacles of its host. *Aclyvolvula lanceolata*, in contrast, has blunt papillae on its mantle, and these can sometimes be of contrasting colour (Schiaparelli *et al.*, 2005: fig. 3m; Lorenz & Fehse, 2009: figs A350, 351, 355).

Remarks on distribution and host species of Aclyvolvinae

The distribution of ovulid species reflects the distribution and abundance of their host species. *Aclyvolvula* species are typically

associated with hosts belonging to the family Ellisellidae (primarily *Ctenocella*, *Dichotella*, *Ellisella* and *Junceella*). Members of the Ellisellidae are found in the Indo-Pacific in both shallow and deep water, and thus species of *Aclyvolvula* also occur in these habitats. The collections of the NBC also contain a shell of *A. lanceolata* from the Persian Gulf (RMNH.MOL.187230). Our molecular data for *A. nicolamassierae* from the Red Sea showed no obvious genetic difference between it and *A. lanceolata* from Indonesia and Malaysia and hence we regard these taxa as synonymous. As a result, the distribution of *A. lanceolata* spans the entire Indo-Pacific.

Hiatavolvula depressa is only known to occur on the octocoral *Aleritorgia* (Anthothelidae). This highly specific association explains the absence of *H. depressa* from the Indian Ocean and Red Sea, where *Aleritorgia* is absent.

Species of *Kuroshiovolvula* are only known to be found in association with hosts belonging to the genus *Plumarella* (Table 2), although specimens of these ovulids are scarce in collections and data on hosts are limited. According to Fabricius & Alderslade (2001) there is only one *Plumarella* species known from shallow waters in Australia, while all other species are from deeper and colder water. *Plumarella* is considered to have a very limited distribution range and it is unclear to what extent this has affected the distribution of *Kuroshiovolvula*.

Doubtful host records

Except for *Echinogorgia* (Plexauridae), *Melithaea* (Melithaeidae) and *Muricella* (Acanthogorgiidae), all host genera of *A. coarctata* (Table 2) are representatives of the family Ellisellidae. The host *Melithaea japonica* was recorded by Yamamoto (1973: as *M. flabellifera*). However, the photographs given by the author show that the ovulid is not *A. coarctata* but *Prosimmia* cf. *draconis* Cate, 1973, for which *Melithaea*

Table 2. Octocoral host species and distribution records for species of *Aclyvolva*, *Hiatavolva* and *Kuroshiovolva* (ovulid names are based on the taxonomic changes in this paper).

Ovulid species	Octocoral host genera/species	Distributional records	References
<i>Aclyvolva coarctata</i>	<i>Ctenocella</i> ; <i>Dichotella</i> ; <i>Echinogorgia</i> ?; <i>Ellisella</i> sp; <i>Meliithaea</i> ?; <i>Muricella</i> ?; <i>Verrucella</i> ; <i>Viminella</i>	Indian Ocean (E Africa, Réunion); Central Indo-Pacific (Australia, Indonesia, Japan, Malaysia, Philippines)	Mase (1989); Schiaparelli <i>et al.</i> (2005); Lorenz & Fehse (2009); Reijnen (2010); this study
<i>Aclyvolva lanceolata</i>	<i>Ctenocella</i> ; <i>Dichotella</i> ; <i>Ellisella</i> ; <i>Junceella</i> ; <i>Verrucella</i> ; <i>Viminella</i>	Indo-Pacific (E Africa, Australia, Indonesia, Malaysia, Persian Gulf, Philippines, Red Sea, Réunion)	Schiaparelli <i>et al.</i> (2005); Lorenz & Fehse (2009); Reijnen (2010); this study
<i>Hiatavolva depressa</i>	<i>Alertigorgia orientalis</i> ; <i>A. hoeksemai</i>	Central Indo-Pacific (Australia, Indonesia, Malaysia, New Caledonia)	Lorenz & Fehse (2009); this study
<i>Kuroshiovolva shingoi</i>	<i>Plumarella</i> ; <i>Plumarella cristata</i> (= <i>Acanthoprinnia cristata</i>)	Central Indo-Pacific (Australia, Fiji, Japan, New Caledonia, Philippines)	Lorenz & Fehse (2009)
<i>Kuroshiovolva lacanientae</i>	<i>Plumarella</i> ; <i>Astrogorgia</i> ?	Papua New Guinea, Philippines	Coleman (2003); Lorenz (2009)

See text for discussion of doubtful records (marked with a query).

is the common host genus (Reijnen, 2010). The records of *Muricella* and *Echinogorgia* as host genera (Mase, 1989; Lorenz & Fehse, 2009: see captions A356, A357) are also doubtful. *Muricella* species are notoriously hard to identify (see Reijnen *et al.*, 2011) and based on photographs it seems most likely that the host species is a *Verrucella* species (Ellisellidae). *Verrucella* and *Muricella* both have a planar and reticulated growth form. *Echinogorgia* is easily confused with other gorgonian genera (e.g. *Paraplexaura*) and cannot be identified *in situ*. Moreover, this genus is very uncommon in the Indo-Pacific. The only way of confirming these records is to examine tissue samples of the host.

Kuroshiovolva lacanientae has likely been recorded from an *Astrogorgia* species by Coleman (2003); *Astrogorgia* usually hosts the ovulid *Phenacovolva rosea* (A. Adams, 1855) and cannot be confidently identified in the field without examination of the sclerites, so this record is also doubtful.

Table 2 summarizes the known host records of *Aclyvolvinae s. l.*

Systematics and synonymy

Based on the phylogenetic and morphological analyses presented above (Figs 4–6), we consider *A. lamyi* and *A. nicolamassierae* to be junior synonyms of *A. lanceolata*. *Hiatavolva coarctata* is transferred to the genus *Aclyvolva*, with *A. rugosa* placed in synonymy. *Aclyvolva lanceolata* is the type species of the type genus of *Aclyvolvinae*, hence the species in this clade (*A. lanceolata* and *A. coarctata*) now compose *Aclyvolvinae s. s.* (Fig. 5). Of the genera formerly considered to belong to *Aclyvolvinae s. l.*, *Hiatavolva* is considered a monotypic genus (*H. depressa*), while *Kuroshiovolva* has two valid species (*K. shingoi* and *K. lacanientae*). The subfamilies to which *Hiatavolva* and *Kuroshiovolva* should be reassigned could not be determined as substantial revisions to the higher taxonomic levels in the *Ovulidae* are needed.

The formal systematics and synonymy are therefore revised as follows:

OVULIDAE Fleming, 1822

ACLIVOLVINAЕ Fehse, 2007

Aclyvolva Cate, 1973

Diagnosis: Shell elongate, narrow, rather cylindrical. Posterior terminal narrow, anterior broader. Canals open. Tips of terminals usually pointed but can also be blunt or have indented terminal tips. Aperture narrow and widest in the fossular section, abruptly con-

stricting to form the siphonal canal. Funiculum absent. (Modified from Lorenz & Fehse, 2009.)

Remarks: The diagnosis has been extended with characters used to distinguish *Aclyvolva* from *Hiatavolva*. The shape and retractile properties of the mantle papillae can be used to separate the two *Aclyvolva* species in life. *Aclyvolva lanceolata* has blunt papillae that do not resemble octocoral tentacles (Fig. 1A, B; Schiaparelli *et al.*, 2005: fig. 3i, l, m), whereas *A. coarctata* has compound papillae that can mimic the host's polyps and tentacles (Fig. 1C, D (extended); Schiaparelli *et al.*, 2005: fig. 4h, i (extended), 4l (retracted)). All known hosts of the genus *Aclyvolva* belong to the gorgonian family Ellisellidae (Table 2; Supplementary Material Table S1; Coleman, 2003; Schiaparelli *et al.* 2005; Lorenz & Fehse, 2009; Reijnen, 2010).

Aclyvolva lanceolata (Sowerby II, 1848)

(Figs 1AB, 2A–F)

Ovulum lanceolatum Sowerby II, 1848: 135.

Ovula lanceolata—Weinkauff, 1881: 207, pl. 52, figs 10, 11.

Neosimnia lanceolata—Allan, 1956: 127.

Aclyvolva lanceolata Lorenz & Fehse, 2009: 133, pl. 189: 1–7, 16, A350.

Aclyvolva aff. *lanceolata*—Lorenz & Fehse, 2009: 133, pl. 189: 9–11.

Aclyvolva cf. *lanceolata*—Lorenz & Fehse, 2009: 133, pl. 189: 8, A351.

Wong, 2011: figs 18a–d, 27 g, h.

Neosimnia lamyi Schilder, 1932: 54, pl. 4, fig. 44.

Aclyvolva cf. *lamyi*—Schiaparelli *et al.*, 2005: fig. 3h, i, l, m.

Lorenz & Fehse, 2009: 134, pl. 190: 1, 3–5, A352.

Aclyvolva lamyi—Lorenz & Fehse, 2009: 134, pl. 190: 2, 6–10, A353–355. Wong, 2011: figs 18e–t, 27a–f.

Aclyvolva aff. *lamyi*—Lorenz & Fehse, 2009: 134, pl. 190: 11, 12.

Aclyvolva nicolamassierae Fehse, 1999: 51, pl. 2, figs 1, 2.

Lorenz & Fehse, 2009: 134, pl. 189: 12–15.

Hiatavolva coarctata—Lorenz & Fehse, 2009: 135, pl. 191, A360, A361 (in part; includes *A. coarctata*; not Sowerby II in Adams & Reeve, 1848).

Aclyvolva coarctata (Sowerby II in Adams & Reeve, 1848) n. comb.

(Figs 1C, D, 3A–D)

Ovulum coarctatum Sowerby II in Adams & Reeve, 1848: 21, pl. 6, fig. 2a, b.

Ovula coarctata—Weinkauff, 1881: 188, pl. 48, figs 9, 12.

Prosimnia (Prosimnia) coarctata—Kuroda, 1958: 169.

Hiata coarctata—Mase, 1989: pl. 10, 22a, d.

- Phenacovolva coarctata*—Liltved, 1989: 132.
Hiata volva coarctata—Lorenz & Fehse, 2009: 135, pl. 191: 1–10, A356–A359 (in part; includes *A. lanceolata*: figs A360, A361).
Hiata rugosa Cate & Azuma in Cate, 1973: 87, fig. 197.
Hiata volva rugosa—Lorenz & Fehse, 2009: 135, pl. 191: 11–17, A362–A365.
Acyvolva lanceolata—Schiaparelli *et al.*, 2005: fig. 4f–i, 1 (not Sowerby II, 1848).

Subfamily incertae sedis

Hiata volva Cate, 1973

Diagnosis: Shell elongate, narrow, almost cylindrical, solidly formed. Terminals evenly narrow towards each canal, gently recurved. Canals open. Tips of terminals indented. Funiculum indistinct or absent. (Modified from Cate, 1973 and Lorenz & Fehse, 2009.)

Remarks: *Hiata volva depressa* is the only ovulid species known to live on the gorgonian genus *Alertigorgia* (Table 2; Coleman, 2003).

Hiata volva depressa (Sowerby III, 1875)

(Figs 1E, 3E)

- Ovulum depressum* Sowerby III, 1875: 128, pl. 24, fig. 1.
Phenacovolva depressa—Iredale, 1935: 105.
Neosimnia (Pellasimnia) depressa—Allan, 1956: 130.
Hiata depressa—Cate, 1973: 87, fig. 194.
Hiata volva depressa—Lorenz & Fehse, 2009: 135, pl. 192: 1–6, A366.

Subfamily incertae sedis

Kuroshiovolva Azuma & Cate, 1971

Diagnosis: Shells have more or less parallel sides, terminal ends almost squarely blunt (having the form of a razor clam); straight apertures, open at either end. (Modified from Cate, 1973.)

Remarks: *Plumarella* is the primary coral host genus for this genus (Table 2).

Kuroshiovolva shingoi Azuma & Cate, 1971

(Fig. 1F)

- Kuroshiovolva shingoi* Azuma & Cate, 1971: 266, text figs 14, 20–23. Lorenz & Fehse, 2009: 136, pl. 192: 7–13, A367, A368. Lorenz, 2009: figs 1(right), 3.

Kuroshiovolva lacanientae Lorenz, 2009

- Kuroshiovolva lacanientae* Lorenz, 2009: 38, figs 1(left), 2, 4.

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