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# Chapter 1

## Host specificity and phylogenetic relationships among Atlantic Ovulidae (Mollusca: Gastropoda)

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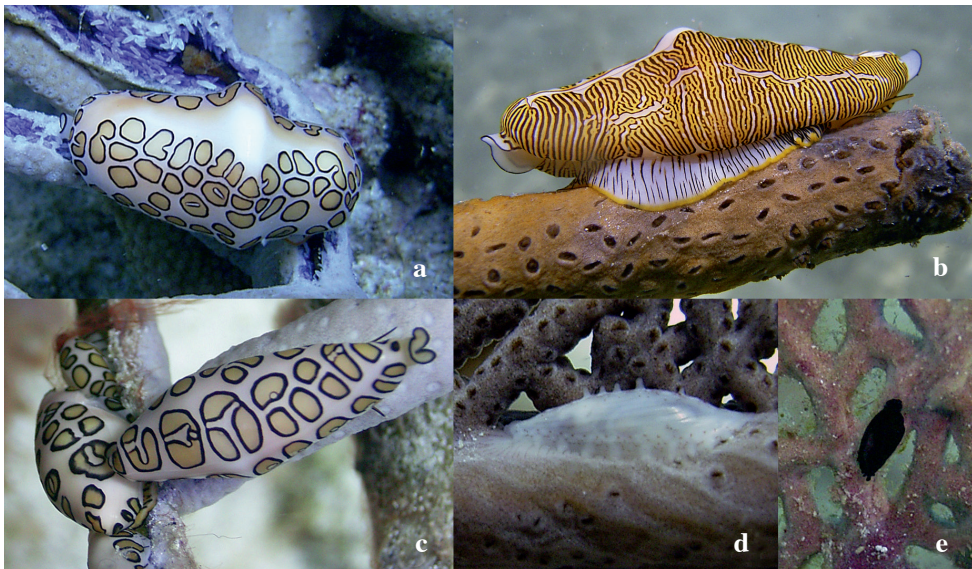
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### Abstract

Ovulid gastropods and their octocoral hosts were collected along the leeward coast of Curaçao, Netherlands Antilles. New molecular data of Caribbean and a single Atlantic species were combined with comparable data of Indo-Pacific Ovulidae and a single East-Pacific species from GenBank. Based on two DNA markers, viz. COI and 16S, the phylogenetic relationships among all ovulid species of which these data are available are reconstructed. The provisional results suggest a dichotomy between the Atlantic and the Indo-Pacific taxa. Fully grown *Simnialena uniplicata* closely resembles juvenile *Cyphoma gibbosum* conchologically. *Cymbovula acicularis* and *C. bahamaensis* might be synonyms. The assignments of Caribbean host species for *Cyphoma gibbosum*, *C. signatum*, *Cymbovula acicularis* and *Simnialena uniplicata* are revised.

## Introduction

Ovulid snails are obligate associates of Cnidaria. As far as known, most occur associated with octocorals (Anthozoa: Octocorallia: Alcyonacea), but in both the Caribbean and the Indo-Pacific some ovulid species feed on antipatharians (Anthozoa: Hexacorallia: Antipatharia) (Tazioli *et al.*, 2007). The species of *Pedicularia* Swainson, 1840, that have been classified with the Ovulidae for a long time (Goud and Hoeksema, 2001), live on stylanderid corals (Hydrozoa: Athecatae: Filefera). *Pedicularia* differs from the undisputed ovulids in radula morphology (Simone, 2004); it is now classified in the separate family Pediculariidae (Fehse, 2007; Lorenz and Fehse, 2009). Thirty-seven species of Ovulidae Fleming, 1822, are known from the Caribbean and Atlantic area (Lorenz and Fehse, 2009). The dominant genus in the Caribbean is *Cyphoma* Röding, 1798, with 14 species of which *Cyphoma gibbosum* (Linnaeus, 1758) is the most common. Due to the low number of ovulid species in the Caribbean and the well-known diversity of Octocorallia (Bayer, 1961) parasite/host relationships are most easily studied here. It has been hypothesized that colour patterns and texture of ovulid mantles may either mislead potential predators by mimicking its host's branches and polyps, or that its colour acts as a warning of unpalatability (aposematic species) (Rosenberg, 1992; Schiaparelli *et al.*, 2005). Snails closely resembling coral branches and polyps of their host may be camouflaged in such a way that they are almost undetectable for predators, such as shown by the Caribbean *Cymbovula acicularis* (Lamarck, 1810), which uses its mantle colour and protrusions to mimic the branches and polyps of its gorgonian host (Fig. 1d). As a first step to a better understanding of the associations between alcyonaceans and ovulids, Yamamoto (1972) and Schiaparelli *et al.* (2005) studied the snails and their



**Fig. 1.** Species in situ. a) *Cyphoma gibbosum* b) *C. signatum* c) *C. gibbosum* (juvenile) d) *Cymbovula acicularis* e) *Simnialena uniplicata* (juvenile).

hosts in the Indo-Pacific. In the present paper we primarily deal with the Ovulidae and their octocoral hosts of the Caribbean island of Curaçao. Our new data on species associations are used to investigate whether the gastropod species should be considered generalists or specialists. Based on of the molecular data from this study and GenBank, a provisional molecular phylogeny reconstruction of Ovulidae is presented.

## Material and methods

In April - June 2005, both ovulid snails and their octocoral hosts were sampled using SCUBA diving at 32 localities off the leeward western coast of Curaçao, Netherlands Antilles. Observations on the windward eastern shore were hampered by exposure to

**Table 1.** Newly sequenced species with GenBank accession numbers and sequence data obtained from GenBank, referring to (S) Schiaparelli *et al.* (2005) and (M) Meyer (2003).

Species	16S accession number	CO-I accession number
<i>Adamantia florida</i> (Kuroda, 1958)	AY 161396 (M)	AY 161629 (M)
<i>Calpurnus lacteus</i> (Lamarck, 1810)	AY 161398 (M)	AY 161631 (M)
<i>Calpurnus verrucosus</i> (Linnaeus, 1758)	AY 161397 (M)	AY 161630 (M)
<i>Crenavolva</i> cf. <i>rosewateri</i> Cate, 1973	AY 161394 (M)	AY 161627 (M)
<i>Crenavolva tokuoi</i> Azuma, 1989	AY 161390 (M)	AY 161623 (M)
<i>Cyphoma gibbosum</i> Linnaeus, 1758	AY 161400 (M)	AY 161633 (M)
<i>Cyphoma gibbosum</i> Linnaeus, 1758	GU 363427	GU 363439
<i>Cyphoma gibbosum</i> Linnaeus, 1758	GU 363428	GU 363440
<i>Cyphoma gibbosum</i> Linnaeus, 1758	GU 363429	GU 363441
<i>Cyphoma gibbosum</i> Linnaeus, 1758	GU 363430	GU 363442
<i>Cyphoma gibbosum</i> Linnaeus, 1758	GU 363431	GU 363443
<i>Cyphoma gibbosum</i> Linnaeus, 1758	GU 363432	GU 363444
<i>Cyphoma gibbosum</i> Linnaeus, 1758	GU 363433	GU 363445
<i>Cymbovula acicularis</i> (Lamarck, 1810)	GU 363434	GU 363446
<i>Cymbovula acicularis</i> (Lamarck, 1810)	GU 363436	GU 363448
<i>Cymbovula acicularis</i> (Lamarck, 1810)	GU 363437	GU 363449
<i>Cypraea tigris</i> (Linnaeus, 1758)	AY 161489 (M)	AY 161722 (M)
<i>Dentiovula takeoi</i> Cate & Azuma, 1973	AY 534354 (M)	AY 534431 (M)
<i>Jenneria pustulata</i> Lightfoot, 1786	AY 161402 (M)	AY 161635 (M)
<i>Neosimnia arcuata</i> (Reeve, 1865)	AY 161401 (M)	AY 161634 (M)
<i>Ovula ovum</i> (Linnaeus, 1758)	AY 161399 (M)	AY 161632 (M)
<i>Phenacovolva tokioi</i> (Cate, 1973)	AY 161393 (M)	AY 161626 (M)
<i>Phenacovolva weaveri</i> Cate, 1973	AJ 868565 (S)	AY 161628 (M)
<i>Primovula concinna</i> Adams & Reeve, 1848	AY 534353 (M)	AY 534430 (M)
<i>Prionovolva brevis</i> Sowerby I, 1828	AY 161391 (M)	AY 161624 (M)
<i>Prosimmia semperi</i> (Weinkauff, 1881)	AJ 868548 (S)	AY 534432 (M)
<i>Simnia patula</i> (Pennant, 1777)	GU 363438	GU 363450
<i>Simnialena uniplicata</i> (Sowerby 2nd, 1848)	GU 363435	GU 363447
<i>Volva volva</i> (Linnaeus, 1758)	AY 534352 (M)	AY 534429 (M)



**Fig. 2.** Shells in dorsal and ventral view. a-d) *Cymbovula acicularis* (juvenile and adult); e) *Cyphoma signatum*; f-g) *Simnialena uniplicata* (juvenile); h) *Simnina patula*; i) *Cyphoma gibbosum*. Scale bars 1 cm (left, for a-d and f-h; right, for e and i).

the open sea. All snails and hosts were photographed in situ and collected by hand, and subsequently stored as vouchers in the collection of NCB Naturalis. Three species of the Atlantic ovulid *Simnina patula* (Pennant, 1777), dredged from the Dutch North Sea bottom by fishermen, were added as additional samples for the phylogeny reconstruction. For the Ovulidae the nomenclature accepted by the ‘Checklist of European Marine Mollusca’ (CLEMAM, 2008) and Cate (1973) was followed, except for *Neosimnina aequalis* sensu stricto (not Sowerby II, 1832), which is referred to as *N. arcuata* (Reeve, 1865), in accordance with Lorenz and Fehse (2009). The status of the former nominal taxon will remain uncertain as long as no valid lectotype selection has taken place, but this cannot affect the use of the latter name.

### *Molecular analyses*

For molecular phylogeny reconstructions, tissue samples from the foot of the snails were used to extract DNA with the E.Z.N.A. Mollusc DNA Kit (Omega Biotek). The primer sets published by Meyer (2003) and Schiaparelli *et al.* (2005) were used to amplify the mtDNA markers COI and 16S marker, respectively. The PCR reaction mixtures were composed after Gittenberger *et al.* (2006). For 16S, 0.005 ml MilliQ was replaced by an equal volume of Qsolution (Qiagen). The annealing temperature used for 16S was set at 52°, whereas for COI a ramp was used, starting at 40° and ending at 44°, increasing with 0.1° s<sup>-1</sup>. Sequencing was performed on a MegaBace 1000, 96 capillary

**Table 2.** Host-species associations between Ovulidae and Octocorallia.

Octocoral taxa		<i>Cyphoma gibbosum</i>	<i>Cyphoma signatum</i>	<i>Cymbovula acicularis</i>	<i>Sinialena uniplicata</i>
<b>Briareidae</b>					
<i>Briareum</i>	<i>asbestinum</i> (Pallas, 1766)	3	-	-	-
<b>Gorgoniidae</b>					
<i>Gorgonia</i>	<i>flabellum</i> Linnaeus, 1758	3	-	2	2
	<i>mariae</i> Bayer, 1961	-	-	1	-
	<i>ventalina</i> Linnaeus, 1758	5	-	12	2
<i>Pseudopterogorgia</i>	<i>acerosa</i> (Pallas, 1766)	4	-	5	-
	<i>americana</i> (Gmelin, 1791)	11	-	-	-
	<i>bipinnata</i> (Verrill, 1864)	2	-	7	-
	<i>rigida</i> (Bielschowsky, 1929)	2	-	-	-
<i>Pterogorgia</i>	<i>citrina</i> (Esper, 1792)	1	-	-	-
<b>Plexauridae</b>					
<i>Eunicea</i>	<i>calyculata</i> (Ellis & Sollander, 1786)	3	-	-	-
	<i>clavigera</i> Bayer, 1961	4	-	-	-
	<i>knightyi</i> Bayer, 1961	2	-	-	-
	<i>succinea</i> (Pallas, 1766)	1	-	-	-
	<i>tourneforti</i> Milne Edwards & Haime, 1857	6	-	-	-
<i>Muricia</i>	<i>muricata</i> (Pallas, 1766)	2	-	-	-
<i>Plexaura</i>	<i>flexuosa</i> Lamouroux, 1821	7	-	-	-
	<i>homomalla</i> (Esper, 1792)	1	-	-	-
	<i>dichotoma</i> (Esper, 1791)	3	1	-	-
<i>Plexaurella</i>	<i>grisea</i> Kunze, 1916	2	-	-	-
	<i>nutans</i> (Duchassaing & Michelotti, 1860)	1	-	-	-
<i>Pseudoplexaura</i>	<i>porosa</i> (Houttuyn, 1772)	6	-	-	-
Unidentified		3	-	-	-

sequencer at Leiden University, and on an Automatic Sequencer 3730xl by Macrogen, Korea. The raw sequence data were assembled and edited using Sequencher 4.2 (Gene Codes Corporation®) and aligned with ClustalX. The sequences in the COI dataset were all checked for stop codons. All sequences were referenced against GenBank (National Center for Biotechnology Information, NCBI) to ensure that non-targeted DNA had not been sequenced. For the phylogeny reconstruction, 17 GenBank sequences for both 16S and COI (Table 1), representing the same species, were combined with sequences of the Atlantic species.

For the single individual of *Cyphoma signatum*, COI could not be amplified. Therefore, this species was excluded from the dataset. The final alignment consisted of 28 sequences containing 961 base pairs. Newly sequenced species are deposited in



**Table 3.** Overview of collected Octocorallia that were not found to be parasitized by Oculididae.

Anthothelidae	<i>Erythropodium caribaeorum</i> (Duchassaing & Michelotti, 1860)
Ellisellidae	<i>Ellisella barbadensis</i> (Duchassaing & Michelotti, 1864) <i>Ellisella elongata</i> (Pallas, 1766)
Plexauridae	<i>Eunicea calyculata coronata</i> Bayer, 1961 <i>Eunicea fusca</i> (Duchassaing & Michelotti, 1860) <i>Eunicea laciniata</i> Duchassaing & Michelotti, 1860 <i>Eunicea mammosa</i> Lamouroux, 1816 <i>Eunicea succinea plantaginea</i> (Lamarck, 1815) <i>Eunicea</i> sp. A <i>Muricea atlantica</i> (Kükenthal, 1919) <i>Muricea laxa</i> Verrill, 1864 <i>Plexaura nina</i> Bayer & Deichman, 1958 <i>Plexaura</i> sp. A <i>Pseudoplexaura flagellosa</i> (Houttuyn, 1772) <i>Pseudoplexaura</i> sp. A <i>Pseudoplexaura</i> sp. B <i>Pseudoplexaura</i> sp. C
Gorgoniidae	<i>Pseudopterogorgia</i> sp. A <i>Pseudopterogorgia</i> sp. B <i>Pterogorgia guadelupensis</i> Duchassaing & Michelin, 1846

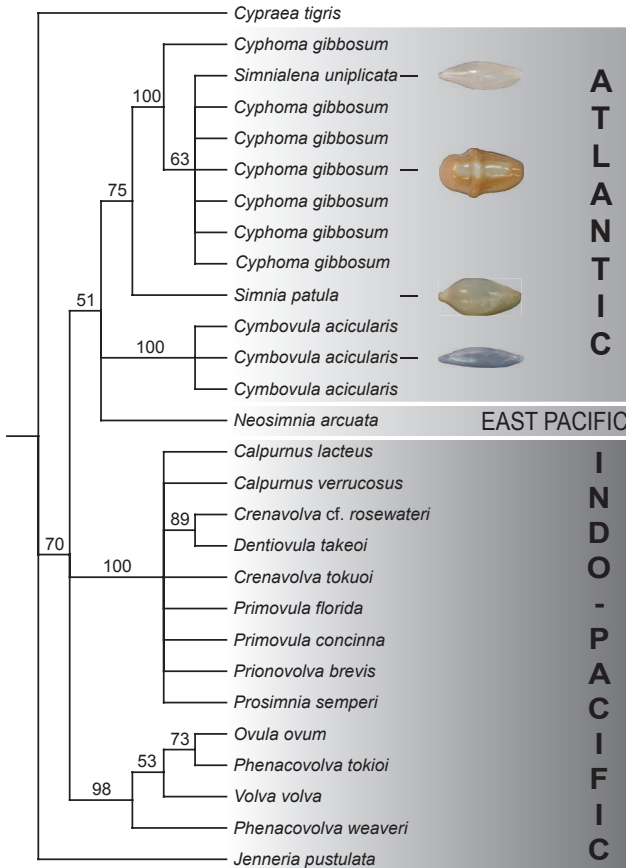
GenBank under accession numbers GU363427 – GU363450. Within 16S highly variable regions exist, formed by either insertions or deletions. Aligning this region proved to be very difficult and the unalignable region, consisting of 75 base pairs in length (position 166 till 241), was deleted. To check whether both datasets could be combined, an incongruence length difference test (ILD-test) was performed. This test resulted in a P-value ( $P = 1.00$ ) allowing the data combination. To determine the optimal evolutionary models the combined molecular dataset was subjected to Modeltest (Posada and Crandall, 1998). This resulted in the Generalised Time Reversible evolutionary model + invariable sites + gamma (GTR I+G). The obtained parameters were used to run a maximum likelihood search in PAUP\* 4.0b (Swofford, 2003). No fewer than a 100 bootstrap replicates were used to evaluate the robustness of the nodes. The search was carried out with tree bisection-reconnection (TBR). Gaps were in all cases treated as missing character and not as a fifth character state. The selected outgroup sequence was that of the cypraeid *Cypraea tigris* (Linnaeus, 1758).

## Results

### *Species and associations*

A total of 104 samples of oculids was collected, representing viz. *Cyphoma gibbosum* (Linnaeus, 1758), *Cyphoma signatum* Pilsbry and McGinty, 1939, *Simnialena uniplicata*





**Fig. 3.** Maximum likelihood analysis for combined Atlantic and Indo-Pacific Ovulidae based on 16S and CO-I data. The values above the branches represent bootstrap values (100 replicates).

(Sowerby II, 1848) and *Cymbovula acicularis* (Lamarck, 1810) (Fig. 2, including specimen resembling the so-called *C. bahamaensis*).

The 72 snails of *Cyphoma gibbosum* that could be studied were found with 21 alcyonacean species, representing nine genera. The 27 individuals of *Cymbovula acicularis* were found in association with five gorgoniid species, belonging to two genera. *Simnialena uniplicata* occurred with two congeneric host species, but since only four individual snails were found, it would be premature to derive any conclusions about host specificity. Unfortunately, only a single specimen of *Cyphoma signatum* could be studied, which was associated with *Plexaurella dichotoma* (Esper, 1791). This gorgonian species was also mentioned by Botero (1990), who additionally reported the congeneric *P. nutans* as a host for *C. signatum*. Due to the poverty of its records, the host preferences of this ovulid remain largely unknown. From a total of 46 octocoral species recorded at Curaçao, 26 (57%) were found to be occasionally parasitised by one or more ovulid species (Table 2).

Not all encountered Octocorallia species were found associated with ovulids. Additionally, a list was composed (Table 3) of encountered Octocorallia without associated ovulids.

### *Phylogeny reconstruction*

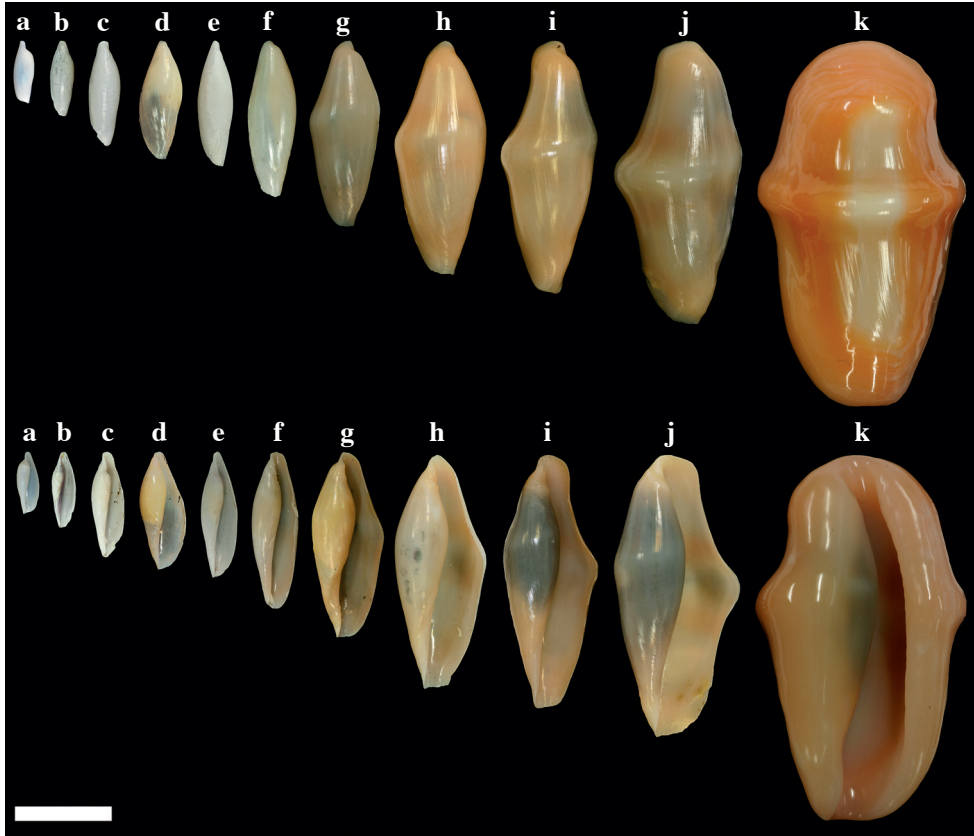
*Cyphoma gibbosum* is a common Caribbean species that is easily recognized by its colour pattern and morphology. To exclude possible sibling species occurring on, for example, different hosts or at other localities at Curaçao, several individuals from different Octocorallia species and from different localities along the coast were sequenced. Based on the molecular data no sibling species occurrence was detected. In Fig. 3 the results of the combined dataset subjected to a maximum likelihood analysis (ML) with bootstrap values is presented.

The phylogeny reconstruction indicates that there is a separation between the Atlantic and the Indo-Pacific clade (moderately supported, bootstrap value 70), although the East-Pacific species *Neosimnia arcuata* clusters with the Atlantic clade (poorly supported, bootstrap value 51). *Simnialena uniplicata* (Fig. 2f-g), *Neosimnia arcuata* and *Cymbovula acicularis* (Fig. 2a-d) are characterized conchologically by long and slender shells, but in the cladogram *S. uniplicata* does not appear as sister species to either *N. arcuata* or *C. acicularis*. Instead, it forms a highly supported (bootstrap value 100) clade with the *Cyphoma* group. The individual sequence of the ovulid specimen resembling *Cymbovula bahamaensis* (Fig. 2c) forms a highly supported clade (bootstrap value 100) with the included *C. acicularis* species.

## Discussion

### *Associations*

Reported host preferences of *Cyphoma gibbosum* (Bertsch, 1984; Lasker *et al.*, 1988; Botero, 1990; Nowlis, 1993; Chiapponne *et al.*, 2003) are partly confirmed and supplemented with new observations of associations of this species, showing once more that *C. gibbosum* is a generalist parasite (Table 2). *Cymbovula acicularis* turned out to be another generalist. It remains unclear why 43% of the encountered alcyonacean species did not appear as hosts for ovulids at the time of our fieldwork (Table 3). It is known that Octocorallia may produce secondary metabolites as protection against predation (Ciereszko and Schneider, 1987; Chiapponne *et al.*, 2003), but the effect of this defence strategy on ovulids is still largely unexplored. Other factors, such as the nutritional value of the corals (O'Neal and Pawlik, 2002) and the unpalatability of sclerites (Alstyne and Paul, 1992), may also influence host choices. An overview of natural products produced by West Indian gorgonian octocorals reveals that many types of secondary metabolites are found. The largest class of metabolites encountered in Caribbean alcyonaceans are diterpenoids, followed by the sesquiterpenes (Rodriguez, 1995). For some of these compounds that are obtained from alcyonaceans, such as *Erythropodium caribaeorum*, feeding experiments were performed, resulting in the observation that coral extracts are deterrent to fish. Also, crude extracts from the gorgonian *Gorgonia ventalina*, containing terpenoids, were used in feeding experiments with *C. gibbosum*. As a result, *C. gibbosum* consumed only 49% of an artificial diet containing terpenoids (Alstyne and Paul, 1992). The sclerites of the gorgonian species that we found as hosts for *Simnialena uniplicata* and *Cymbovula acicularis* turned out to be relatively small. The sclerites of *Gorgonia* spp. and *Pseudopterogorgia* spp. have average sizes of 0.10 mm



**Fig. 4.** Shells in dorsal and ventral view. *Simnialena uniplicata* (d) compared to a series of shells of *Cyphoma gibbosum* (a-c, e-k) in different developmental stages. Scale bar 1 cm.

and 0.10-0.15 mm, respectively, whereas 0.30-1.00 mm is common for other Octocoralia species, like *Eunicea* and *Pseudoplexaura* species (Bayer, 1961). For *Cyphoma gibbosum*, however, the sclerites' size seems to be irrelevant for host selection, since this species is found on corals containing either small or large sclerites. By analysing faecal pellets which all contained sclerites, we conclude that *C. gibbosum* at least ingests sclerites instead of eating around them.

#### *Paedomorphosis*

In the phylogeny reconstruction, *Simnia patula* is the sister group of the combined *Simnialena uniplicata* and *Cyphoma* species group. This is surprising because, at first sight, fully grown individuals of *Cyphoma* species clearly differ from *S. uniplicata* in shell morphology. However, when shells of *S. uniplicata* are compared to a series of shells of *C. gibbosum* in various growth-stages (Fig. 4), it turns out that *S. uniplicata* closely resembles juvenile *C. gibbosum* (as well as juvenile ovulids in general).

Both species lack a clear funiculum (narrow ridge of callus at the ventral side of the shell close to the aperture) and have rounded, tapering ends, character states that are absent in fully-grown *C. gibbosum*. Identification of juvenile ovulid shells is difficult, if not impossible, due to a lack of diagnostic shell characters. However, some specimens that were collected alive showed a mantle colour pattern diagnostic for *C. gibbosum*, viz. bright orange spots, encircled with a black line at a whitish background (Fig. 1). *Cymbovula acicularis* had a nearly transparent mantle, sometimes with white protuberances. In *Simnialena uniplicata* the mantle is entirely black, whereas in *Cyphoma signatum* it had a distinct yellow/black fingerprint pattern. This leads to the conclusion that the mantle colour and pattern may be diagnostic in Ovulidae (Mase, 1989) and that this character can be used to distinguish fully grown *Simnialena uniplicata* from juvenile *C. gibbosum*. Therefore, we hypothesize that *S. uniplicata* exemplifies paedomorphosis.

#### *Systematics, biogeography and nomenclature*

According to the principles of phylogenetic systematics, the species referred to as *Simnialena uniplicata* should be called *Cyphoma uniplicata* (Fig. 3). Meanwhile, the status of the nominal genus *Simnialena* Cate, 1973, with its insufficiently known type species *Simnialena marferula* Cate, 1973, remains unclear. According to Lorenz and Fehse (2009: 105), '*S. marferula* is a close relative of *S. uniplicata*'. This conclusion, on which we cannot elaborate here, is based on similarities in shell morphology. The DNA sequences of specimens belonging to *Cymbovula acicularis* and specimens that agree with the description of *C. bahamaensis* (Figs 2c-d) are almost identical. As a consequence, these nominal taxa should most probably be considered synonyms, as has also been suggested by Lorenz and Fehse (2009) based on morphological data. There is a moderately strong supported dichotomy between the Atlantic and the Indo-Pacific taxa, with *Neosimnia arcuata* from the East-Pacific having an aberrant, but poorly supported, position in the cladogram, where it clusters with the Atlantic taxa. Together, these species represent the Simniinae Schilder, 1927. Furthermore, two undisputed clades were found among the Indo-Pacific taxa, supporting the occurrence of the subfamilies Prionovolvinae Fehse, 2007, and Ovulinae Fleming, 1822, respectively. In order to get a better understanding of the phylogeny and parasite/host associations of the Atlantic Ovulidae, additional shells and DNA material are needed. DNA obtained from other ovulids occurring in the Atlantic area (e.g. *Cyphoma macumba* Petuch, 1979; *C. versicolor* Fehse, 2003; *C. mcgintyi* Pilsbry, 1939) may elucidate the taxonomical position of the genus *Cyphoma* as a monophyletic group. However, several ovulid species are rare and generally only their shells are found, which hampers further investigations.

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