Phylogenetic ecology of octocoral - gastropod associations

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Chapter 2

Coat of many colours - DNA reveals polymorphism of mantle patterns and colouration in Caribbean *Cyphoma* Röding, 1798

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Abstract

The associated fauna of corals form a major part of the biodiversity on reefs, and many taxa live in close association with a host organism. In the Caribbean the enigmatic gastropod genus *Cyphoma* is commonly observed, where it lives in association with a range of gorgonian hosts. Each species in the genus *Cyphoma* has a unique, characteristic mantle pattern and colouration, which easily separates the nominal species. Because of its abundance and recognisability *C. gibbosum* has been used as a model organism in several studies concerning allelochemicals, reef degradation, and physical defence mechanisms. Molecular analyses based on four molecular markers (COI, 16S, H3 and 28S) on four *Cyphoma* species (*C. gibbosum*, *C. mcgintyi*, *C. signatum* and an undescribed black morph) from three localities throughout the Caribbean show that they actually represent morphological varieties of a single, genetically homogeneous species. As a result *C. mcgintyi* and *C. signatum* are synonymised with the type species *C. gibbosum*. The striking morphological differences in mantle pattern and colouration are hypothesised to be the effect of an evolutionary process. Three possible scenarios for the observed morphological variety are discussed: rapid diversification, supergenes or discontinuous variation.

Introduction

Biodiversity on reefs is dominated by highly diverse invertebrate taxa that are understudied and incompletely described (Reaka-Kudla, 1997). Many of these taxa live in association with corals on which they rely for food, habitat and/or settlement cues. Arthropods are the most numerous associated taxa on [stony] corals, followed by molluscs (Stella *et al*., 2011). Goh *et al*. (1999) reported on 30 species (belonging to 17 families) living in association with gorgonians in Singapore. For the molluscs, the gorgonian associated fauna included bivalves (e.g. *Pteria*), nudibranchs (e.g. *Phyllodesmium*, *Tritonia*), and gastropods (Ovulidae). The widespread family Ovulidae occurs in all temperate and tropical oceans and all but one species (*Volva volva* (Linnaeus, 1758)) live intrinsically associated on Octocorallia and Antipatharia (Cate, 1973; Lorenz and Fehse, 2009). Ovulid snails roam the branches of their host corals and feed on the polyps and tissue. This feeding behaviour can leave big feeding scars on their hosts (Gerhart, 1990). Ovulids have a mantle, which can cover their entire shell; the different colours, patterns and appendices provide camouflage for their host or, reversely, advertise their toxicity with conspicuous, aposematic mantle patterns and colourations (Rosenberg, 1992).

The best-known Atlantic ovulid species *Cyphoma gibbosum* (Linnaeus, 1758) is better known under its vernacular name 'Flamingo tongue'. These large, brightly coloured snails can easily be detected on various gorgonian species throughout the Caribbean (Simone, 2004; Lorenz and Fehse, 2009; Reijnen *et al.,* 2010; Humann *et al*., 2013). *Cyphoma gibbosum* is an easy to recognize species that can locally occur in high densities (Chiappone *et al.,* 2013), and is therefore often used as a model organism. It has been used in studies dealing with allelochemicals and physical defence systems (Van Alstyne and Paul, 1992; Vrolijk and Targett, 1992; Wahlen *et al.,* 2010), studies on their association with fungal diseases in Caribbean gorgonians (Rypien and Baker, 2009) and research on reef degradation and predation (Gerhart, 1990; Burkepile and Hay, 2007; Evans *et al.,* 2013). The appeal of *C. gibbosum* as a model organism or "marine lab rat" is not shared by the other nominal *Cyphoma* species such as *C. signatum* Pilsbry and McGinty, 1939 or *C. mcgintyi* Pilsbry, 1939.

The genus *Cyphoma* has 14 extant species recognised by Lorenz and Fehse (2009) and 13-15 extant species according to Rosenberg (2015). Most *Cyphoma* species are considered to be relatively rare (Lorenz and Fehse 2009) and as a result there are fewer studies on these nominal *Cyphoma* species. Botero (1990), Ruesink and Harvell (1990), and Reijnen *et al*. (2010) studied the host species of *C. signatum*, whereas Ghiselin and

Fig. 1. Sequenced nominal *Cyphoma* species showing their different mantle patterns and colour varieties. a) *Cyphoma gibbosum* on *Pseudoplexaura* sp. b) *C. gibbosum* on *Pseudoplexaura* sp. c) *C. gibbosum* with atypical mantle pattern (only dots around mantle edges) on *Briareum asbestinum* d) *C.* cf. *allenae* on an *Antillogorgia americana* e) *C. signatum* on *Plexaurella dichotoma* f) Juvenile *C. signatum* on *Gorgonia ventalina* g) *Cyphoma* sp. (black morph) on *Eunicea tourneforti* h) *C. mcgintyi* from Florida, USA. Photos: a-g) B.T. Reijnen, all from Curaçao; h) Florida Museum of Natural History. ▶


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Wilson (1966) studied the anatomy, natural history and reproduction of this species. Apart from the before mentioned studies there are no records of *Cyphoma* species, other than *C. gibbosum*, in scientific literature. The most recent addition to the genus is *Cyphoma eludens* Lorenz and Braun, 2015. This species was described from St. Helena and the Canary Islands in the Atlantic. Two *Cyphoma* species are not found in the Atlantic Ocean but instead have an East Pacific distribution, namely *C. emarginata* (Sowerby I, 1830) and *C. arturi* Fehse, 2006. All other *Cyphoma* occur in the Atlantic on shallow reefs (intertidal) and in deep water (1200 m), from Florida to southern Brazil, and from the Caribbean to the Canary Islands and St. Helena (Lorenz and Fehse, 2009; Humann *et al.*, 2013).

The majority of the *Cyphoma* species can easily be identified *in situ* with the help of the characteristic patterns and colouration of their mantle, which are considered species specific in Ovulidae (Mase, 1989; Fig. 1). In contrast, interpretation of the morphological anatomical features in *Cyphoma* can be troublesome (Simone, 2004). This is supported by Lorenz and Fehse (2009: 90) who state: “Some of the species of this genus are difficult to identify with only the shells at hand” and “Most species are characterized mainly by the colour pattern of the mantle lobes and the foot”. For example, *C. signatum* and *C. mcgintyi* can easily be differentiated based on their respective colour patterns (fingerprint pattern vs. black dots), but based on just shell morphological features these species can hardly be distinguished. There are also exceptions where shell morphological features are clear in separating species, for example between *C. gibbosum* and *C. signatum*. The differences in shell outline (oval vs. rhomboid) and shell colour (often orange in *C. gibbosum*) easily separate the two (Fig. 1).

Besides the typical species-specific mantle patterns some unusual *Cyphoma* morphotypes have been recorded (e.g. Lorenz and Fehse, 2009: A202-204; Humann *et al.*, 2013: 175). Because of their unusual appearance and apparent rarity these morphotypes have not yet been identified to species level, or formally described as separate species, and so far remain mysterious members of the genus *Cyphoma*. Contrasting features between nominal species identifications and morphological features such as colour patterns are not uncommon. Examples are not just from the marine realm, but also from terrestrial and aquatic environments e.g. *Caridina* fresh water shrimps and *Tylomelania* fresh water snails (Rintelen *et al.*, 2004, 2007), *Heliconius* butterflies (Joron *et al.*, 2011) and *Cepea* land snails (Richards *et al.*, 2013).

To investigate the morphological difference in shell shape, mantle patterns and colouration in *Cyphoma* spp. more closely, we re-used data obtained for a previous study on *Cyphoma* (Reijnen *et al.*, 2010) and supplemented this dataset with an additional 26 specimens belonging to three ovulid species and one unidentified morphotype. Two additional markers were supplemented to the previous data set. Here we show the results of phylogenetic analyses based on four molecular markers (COI, 16S mtDNA; 28S and H3 nDNA) tested on three nominal *Cyphoma* species and one undescribed colour variety (Fig. 2g), as well as three temperate Atlantic representatives of the subfamily Simniinae (*Cymbovula acicularis*, *Neosimnia spelta*, *Simnia patula*).

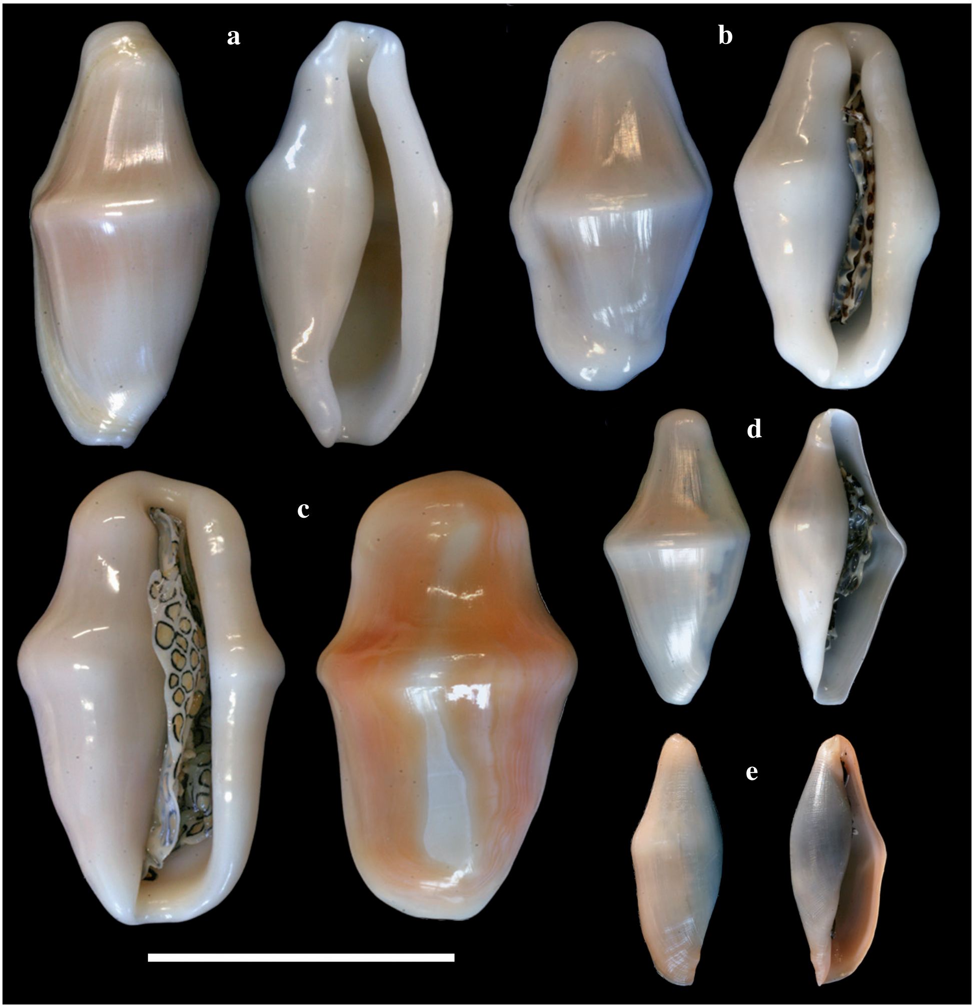

Fig. 2. Dorsal and ventral views of the shells from nominal species in this study. a) *Cyphoma signatum* (RMNH.Mol.100828) b) *C. mcgintyi* (UF.446893a) c) *C. gibbosum* (UF.446879) d) *C. mcgintyi* (UF.446893b; juvenile) e) *C.* sp. (black morph) (RMNH.Mol.337800).

Material and methods

Collecting

Cyphoma specimens and their host corals were collected during fieldwork at the leeward side of Curaçao (Dutch Caribbean) in 2005 and 2013, and from St. Eustatius in 2015 (Fig. 3).

When possible, *in situ* photographs were made to document the mantle patterns and colouration. Subsamples were taken from the host corals for their identification based on sclerite morphology. All specimens were preserved in 80% ethanol and deposited in

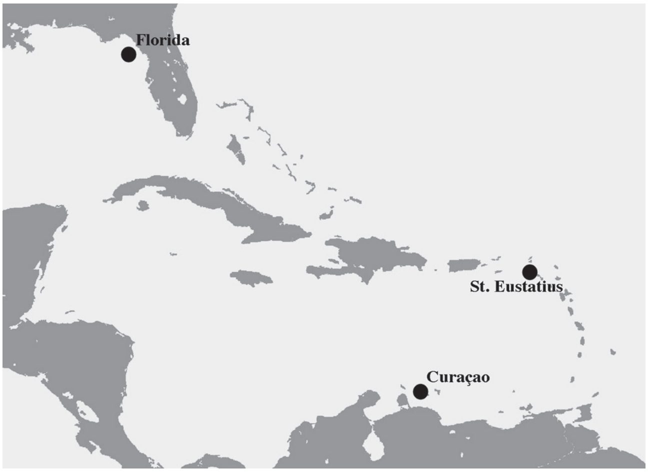

Fig. 3. Sites from which the *Cyphoma* spp. and other ovulids were collected in the Caribbean.

the mollusc collection of Naturalis Biodiversty Center, Leiden, The Netherlands (collection coded as RMNH.Mol). Three samples of *Cyphoma mcgintyi* and one additional sample of *C. gibbosum* were obtained from the Florida Museum of Natural History (FLMH; Suppl. Mat. 1). Identifications of the snails were based on Kaicher (1991), Fehse (2003), Lorenz and Fehse (2009) and Humann *et al*., (2013), their octocoral hosts were identified with the help of Bayer (1961).

Molecular analyses

Soft tissue from the foot or mantle was used for DNA extractions. Samples were either extracted individually with the DNAeasy Blood & Tissue kit, or as a part of the 'barcoding initiative' at Naturalis Biodiversity Center with the Machery-Nagel DNA extraction kit on a KingFisher Flex extraction robot. Extraction was performed according to the respective protocols, except for the lysis times, which were performed overnight (approx. 17 h.) and the final elution volume that was decreased to 100 μL and 150 μL respectively. Before PCR amplification, extracts were diluted 100 to 300 times to lower the ratio of inhibitors versus DNA. Each PCR reaction contained 2.5 μl CoralLoad PCR buffer, 0.5 μl dNTP's, 1.0 μl for each primer (Table 2), 0.3 μl Taq polymerase, 18.7 μl PCR water and 1.0 μl template. For the 28S marker, 5 μl of PCR water was replaced with 5.0 μl Q-solution. Each PCR program consisted of initial denaturation for 3 min at 95° C, followed by 39 cycles of 10 sec 95° C, specific annealing temperature (Table 1) for 1 min, with an extension of 1 min. A final extension of 10 min was used as a final step in the PCR programme. PCR amplification was performed on a C1000 Touch Thermal Cycler (Bio-RAD). Sequencing of the PCR products was performed at either Macrogen Europe or at BaseClear on an ABI Automated Sequencer 3730xl capillary sequencer. Sequences were edited in Sequencher 4.10.1.

All novel sequences are uploaded to GenBank, accession numbers: KT372440 – KT372515. Additional sequences of Caribbean ovulids (Reijnen *et al*., 2010) were downloaded from GenBank (Suppl. Mat. 1) and aligned on the GUIDANCE server

Table 1. Primer information of the markers used in this study, including annealing temperatures, sequenced regions and fragment sizes.

Primer names	Primer sequence	Region	Annealing T	Fragment size (bp)	Reference
H3F	ATGGCTCGTACCAAGCAGACVGC	Histone H3 (nuclear)	50	~ 380	Colgan *et al*., 2000
H3R	ATATCCTTRGGCATRATRGTGAC	Histone H3 (nuclear)	50	~ 380	Colgan *et al*., 2000
LSU5	TAGGTCGACCCGCTGAAYTTAAGCA	28S (nuclear)	50	~ 800	Littlewood *et al*., 2000
LSU 800rc	GACTCCTTGGTCCGTGTTTC	28S (nuclear)	50	~ 800	Reijnen, subm.
16Sar	CGCCTGTTTATCAAAAACAT	16S (mitochondrial)	52	~ 540	Palumbi *et al*., 1996
16Sbr	CCGGTCTGAACTCAGATCACGT	16S (mitochondrial)	52	~ 540	Palumbi *et al*., 1996
LCO-1490	GGTCAACAAATCATAAAGATATTGG	COI chondrial)	50	~ 660	Folmer *et al*., 1994
HCO-2198	TAAACTTCAGGGTGACCAAAAATCA	COI (mitochondrial)	50	~ 660	Folmer *et al*., 1994

Table 2. Genetic variation (%) of Atlantic Ovulidae between and within nominal *Cyphoma* species groups.

Between groups (no. of specimens)	1	2	3	4	6	7	8	9	Within groups	
1. *Cyphoma gibbosum* (n=19)									*Cyphoma gibbosum*	0.1
2. *Cyphoma* sp. (n=2)	0.1								*Cyphoma* sp.	0.1
3. *Cyphoma signatum* (n=6)	0.2	0.2							*Cyphoma signatum*	0.2
4. *Cyphoma*. *mcgintyi* (n=3)	0.3	0.4	0.3						*Cyphoma mcgintyi*	0.2
5. *Simnialena uniplicata* (n=1)	0.3	0.4	0.2	0.6					*Simnialena uniplicata*	-
6. *Cymbovula acicularis* (n=12)	6.2	6.6	6.7	7.1	7.8				*Cymbovula acicularis*	0.2
7. *Neosimnia spelta* (n=1)	6.5	7.1	6.7	7.7	7.3	7.4			*Neosimnia spelta*	-
8. *Simnia patula* (n=1)	7.7	8.7	8.1	9.5	8.9	9.2	7.6		*Simnia patula*	-
9. Outgroup (n=1)	9.3	9.6	10.3	10.1	11.6	10.2	12.0	12.7	Outgroup	-

(Penn *et al*., 2010) using the MAFFT algorithm (alignment score: 0.792612). Gene regions that could not be amplified for certain specimens were replaced by N's in the final alignment.

The final alignment contains 46 specimens and the concatenated dataset is 2,355 base pairs in length including insertions and/or deletions. The Indo-Pacific species *Ovula ovum* (Linnaeus, 1758) was selected as outgroup. The dataset of the individual markers were subjected to the model-testing algorithm in jModeltest and MEGA6 (Tamura *et al*., 2013), and were based on the uncorrected Akaike Information Criterion (AIC). Bayesian analyses were performed in MrBayes 3.2.0 (Ronquist and Huelsenbeck, 2003) and were run for 4,000,000 generations with six chains. Data was sampled every 100 generations. The final split frequency between the two independent runs was < 0.01. Garli2.0 (Zwickl, 2006) was used to determine the phylogenetic relationships based on the maximum likelihood approach. The phylogeny was reproduced using 1,000 bootstrap iterations. Species delimitation was tested with an Automatic Barcode Gap Discovery tool (ABGD; Puillandre *et al*., 2011). Default settings were used and analysis was performed with the Jukes-Cantor (JC69) algorithm. Genetic distances were calculated in MEGA6.

Results

The phylogram (Fig. 4) shows three groups containing from top to bottom: 1) *Cyphoma* spp. including *Cyphoma* sp. (RMNH.Mol.100770) which was formerly identified as *Simnialena uniplicata* (Reijnen *et al*., 2010), 2) *Cymbovula acicularis*, 3) *Neosimnia spelta* and *Simnia patula*. All groups are well supported by the Bayesian and maximum-likelihood analysis analyses. Phylogenetic relationships between *Cymbovula acicularis* and the group containing *Neosimnia spelta* and *Simnia patula* have low support values (57/87). Within the clade containing the nominal *Cyphoma* species there is no clustering observed concordant with the respective species identifications (*C. gibbosum*, *C. signatum*, *C. mcgintyi*, *Cyphoma* sp. black morph). There is however a small cluster of specimens that is highly supported (95/85), which contains all three *C. mcgintyi* specimens and one representative of *C. signatum*, but the branch lengths are short.

In the alignment only five nucleotide sites out of 2,347 positions support the grouping of these four specimens. One of these nucleotide sites is within the non-coding 16S region, while the other four are situated in the coding COI region. Each of these four nucleotide sites are on the third codon position of the protein translation and hence do not change the translation of the protein coding alignment when compared with the other *Cyphoma* spp. All other nominal *Cyphoma* species are distributed randomly throughout the group and do not show phylogenetic affinities based on morphological characters.

To investigate the observed random positioning of the nominal *Cyphoma* species in more detail, the genetic distances between and within the nominal *Cyphoma* species were calculated (Table 2). Genetic distance values within species were almost as low as between *Cyphoma* species (within range: 0.1-0.2%; between range: 0.1-0.4%). When distances values were calculated between *Cyphoma* spp. and *Cymbovula acicularis*,

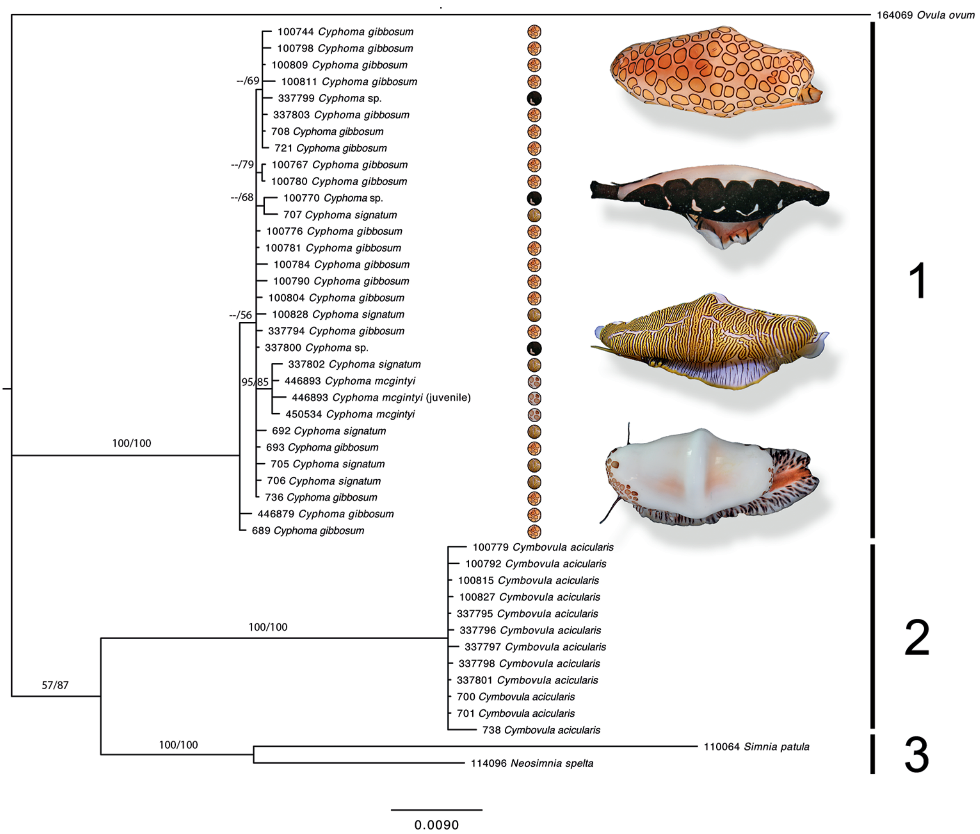

Fig. 4. Phylogram based on the Bayesian consensus tree showing Bayesian inference support values (left) and Maximum likelihood bootstrap support values (right). For the *Cyphoma* species their respective characteristic mantle patterns are depicted per specimen including photographs of the live animals (not to scale).

Simnia patula or *Neosimnia spelta* genetic distance values were notably higher (0.1-0.4% between *Cyphoma* spp. vs. 6.5-8.1% between *Cyphoma* spp. or *Cymbovula acicularis*, *S. patula* and *N. spelta*).

To test whether the phenotypic differences in colours and patterns comprise different nominal *Cyphoma* species, an ABGD species delimitation analysis was performed. This test resulted in five groupings: 1) *Cyphoma gibbosum*, *C. mcgintyi*, *C. signatum*, *Cyphoma* sp. black morph, *Simnialena uniplicata* 2) *Cymbovula acicularis* 3) *Neosimnia spelta* 4) *Simnia patula* 5) *Ovula ovum*. The ABGD results are therefore congruent with the results from the phylogenetic analyses and do not differentiate the nominal *Cyphoma* species (with their unique mantle patterns and colouration) in separate species groups.

The earlier identification of *Simnialena uniplicata* in Reijnen *et al.*, (2010) most likely constitutes a misidentification. In the phylogenetic analyses *S. uniplicata* clusters with all other *Cyphoma* species, without significant genetic difference between these specimens. Clear diagnostic characters are missing, because of the juvenile state of this particular specimen. Based on the photograph in Reijnen *et al*. (2010: Fig. 1e), a similar phenotype as *Cyphoma* sp. black morph is apparent and therefore this specimen is now identified as such.

Discussion

Mantle colouration and mantle patterns in Ovulidae were long thought to be diagnostic species characters and were used as such by several authors (Mase, 1989; Reijnen *et al*., 2010; Lorenz and Fehse, 2012; Lorenz and Brown, 2015). Mase (1989) did not only look at the shell and mantle, but also patterns and colours on the foot, antenna and siphon of Japanese ovulids. The very distinct and recognisable mantle patterns such as the yellow/black fingerprint pattern in *C. signatum* and the orange coloured dots in *C. gibbosum* have so far been used to separate these species. Nevertheless, the morphological characters (mantle patterns, mantle colouration, shell morphological features) in *Cyphoma* do not correspond with the observed genetic results. The genetic distance values between nominal *Cyphoma* species (Table 2) are comparable with the genetic variation found in Indo-Pacific *Crenavolva* (Reijnen, 2015). In that specific case *Crenavola chiapponii* was synonymised with *C. aureola* based on genetic data and morphological similarity. The discrepancy between the morphological data and the molecular results in this study are difficult to reconcile. However, various scenarios can explain the findings presented here. Possible hypotheses for example are: rapid diversification, supergenes or discontinuous variation.

In a scenario of rapid divergence, trophic specialisation is frequently a key feature that characterises sister species (Vailant *et al*., 2011). Such trophic specialisation is not known in the *C. gibbosum* “complex”. Nominal *Cyphoma gibbosum* is a generalist predator that has been found associated with at least 21 different host species from at least nine different genera (Reijnen *et al*., 2010). Morphotypes resembling nominal *Cyphoma signatum* are uncommon on most reefs and as a result ecological data is rare for this species. Most specimens are found on the genus *Plexaurella*, yet a juvenile resembling *C. signatum* was observed on *Gorgonia ventalina* (Fig. 1f) suggesting that *C. signatum* is a less species-specific feeder on octocorals than previously assumed.

The second scenario is that the phenotypic diversity in *Cyphoma gibbosum* is regulated by a supergene. A supergene consists of multiple strongly linked loci that determine the phenotype, without difference in the studied molecular markers (this study 28S, 16S, H3, COI) (Joron *et al*., 2006, 2011). The typical *Cyphoma gibbosum* orange-spotted-morph would be the general phenotype and rare phenotypes, such as the yellow fingerprint pattern in *C. signatum*, the less common morph (Cook, 2005). In case of the shell morphological features it is more difficult to reconcile the data. Reijnen (2015) showed that in Ovulidae minor shell morphological characters used for separating nominal species should be considered a morphological variety within a single species. The presence of different morphotypes in a species is not unique within the family Ovulidae. Schiaparelli *et al*. (2005) recognised different morphotypes for one Atlantic/

Mediterranean and four Indo-Pacific species (*Neosimnia spelta*, *Pellasimnia brunneiterma*, *Dentiovula dorsuosa*, *Diminovula punctata* and *Habuprionovolva aenigma*, respectively) and could not discriminate between the morphs based on 16S molecular data.

A third hypothesis is that *Cyphoma gibbosum* is "caught in speciation" which is reflected by the discontinuous variation in morphology, but (not yet) in the studied genes. This hypothesis is supported by the idea that "phenotype precedes genotype" is a common mode of evolution (Palmer, 2004). A similar case was observed in the shrimp species *Conchodytes meleagrinae* (Fransen and Reijnen, 2013). Shrimp specimens from different bivalve hosts showed very dissimilar colour patterns and were thought to be distinct species. Molecular analyses showed that based on their genetic barcodes these species could not be distinguished from each other. It was therefore hypothesised that this species recently speciated.

It is unclear which of the proposed hypotheses is the most parsimonious and fits the observed results for the genus *Cyphoma* the best. What should be considered is that morphological features in the Ovulidae are probably more plastic than previously thought (Schiaparelli *et al.*, 2005; Reijnen, 2015). The closely related cowrie family Cypraeidae shows a contrasting pattern where multiple cryptic lineages have been uncovered by genetics (Meyer, 2003; Moretzsohn, 2014), as is the case in many other taxa (Bickford *et al.*, 2007). Reports of distinct morphospecies attributed to a single, genetically homogeneous species are available, but far less common. Several reports are however available, for example: polychaetes (Willette *et al.*, 2015, and references therein), sea stars (Harley *et al.*, 2006), and caridean shrimps (Bauer, 2004).

Taxonomic account

Resulting from the molecular results and species delimitation analysis as presented before, *Cyphoma signatum* and *C. mcgintyi* need to be synonymised with *Cyphoma gibbosum*. The synonymy of this species is as follows:

Family Ovulidae Fleming, 1822
Genus *Cyphoma* Röding, 1798
Cyphoma gibbosum (Linnaeus, 1758)

Bulla gibbosa Linnaeus, 1758: 726
? *Cyphoma dorsatum* Röding, 1798: 21
? *Ovula pharetra* G. Perry, 1811: pl. 53, fig. 2
? *Ovula rostrata* Mörch, 1877: 53
? *Cyphoma precursor* Dall, 1897
Cyphoma alleneae Cate, 1973: 67-68, figs 151, 151c
Cyphoma signata Pilsbry & McGintyi, 1939: 3, pl. 1, fig. 1, 1a, 2, 2a, 9, 10
Cyphoma mcgintyi Pilsbry, 1939: 108
? *Cyphoma macumba* Petuch, 1979: 515-517, figs. 1c-d, 2b-c
Simnialena uniplicata. — Reijnen *et al.*, 2010: fig. 1e, 2f-g

Remarks: Ghiselin and Wilson (1966) already mentioned that there are no striking morphological differences between *C. gibbosum* and *C. signatum* when it comes to the external morphology and mantle cavity. The radular morphology of *C. gibbosum* and other Atlantic ovulids was studied by Bandel (1984) and Simone (2004) and both concluded that radular morphology does not differ significantly between ovulid species. Reid (2000) warns about using radular morphology as a morphological character, because of ecophenotypic plasticity, convergence and intraspecific variation.

Simone (2004: 88) included *C. allenae* in the synonymy of *C. gibbosum*, albeit without further discussion. Additionally, he discussed the taxonomy and systematics of other *Cyphoma* species such as *C. intermedium*, *C. macumba* and *C. signatum*. According to Rosenberg (1996, in Simone 2004) and Simone (2004) *C. macumba* is a possible synonym of *C. signatum*. Simone (2004) investigated the type species and did not observe clear morphological differences based on the shells alone. Nevertheless, Lorenz and Fehse (2009) separated the species based on their mantle features and a minor shell morphological feature (callus-denticles on the outer labrum). Here we provisionally follow Simone's (2004) suggestion that *C. macumba* is a synonym of *C. signatum*, and hence *C. gibbosum*. Rosenberg (2009) includes the following synonymies of *C. gibbosum*: *Cyphoma dorsatum* Röding, 1798, *Ovula pharetra* G. Perry, 1811, *Ovula rostrata* Mörch, 1877, and *Cyphoma precursor* Dall, 1897. We provisionally included these synonymies here as well.

Simone (2004) also investigated the anatomy of *Cyphoma intermedium* (Sowerby I, 1828). The discussion of this species starts with the statement that this species has a highly variable shell and that it closely resembles some more recently described *Cyphoma* species (*C. kathiewayae*, *C. guerrini*, *C. macumba*, *C. rosenbergi*). According to Simone these species all fit in the range of the two most extreme phenotypes of *C. intermedium* and are therefore likely synonyms of the latter.

Variability of morphological characters, in combination with molecular data, should be taken into account in future research on Ovulidae. Unnecessary profusion of species names and other taxonomical problems can be avoided by assessing both morphological and molecular data. It is very likely that more synonymies, rather than species, need to be uncovered in the taxonomy of the Ovulidae.

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Suppl. Mat. 1. Species information and GenBank accession codes including references, an asterisk marks GenBank accession numbers which were previously misidentified in Reijnen *et al*., 2010.

Species	Code	Location (locality code)	Longitude / Latitude	Depth (m)
Cymbovula acicularis (Lamarck, 1810)	RMNH.Mol. 100779	Curaçao, Barank’i Karanito, CUR.15	12°02’13.5”N 68°48’14.2”W	25
Cymbovula acicularis (Lamarck, 1810)	RMNH.Mol.100792	Curaçao, Daaibooi, CUR.19	12°12’42.9”N 69°05’0°5.4”W	6
Cymbovula acicularis (Lamarck, 1810)	RMNH.Mol.100815	Curaçao, Caracasbaai, CUR.17	12°04’25.8”N 68°51’46.5”W	17
Cymbovula acicularis (Lamarck, 1810)	RMNH.Mol.337795	Curaçao, Blauwbaai, CAO.04	12°08’05.7”N 68°59’03.5”W	10
Cymbovula acicularis (Lamarck, 1810)	RMNH.Mol.337796	Curaçao, St. Marie, CAO.06	12°11’53.0”N 69°04’45.1”W	14
Cymbovula acicularis (Lamarck, 1810)	RMNH.Mol.337801	Curaçao, Marie Pampoen, CAO.21	12°05’26.7”N 68°54’17.8”W	10
Cymbovula acicularis (Lamarck, 1810)	RMNH.5004206	St. Eustatius, The Blocks, EUX015	17°27’50.9”N 62°59’06.8”W	-
Cymbovula acicularis (Lamarck, 1810)	RMNH.5004207	St. Eustatius, The Blocks, EUX015	17°27’50.9”N 62°59’06.8”W	-
Cymbovula acicularis (Lamarck, 1810)	RMNH.5004208	St. Eustatius, Blind Shoal, EUX039	17°30’37.1”N 63°00’27.1”W	-
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.Mol.100767	Curaçao, Blauwbaai, CUR.10	12°22’29.9”N 68°59’27.7”W	8
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.Mol.100776	Curaçao, Playa Jeremy, CUR.12	12°19’44.1”N 69°09’00.2”W	17
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.Mol.100780	Curaçao, Barank’i Karanito, CUR.15	12°02’13.5”N 68°48’14.2”W	10
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.Mol.100781	Curaçao, Barank’i Karanito, CUR.15	12°02’13.5”N 68°48’14.2”W	9
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.Mol.100804	Curaçao, Sint Michielsbaai, CUR.21	12°08’50.9”N 68°59’56.6”W	14
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.Mol.100809	Curaçao, Superior Producer, CUR.22	12°05’21.5”N 68°56’35.5”W	18
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.Mol.100811	Curaçao, Superior Producer, CUR.22	12°05’21.5”N 68°56’35.5”W	5
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.Mol.337794	Curaçao, Waterfabriek I, CAO.02	12°06’31.0”N 68°57’01.2”W	-
Cyphoma gibbosum (Linnaeus, 1758)	UF.446879	USA, Florida, N of St. Petersburg	28°35’55.7”N 84°15’41.4”W	27
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.5004209	St. Eustatius, Aquarium, EUX012	17°30’22.6”N 63°00’22.0”W	-
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.5004210	St. Eustatius, Aquarium, EUX012	17°30’22.6”N 63°00’22.0”W	-
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.5004211	St. Eustatius, Blairs Reef, EUX019	17°28’13.6”N 62°59’30.2”W	-
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.5004212	St. Eustatius, Twelve Guns, EUX029	17°28’12.8”N 62°58’58.7”W	-
Cyphoma gibbosum (Linnaeus, 1758)	RMNH.5004213	St. Eustatius, Blue Bead Hole II, EUX037	17°28’37.4”N 62°59’29.6”W	-
Cyphoma mcgintyi (Pilsbry, 1939)	UF.446893a	USA, Florida, N of St. Petersburg	28°32’16.1”N 84°16’21.7”W	26

Host organism	COI	16S	28S	H3	Reference
Antillogorgia acerosa (Pallas, 1766)	GU363447 *	GU363434	-	KT372494	Reijnen *et al.*, 2010; this publication
Gorgonia ventalina (Linnaeus, 1758)	GU363448	GU363436	KT372474	KT372497	Reijnen *et al.*, 2010; this publication
Antillogorgia bipinnata (Verrill, 1864)	GU363449	GU363437	-	KT372501	Reijnen *et al.*, 2010; this publication
Gorgonia ventalina (Linnaeus, 1758)	KT372445	KT372460	KT372481	KT372507	this publication
Antillogorgia acerosa (Pallas, 1766)	KT372446	KT372461	KT372482	KT372508	this publication
Antillogorgia acerosa (Pallas, 1766)	KT372449	KT372464	KT372485	KT372511	this publication
-	KX360175	KX360216	KX360199	KX360187	this publication
-	KX360176	KX360217	KX360200	KX360188	this publication
Antillogorgia	KX360177	KX360218	KX360202	KX360189	this publication
Antillogorgia acerosa (Pallas, 1766)	KT372440	-	KT372470	KT372491	this publication
Briareum asbestinum (Pallas, 1766)	-	KT372455	KT372471	KT372493	this publication
Gorgonia ventalina (Linnaeus, 1758)	GU363440	GU363428	KT372472	KT372495	Reijnen *et al.*, 2010; this publication
Plexaurella nutans (Duchassaing & Michelotti, 1860)	GU363444	GU363432	KT372473	KT372496	Reijnen *et al.*, 2010; this publication
Pseudoplexaura porosa (Houttuyn, 1772)	GU363443	GU363431	KT372475	KT372498	Reijnen *et al.*, 2010; this publication
Antillogorgia americana (Gmelin, 1791)	GU363446 *	GU363433	KT372476	KT372499	Reijnen *et al.*, 2010; this publication
Muricea muricata (Pallas, 1766)	GU363441	GU363429	KT372477	KT372500	Reijnen *et al.*, 2010; this publication
Eunicea sp.	KT337794	KT372459	KT372480	KT372506	this publication
unknown	KT372451	KT372466	KT372487	KT372513	this publication
-	KX360170	KX360209	KX360192	KX360180	this publication
-	-	KX360211	KX360194	KX360181	this publication
Plexaura nina/homomalla	KX360172	KX360213	KX360196	KX360183	this publication
-	-	KX360206	-	KX360179	this publication
-	KX360171	KX360212	KX360195	KX360182	this publication
unknown	KT372452	KT372467	KT372488	KT372514	this publication

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Species	Code	Location (locality code)	Longitude / Latitude	Depth (m)
Cyphoma mcgintyi (Pilsbry, 1939)	UF.446893b	USA, Florida, N of St. Petersburg	28°32’16.1”N 84°16’21.7”W	26
Cyphoma mcgintyi (Pilsbry, 1939)	UF.450534	USA, Florida, NNW of St. Petersburg, S of Big Bend area	28°39’04.0”N 84°23’03.8”W	26-30
Cyphoma signatum Pilsbry & McGinty, 1939	RMNH.Mol.100828	Curaçao, Marie Pampoen /Carpile, CUR.05	12°05’42.1”N 68°54’43.0”W	5
Cyphoma signatum Pilsbry & McGinty, 1939	RMNH.Mol.337802	Curaçao, Marie Pampoen, CAO.21	12°05’26.7”N 68°54’17.8”W	8
Cyphoma signatum Pilsbry & McGinty, 1939	RMNH.5004214	St. Eustatius, Aquarium, EUX012	17°30’22.6”N 63°00’22.0”W	-
Cyphoma signatum Pilsbry & McGinty, 1939	RMNH.5004215	St. Eustatius, Shark Reef, EUX018	17°30’37.1”N 63°00’27.1”W	-
Cyphoma signatum Pilsbry & McGinty, 1939	RMNH.5004216	St. Eustatius, Blairs Reef, EUX019	17°28’13.6”N 62°59’30.2”W	
Cyphoma signatum Pilsbry & McGinty, 1939	RMNH.5004217	St. Eustatius, Shark Reef, EUX018	17°30’37.1”N 63°00’27.1”W	-
Cyphoma sp.	RMNH.Mol.337799	Curaçao, Holiday Beach, CAO.11	12°06’34.1”N 68°56’49.3”W	4
Cyphoma sp.	RMNH.Mol.337800	Curaçao, Holiday Beach, CAO.11	12°06’34.1”N 68°56’49.3”W	4
Neosimnia spelta (Linnaeus, 1758)	RMNH.Mol.114096	Spain, Begur, Aigua Blava	41°56’08,0”N 03°13’04,9”E	<15
Ovula ovum (Linnaeus, 1758)	RMNH.Mol.164069	Malaysia, Borneo, Kapalai Island, SEM.10	04°13’04,8”N 118°40’20,1”E	3
Simnia patula (Penant, 1777)	RMNH.Mol.110064	North Sea, S side of Doggersbank	54°20’N 02°20’E	-
Simnialena uniplicata (Sowerby II, 1848)	RMNH.Mol.100770	Curaçao, Marie Pampoen/Carpile, CUR.05	12°05’42.1”N 68°54’43.0”W	18

Host organism	COI	16S	28S	H3	Reference
unknown	KT372453	KT372468	KT372489	KT372515	this publication
unknown	KT372454	KT372469	KT372490	-	this publication
Plexaurella dichotoma (Esper, 1791)	KT372441	KT372456	KT372478	KT372502	this publication
Gorgonia ventalina (Linnaeus, 1758)	KT372450	KT372465	KT372486	KT372512	this publication
-	KX360173	KX360214	KX360197	KX360184	this publication
Plexaurella nutans	-	KX360204	-	-	this publication
Plexaurella dichotoma	-	KX360210	KX360193	-	this publication
Plexaurella nutans	KX360169	KX360205	-	KX360178	this publication
Eunicea tourneforti Milne Edwards & Haime, 1857	KT372447	KT372462	KT372483	KT372509	this publication
Eunicea tourneforti Milne Edwards & Haime, 1858	KT372448	KT372463	KT372484	KT372510	this publication
Leptogorgia sarmentosa (Esper, 1789)	KT372442	KT372457	-	KT372504	this publication
Sarcophyton glaucum (Quoy & Gaimard, 1833)	KT372443	KT372458	KT372479	KT372505	this publication
Alcyonium digitatum Linnaeus, 1758	GU363450	GU363438	-	KT372503	Reijnen *et al*., 2010; this publication
Gorgonia flabellum Linnaeus, 1758	GU363445 *	GU363435	-	KT372492	Reijnen *et al*., 2010; this publication