Phylogenetic ecology of octocoral - gastropod associations

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Chapter 7

Following the octocorals on a snails' pace: the evolutionary history of Octocorallia and Ovulidae

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Abstract

Host-symbiont associations can on an evolutionary time scale affect the evolution of both animal groups. Associations between species that appear intrinsic or obligate are usually thought to be the result of coevolution or cospeciation. This also accounts for Octocorallia hosts and their Ovulidae snail associates. Most Ovulidae species are perfectly well-adapted to the appearance of their host species of Octocorallia by their specialised morphology and colour patterns resulting in camouflage as a defence mechanism against potential predators like fish. Such associations may be thought to result from coevolution or cospeciation. Specialisation related to a specific host assumes obligate relationships formed over long periods of time between the snails and the corals. To investigate the evolutionary histories of both the Octocorallia and the Ovulidae, a tanglegram was made to identify all possible associations based on phylogeny reconstructions of Octocorallia and Ovulidae. Phylogeny reconstructions were based on mitochondrial as well as nuclear markers: 28S, igr-COI, mtMutS, ND6 for the Octocorallia and 16S, 28S, COI, H3 for the Ovulidae. The tanglegram data was subjected to event-based analyses in Jane 4 and CoRe-PA to study the presence of coevolution between Octocorallia and Ovulidae. Both programs identified cospeciation events and statistical tests showed that these results can be considered significant. To calibrate the speciation events, molecular clock analyses were performed on the datasets for both Octocorallia and Ovulidae in BEAST2. Occurrences of fossils for Octocorallia and Ovulidae were used to calibrate nodes in the phylogenies. The analyses showed that the diversification in the Octocorallia started earlier (100-50 mya) than in the Ovulidae (40-15 mya). This excludes strict coevolution or cospeciation and suggests that the association between Octocorallia and Ovulidae is most probably based on sequential evolution, by which the host affects the symbiont but not vice versa. The host-symbiont defence strategies have therefore not an evolutionary background but prove to be the results of adaptation within the symbionts' generation.

Introduction

Coevolution is a widely used umbrella term referring to separate lineages of hosts and symbionts that are mutually influenced by each other's evolution (de Vienne *et al*., 2013). In heterospecific associations, the partner species may change together (coadaptation) and eventually speciate simultaneously (cospeciation) (Lanterbecq *et al*., 2010). Co-evolutionary relationships between host species and symbionts develop by intertwined physiological and ecological interactions over millions of years. These relationships between species can be based on symbiosis, commensalism, mutualism or parasitism.

Coevolution generally encompasses two modes of species interactions, e.g. sequential evolution and strict evolution (also known as 'Fahrenholz rule') (Ridley, 1996; Lanterbecq *et al*., 2010). Alternative to strict evolution, which has been proven to be rare (probably less than 7% convincing cases: De Vienne *et al*., 2012), sequential evolution is a non-reciprocal mode of evolution. In the latter mode of evolution morphological or physiological changes and the phylogeny of the symbionts are influenced by the host evolution but not vice versa. De Vienne *et al*. (2012) concluded that in many instances symbionts/parasites have diverged more recently than their hosts, mostly by host-shift speciation. This result points to sequential evolution as the primary driver of evolutionary diversification in close associations between species.

Cophylogenetic studies on marine species interactions are far less common than on terrestrial systems. Studies dealing with marine species often focus on fish and their parasites, because of the commercial value of the fish (Kirk, 2003). The only published investigations involving marine invertebrate species' interactions are those for myzostomid flatworms, which are obligate associates on crinoids (Lanterbecq *et al*., 2010) and gall crabs (Cryptochiridae) that live in close association with stony corals (van der Meij *et al*., 2015).

The present paper deals with the evolutionary ecology of Octocorallia (Cnidaria) and Ovulidae (Gastropoda) associations. Octocorallia, mainly soft corals and sea fans, are often a dominant and abundant species group in deep and shallow coral reef environments. Worldwide there are approximately 3,200 species, of which almost 3,000 are gorgonians and soft corals (Appeltans *et al*., 2012; van Ofwegen, 2015). This species group plays an important role in the ecosystem because many species depend on octocorals as a host, e.g. crustaceans (Humes, 1990; Buhl-Mortensen and Mortensen, 2004), echinoderms (Neves *et al*., 2007), and gastropods (Goh *et al*., 1999; Mase, 1989; Reijnen *et al*., 2010; chapter 2).

Ovulidae belong to the superfamily Cypraeoidea and are the sister group of the Cypraeidae and Pediculariidae (Meyer, 2003; chapter 5). All Ovulidae (~200 spp.) and Cypraeidae have a retractable mantle that can cover the entire shell. Therefore, in active snails, the shell is hidden underneath the mantle, which is shown to the outside world when the animals are not disturbed. The Ovulidae differ from the Cypraeidae in the fact that almost all species are obligate symbionts of Octocorallia (Lorenz and Fehse, 2009). For survival on their coral hosts, many ovulids have an adapted morphology. In some species the mantle may be provided with mimicked, retractable polyps. Other species have a bright, vividly coloured mantle, advertising their noxious properties

(aposematism), which they may have obtained from their host by feeding (Schiaparelli *et al*., 2005).

Gastropods of some families and genera are found in particular associations with various taxa of marine invertebrates e.g. Phyllidiidae nudibranchs - Porifera (Brunckhorst, 1993), Eulimidae - echinoderms (Warén, 1983; Takano and Kano, 2014), Epitoniidae - Scleractinia (Gittenberger and Gittenberger, 2005; Gittenberger and Hoeksema, 2013), *Leptoconchus* - Scleractinia (Gittenberger and Gittenberger, 2011), but cospeciation has not been demonstrated with coevolutionary analyses in any of these cases so far. Because of the striking similarities between the appearance of the mantle in Ovulidae and that of the octocoral hosts, coevolution between these snails and their hosts is supposed to have occurred. To investigate this hypothesis, phylogeny reconstructions have been made: 1) to assess the interspecific relationships of the Octocorallia and the Ovulidae species; 2) to elucidate cospeciation and host-symbiont switches, and 3) to use molecular clock dating for the speciation events in both species groups.

Material and methods

Collecting and identification

Octocoral specimens and their ovulid symbionts were collected during fieldwork by the author and colleagues in Curaçao, Florida (USA), North Sea, Spain, Saudi Arabia, Oman, Maldives, Malaysia, Indonesia and New Caledonia (Suppl. mat. 1). Octocoral samples are coded RMNH.Coel and Ovulidae samples are coded RMNH.Mol. Unless stated otherwise, voucher specimens are stored in the collections of Naturalis Biodiversity Center (Naturalis).

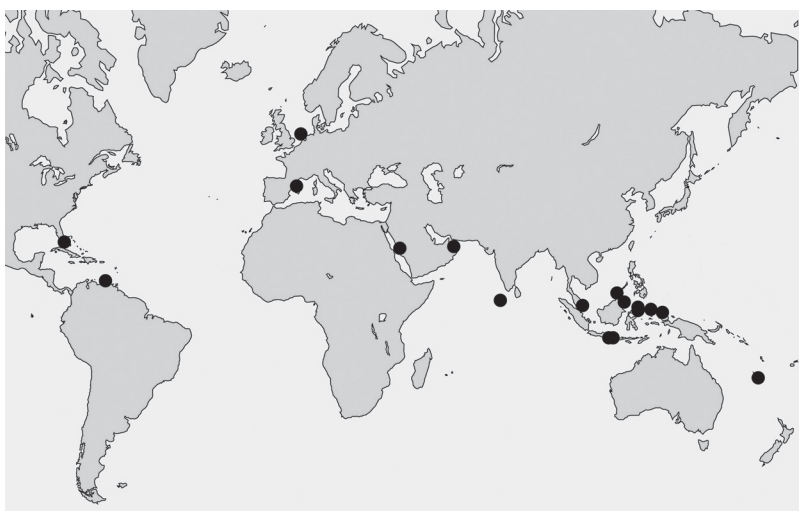


Fig 1. All localities (n=17) where Ovulidae snails were collected. Provenance data can be found in Suppl. mat. 1.

Octocoral species were identified with microscope slides of the sclerites. To make these slides, a small fragment of the tip of a gorgonian was dissolved in a common household bleach solution (approximately 4% active hypochlorite). In case of soft corals, tissue from the base of the colony and of the polyps was used to make such slides. After dissolving the tissue in hypochlorite solution, the residual sclerites were washed with tap water and double distilled water, and eventually embedded in Euparal for visualisation under a microscope (Olympus BX53F). Sclerites were compared with those illustrated by Fabricius and Alderslade (2001) and were identified to genus level and where possible to species level.

Ovulidae specimens were studied under a Leica MZ16 stereomicroscope. Specimens were compared with photographs and species descriptions in the literature (Cate, 1973; Kaicher, 1991; Lorenz and Fehse, 2009).

DNA extraction, sequencing and sequence processing

DNA extractions were performed with the Machery Nagel kit or the Qiagen Tissue kit on a Kingfisher Flex. Tissue samples from the foot or mantle of ovulids, or terminal tips from octocoral colonies, were subjected to tissue lysis overnight (approximately 16 hours). All other DNA extraction steps were performed according to the manufacturers' protocol except for the final DNA elution step. For Ovulidae samples DNA was eluted with 100 μl and for Octocorallia with 150 μl. For both Octocorallia and Ovulidae several dilutions (1:100-1:300) were made for amplification of the targeted gene regions by PCR. For the Octocorallia we targeted the following four genes: COI, mtMutS, 28S, ND6 and for the Ovulidae COI, 16S, 28S and H3. The amplified PCR products were

Table 1. Molecular markers used and additional amplification data for PCR.

	Forward primer	Reverse primer	Targeted area	Annealing temperature	Reference
Ovulidae	LCO-1490	HCO-2198	Cytochrome oxidase I	50	Folmer *et al*., 1994
	16Sar	16Sbr	16S	52	Palumbi, 1996
	H3F	H3R	Histone 3	50	Colgan *et al*., 2000
	LSU5	LSU800rc	28S	50	Chapter 2, 5
Octocorallia	Alc_715_Fw	Alc_1303_Rv	ND6	50	Reijnen *et al*., 2014
	Alc_715_Car	Alc_1303_Car	ND6	50	This chapter
	ND42599F	MUT3458R	mtMutS	48	France and Hoover, 2002; Sánchez *et al*., 2003
	28S-Far	28S-Rar	28S	50	McFadden and van Ofwegen, 2013
	COII8068xF	COIOCTr	Cytochome oxidase I (incl. igr)	58	McFadden *et al*., 2011

sequenced (bidirectional) at Macrogen Europe or BaseClear (both on an ABI 3730XL). Raw sequence data was edited with Sequencher 4.10.1 and Geneious 5.6.4.

For three octocoral host genera sequence data was missing (*Alcyonium digitatum*, *Eunicella singularis* and *Studeriotes* sp.). To include data for these hosts, sequence information was obtained from GenBank (*Alcyonium digitatum*: JX203641 (28S), GQ342381 (COI), AF530498 (ND6); *Eunicella singularis*: AY827538 (COI); *Studeriotes* sp.: GQ342443 (COI), GQ342515 (mtMutS)).

Preliminary alignments were made in BioEdit and some minor adjustments were made manually. Final single gene alignments were constructed with GUIDANCE2 (online server) (Sela *et al*., 2015) using the MAFFT algorithm. For the Octocorallia the alignments contained many indels. To partly overcome the problems of analysing a dataset that contains many indels, columns with a probability lower than 0.9 were deleted from the dataset.

The alignments for each marker were separately tested for the best fitting evolutionary model in jModeltest2.0 by using the AIC (Akaike Information Criterion). Eventually the single marker alignments were concatenated into two datasets: one containing all data for the ovulids and a dataset containing all sequence data for the octocorals.

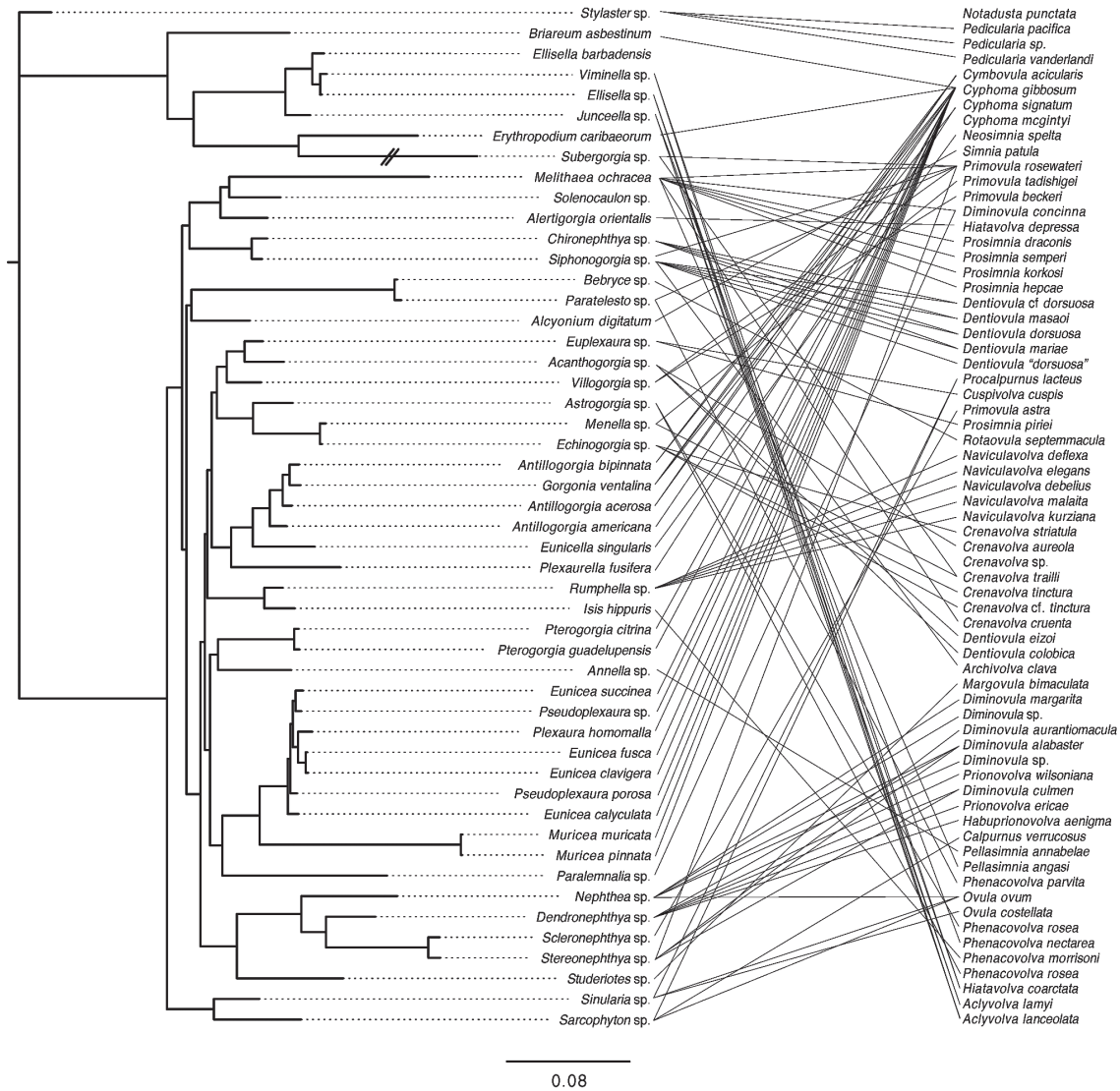
Host and symbiont phylogenetic analyses

Phylogeny reconstructions were performed in MrBayes 3.2.5 (Bayesian inference) and Garli 2.0 (Maximum Likelihood approach). The cypraeid species *Notadusta punctata* was used as an outgroup in the Ovulidae analyses and the lace coral *Stylaster* was selected as outgroup for the Octocorallia. The actual Stylasteridae sequences were not included in the DNA dataset. Firstly, because only non-homologous sequence data was available from GenBank (e.g. mtMuts is only known from bacteria and Octocorallia (McFadden *et al*., 2006)). Secondly because lace corals are from a different class, which would make aligning the Octocorallia dataset even more challenging than it already was. A recently published paper by Zapata *et al*. (2015) shows that Filifera (which includes the Stylasteridae) are eligible as outgroup for Octocorallia. Moreover, by including *Stylaster* as an outgroup for the Octocorallia, the host associations between Pediculariidae and Stylasteridae could also be assessed in the cophylogeny analyses.

MrBayes was run for 4 million generations with evolutionary models set for each

Table 2. Best evolutionary models estimated with the jModeltest analyses for Ovulidae and Octocorallia including their respective AIC scores.

Ovulidae	Evolutionary model	Likelihood score (AIC)	Octocorallia	Evolutionary model	Likelihood score (AIC)
16S	TPM3uf+I+G	13757.7416	28S	GTR+I+G	9785.7953
28S	GTR+I+G	8466.4251	igr-COI	GTR+G	9927.8210
COI	TIM2+I+G	19353.7919	mtMutS	TPM3uf+G	10755.5549
H3	TIM3+I+G	2941.0842	ND6	TIM1+G	6198.1305



of the different gene partitions on both the concatenated dataset of the Octocorallia and Ovulidae. Not all evolutionary models are represented in MrBayes. For some gene regions, instead of the jModeltest result for the particular gene region, the GTR+I+G model was the most appropriate model and was consequently used. The sample frequency was set at 100 and the burnin fraction was 0.25. Average standard deviations of split frequencies were 0.004 for the Ovulidae and 0.007 for the Octocorallia.

Garli is more versatile with implementing evolutionary models. Therefore, the selected models by jModeltest were used in the partitioned garli config file. Garli was run with 500 bootstrap iterations with each two replicates.

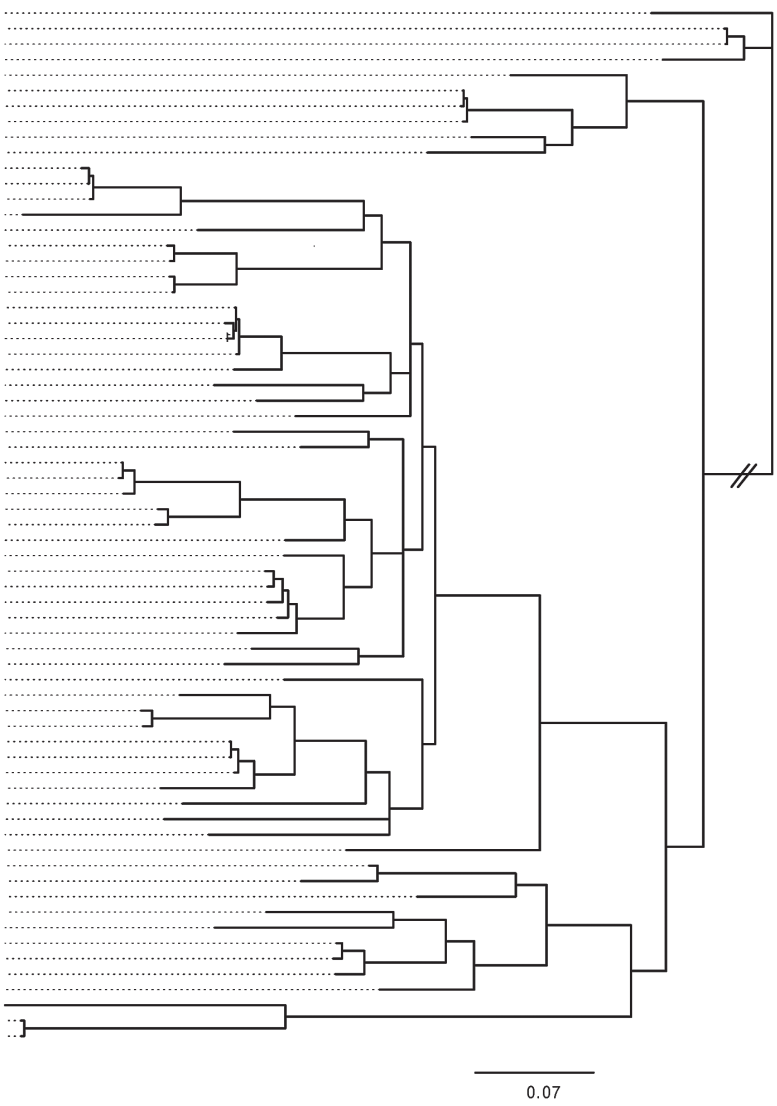


Fig. 2. Tanglegram showing the species associations between Octocorallia (left) and Ovulidae (right).

Cospeciation analyses

The phylogeny reconstructions based on the Bayesian and Maximum Likelihood analyses were used to create a tanglegram providing an overview of the species' associations between the Ovulidae and Octocorallia (Fig. 2).

The tanglegram was redrawn after the host and parasite trees in Jane 4.0 (Conow *et al*., 2010) for an event-based cospeciation analysis (50 host tips and 62 parasite tips). This method uses a parsimonious approach where costs for evolutionary events such as host switching and duplication events of the associated species are superimposed on the host phylogeny. Jane (solve mode) was run for 1,000 generations with 250 populations.

All other settings were default and Jane (stats mode) was run 10 generations with 100 populations and all other settings at default. Likewise the phylogeny reconstructions were used to create tanglegrams in CORe-PA (Merkle *et al*., 2010). Both Jane and CORe-PA are event-based concepts for reconciliation analyses, but in contrast to Jane, CoRe-PA has a different algorithm that uses another parameter-adaptive approach, i.e., no costs have to be assigned to the coevolutionary events in advance (Merkle *et al*., 2010). CORe-PA was run for 10,000 random cycles to investigate coevolutionary patterns between the cladograms of the Octocorallia and Ovulidae.

Molecular clock analysis

The BEAST2 package (Bouckaert *et al*., 2014) was used to calibrate the phylogenies of the Ovulidae and the Octocorallia. BEAUti datasets were set up with the help of fossil data and sequence divergence estimates (only for the Ovulidae) obtained from literature (Table 3 and 4).

For dating the Ovulidae phylogeny the following fossil calibrations were used: *Cyphoma* (20.44 – 15.97 mya); *Pedicularia* (37.8 – 33.9 mya); *Pellasimnia* (28.1 – 23.03 mya) and *Prosimnia* (23.03 – 20.44 mya). For the Octocorallia three calibration points (of 3 genera) were used, viz. *Isis* (83.6 – 72.1 mya), *Melithaea* (66.0 – 61.6 mya) and *Verrucella* (66.0 – 61.6 mya). All calibration points were regarded as 'stem fossils' because of the uncertainty of the exact taxonomic position of the fossils in the phylogeny reconstructions. This is considered the most conservative option for calibrating phylogeny reconstructions with fossil data (Forest, 2009).

Divergence estimates can also be determined, in addition to fossil data, by using the rates of sequence evolution. Such data was not available for the Octocorallia but for molluscs the molecular marker COI is the best-researched gene region for sequence rate estimates. Previously estimated values range between 0.7% / my (Marko *et al*., 2002) to 2.6% / my (Williams and Reid, 2004) for various gastropod groups. For the Ovulidae in particular, such data is not yet available, but can be retrieved from other relatively

Table 3. Records obtained from the literature for Indo-Pacific and Caribbean Octocorallia fossils. Records in **bold** were used for dating the phylogeny of the Octocorallia.

Family/Genus	Epoch	Mya	Reference	Remarks
Acabaria	Danian	66.0 - 61.6	Kusmicheva, 1980.	is *Melithaea* (See Reijnen *et al*., 2014)
Isis	**Campanian**	**83.6 - 72.1**	Kusmicheva, 1980.	
Melithaea	**Danian**	**66.0 - 61.6**	Kusmicheva, 1980.	
Verrucella	**Danian**	**66.0 - 61.6**	Sepkoski, 2002	
Nicella	Maastrichtian	72.1 - 66.0	Kusmicheva, 1980.	
Gorgoniidae	Rupelian	33.9	Kocurko and Kocurko, 1992	Caribbean origin
Plexauridae	Rupelian	33.9	Kocurko and Kocurko, 1992	Caribbean origin
Anthothelidae	Rupelian	33.9	Kocurko and Kocurko, 1992	Caribbean origin

closely affiliated marine gastropod species. One of the most closely related taxa for which a value for the COI region has been estimated is *Nucella* (1.3%; Marko *et al*., 2014). Therefore, this value was used as the substitution rate under the site model for COI in BEAUti. For both species groups the relaxed clock log normal was selected. The Yule model was selected as the speciation model prior with a uniform birth rate and the upper limit set at 10,000. Gene regions were treated as gamma site models and the gamma shape was considered exponential. Substitution rates were also set as gamma distributed.

The .xml files can be obtained from the author. The BEAST2 analyses for the Ovulidae and Octocorallia had problems to converge; therefore a starting tree was added manually to the xml file. The starting tree was obtained by performing an initial analysis with the models and priors set as described above. The initial analysis was run for 15 million generations. The consensus tree (newick) was implemented manually in the xml file created by BEAUti. Final analyses were run for 30 million iterations (Ovulidae) or 50 million iterations (Octocorallia) with samples saved for every 1,000 iterations. The effective sampling size (ESS) values (>100) were investigated in Tracer 1.6 (Rambaut *et al*., 2014).

Table 4. Records of non-Eocypraeinae fossil Ovulidae (and one pediculariid). Records in **bold** were used for dating the Ovulidae phylogeny.

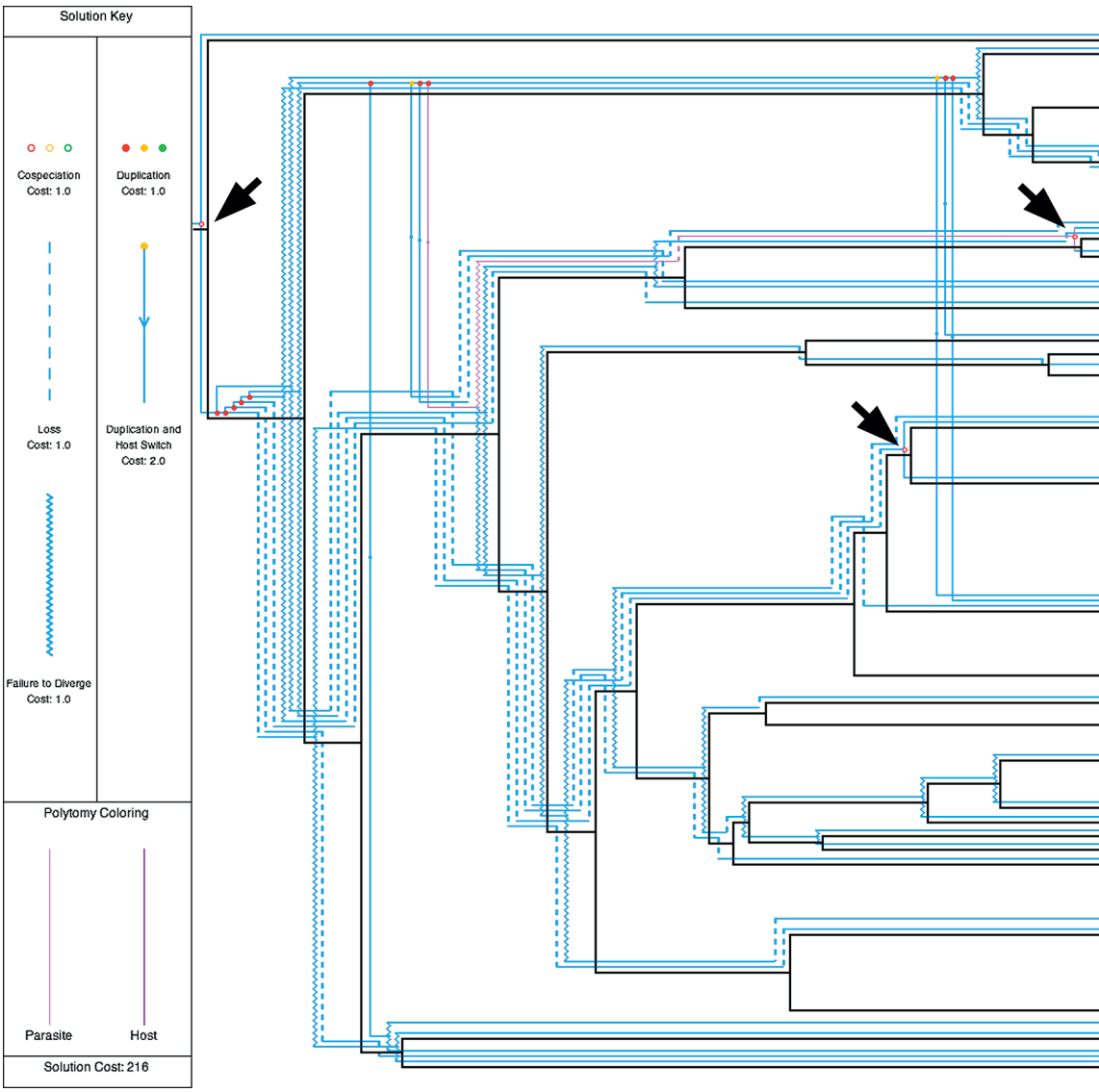
	Genus	Epoch	Mya	Reference
Ovulidae	*Amphiperas*	Langhian and Serravallian	15.97 - 11.63	Darragh, 1985
	Cyphoma	**Burdigalian**	**20.44 - 15.97**	Woodring, 1973
	Globovula	Middle Miocene	15.97 - 11.63	Dharma, 2005
	Jenneria	Bartonian	41.2 - 37.8	Signor, 1990
	Margovula sp. 1	Middle Miocene	15.97 - 11.63	Dharma, 2005
	Margovula sp. 2	Upper Pliocene	3.6 - 2.58	Dharma, 2005
	Neosimnia	Ypresian	56.0 - 47.8	Schilder, 1932
	Pellasimnia	**Chattian;**	**28.1 - 23.03;**	Signor,1990;
		Late Eocene	**37.6 - 33.9**	Beu and Marshall, 2011
	Phenacovolva	Pliocene	5.3 - 2.58	Ladd, 1977
	Primovula	Pliocene	5.3 - 2.58	Ladd, 1977
	Prionovolva	Pleistocene	2.58 - 0.0117	Sepkoski, 2002
	Prionovolva brevis	Upper Pliocene	3.6 - 2.58	Dharma, 2005
	Procalpurnus	Pleistocene	2.58 - 0.0117	Schilder, 1932
	Prosimnia	**Upper Pliocene;**	**3.6 - 2.58;**	Schilder, 1932;
		Lower Miocene	**23.03 - 20.44**	Beu and Marshall, 2011
	Simnia	Langhian and Serravallian	15.97 - 11.63	Schilder, 1932
	Volva	Upper Pliocene	3.6 - 2.58	Schilder, 1932; Dharma, 2005
Pediculariidae	***Pedicularia***	**Priabonian**	**37.8 - 33.9**	Ladd, 1977

Results

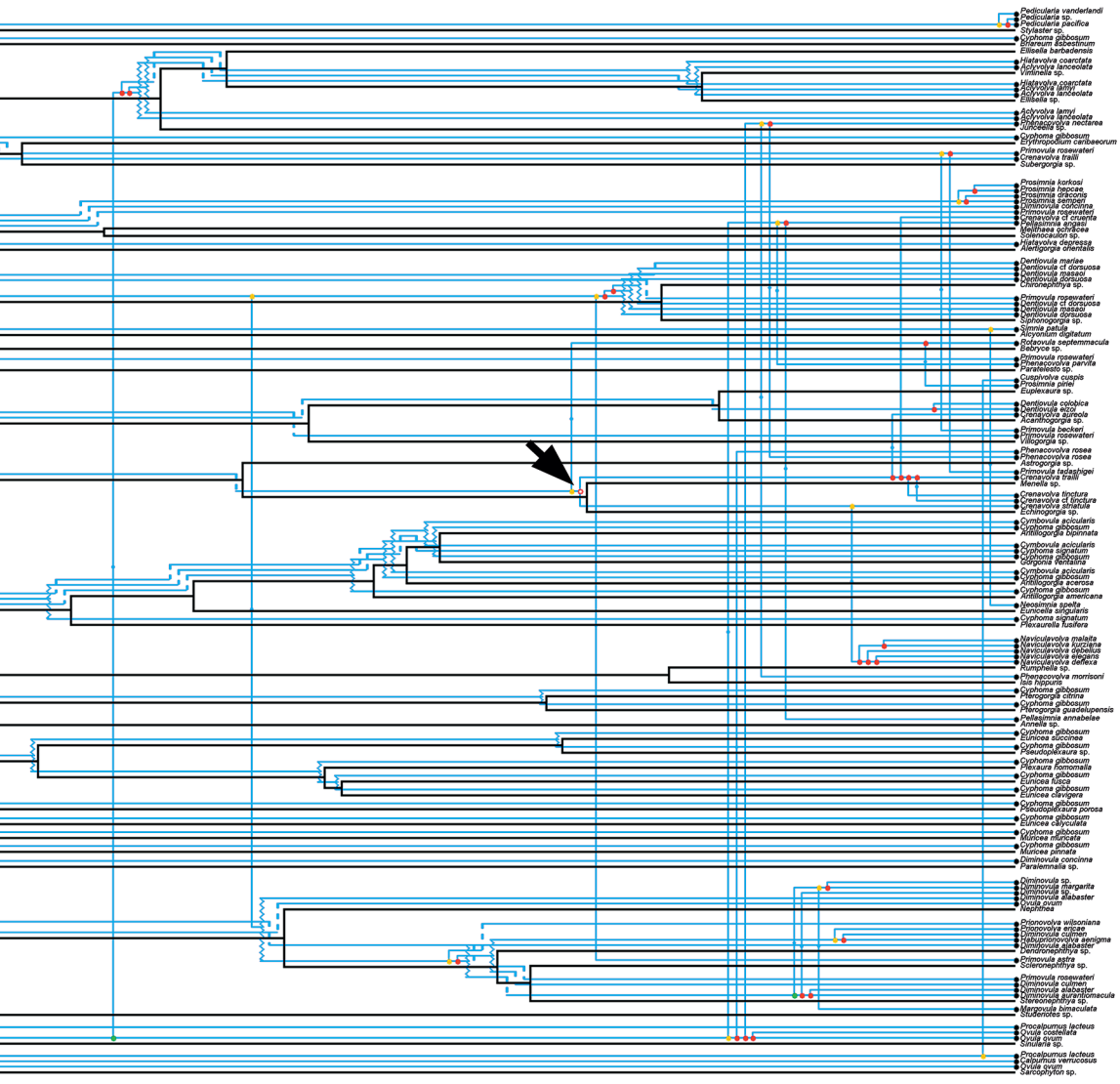
Octocorallia and Ovulidae – host and symbiont phylogenies

The phylogenetic relationships within the Octocorallia have proven to be difficult to assess. Phylogeny reconstructions by McFadden *et al*. (2006) and McFadden and Ofwegen, 2013 based on multiple markers (mtMutS, COI and ND2 or 28S), show that the basic

Fig. 3. Output of isomorphic tree by Jane 4.0 showing all events reflected on an overlay of the parasite-host trees. The black arrows indicate the cospeciation events.



relationships between octocoral genera or even families (sensu Fabricius and Alderslade, 2001) are generally not supported. Adding another molecular marker (ND6) as was performed to identify the evolutionary associations between Octocorallia and the Ovulidae did not improve these results. Yet, some families (Alcyoniidae, Ellisellidae, Nephtheidae [except for *Paralemnalia*] and Nidaliidae) were retrieved as monophyletic groups, in contrast with the results by McFadden and Ofwegen (2013) for the Alcyoniidae and Nephteidae (see also Suppl. mat. 2). The Atlantic genera and species do not form a monophyletic group but cluster together with Indo-West Pacific (IWP) genera in a single clade. These groups are poorly supported, however. To be able to use a phylogeny reconstruction for



the cospeciation analyses the tree should be fully resolved. Therefore the cladogram is represented according to the majority-rule consensus method showing al groupings (even below 50% majority).

For the Ovulidae the increase of molecular data has provided new insights in the relationships between species and some higher taxa (Suppl. mat. 2) as compared to the large-scale phylogeny reconstruction by Schiaparelli *et al*. (2005). The current opinion regarding the subfamily level is largely consistent with that of previous studies except for some species or genera. In contrast to the Octocorallia the Caribbean / Atlantic Ovulidae species form a clade together, which is the sistergroup to all IWP Ovulidae whereas the family Pediculariidae is the sistergroup of all Ovulidae species. According to the molecular data, some Ovulidae species should be classified in other subfamilies than hitherto accepted. The genus *Naviculavolva* allegedly belonging to the Simniinae clustered in the subfamily Prionovolvinae as well as *Hiatavolva depressa* supposedly belonging to the subfamily Aclyvolvinae, was also clustering in the subfamily Prionovolvinae (see chapter 5). The phylogenetic relationships between the species of the Prionovolvinae are still unclear. While aiming at monophyletic groups as genera, extensive revisions are inevitable.

Cospeciation

Almost all ovulids are more or less obligate symbionts with one or more octocoral genera. The tanglegram of the Octocorallia and Ovulidae (Fig. 2) shows that the Caribbean species *Cyphoma gibbosum* and the Indo-Pacific *Primovula rosewateri* are facultative symbionts of Octocorallia. These ovulid species are found associated with eight and six different octocoral genera, respectively. It is striking that the latter two species show aposematic colour patterns and do not rely on camouflage like most other ovulids. Both *P. rosewateri* and *C. gibbossum* are not closely related and occur in different oceans (Indo-West Pacific vs. Atlantic) what indicates that aposematic characters in the Ovulidae have originated at least twice.

In contrast, other species are found to be symbiotic with host species from only a single genus, for example the ovulid species *Dentiovula eizoi* and *D. colobica*. These species were found only on *Acanthogorgia* spp. whilst congeners *D. dorsuosa*, *D. mariae* and *D. masaoi* are obligate symbionts of the octocoral genera *Chironephthya* and *Siphonogorgia*. This would exemplify a typical host-switch event regarding *Dentiovula* spp. but the phylogeny reconstruction shows that these *Dentiovula* species are not closely related to one another. In this case convergent evolution of shell morphology has probably troubled the systematics of this genus. An ovulid genus that is dependent on a single octocoral genus is for example *Naviculavolva*, in which all species are associated with *Rumphella* sp. Furthermore, *Phenacovolva annabelae* was only found on *Annella* and the ovulids of the genus *Prosimnia* are solely associated with *Melithaea* species, except for *Prosimnia piriei* which is an obligate symbiont on *Euplexaura*. *Prosimnia piriei* and the allegedly congeneric species are not closely related and do not share a common ancestor, so this is also not a convincing host-switch event. Anyway, the tanglegram shows that a large number of octocoral genera play an important role as host species for Ovulidae. Specifically the genera from the families Nephtheidae (*Dendronephthya*, *Nephthea*), Nidaliidae (*Siphonogorgia*, *Chironephthya*) and Melithaeidae (*Melithaea*) are important as hosts for

more than one ovulid species. Some of these ovulid species, like *Habuprionovolva aenigma*, solely depend on this single host genus (*Dendronephthya* sp.). Less strict is the association between the octocoral genera in the family Ellisellidae (*Ellisella*, *Junceella*, *Viminella* a.o.), which are important as host genera for *Aclyvolva* species.

All species association data was used for a cospeciation analysis in Jane 4.0. This analysis resulted in nine isomorphic solutions (having the same topological structure when the associated species are superimposed on the host phylogeny) of which one solution contained 115 trees. The costs for all nine isomorphic solutions were 216, and were based on 26 duplications and 39 failures to diverge. Depending on which of the the nine different isomorphic solutions was investigated, there were three or four cospeciation events, 32 or 31 duplications / host switches, and 84 or 85 losses. The selected tree (with the most isomorphic trees) (Fig. 3) had four cospeciation events, 31 duplications / host switches and 85 losses.

Cospeciation events are retrieved for 1) *Stylaster – Pedicularia*, 2) *Melithaea – Prosimnia* spp. and 3) the clade containing *Euplexaura – Cuspivolva cuspis* / *Prosimnia piriei*, *Acanthogorgia – Dentiovula colobica* / *D. eizoi* / *Crenavolva aureola*, *Villogorgia – Primovula beckeri* / *P. rosewateri*, *Phenacovolva rosea – Astrogorgia*, *Primovula tadashigei* / *Crenavolva trailli – Menella*, *Crenavolva* spp. – *Echinogorgia*. The cospeciation event of *Primovula tadashigei* / *Crenavolva trailli – Menella*, *Crenavolva* spp. – *Echinogorgia*) is considered the fourth cospeciation event.

The output data resulting from Jane was tested against randomly generated trees in the 'stats mode'. This test compares the obtained coevolutionary signal from the 'solve mode' versus randomly generated data of the coevolution data. Based on the costs for the obtained optimal coevolutionary analysis versus the randomised data, a cost histogram was generated. This histogram (Fig. 4) shows that randomly generated data has much higher costs (average = 443; median = 436) than the lowest costs observed (216) in the coevolution analysis, showing that there is a coevolutionary signal.

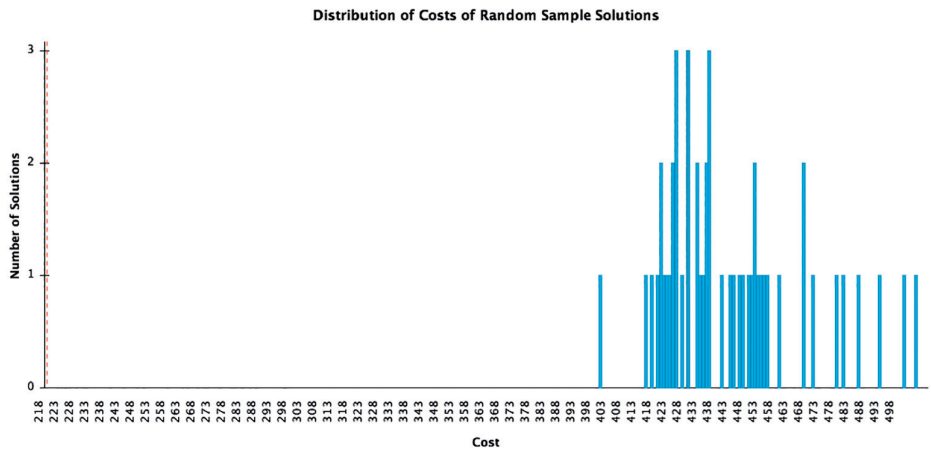
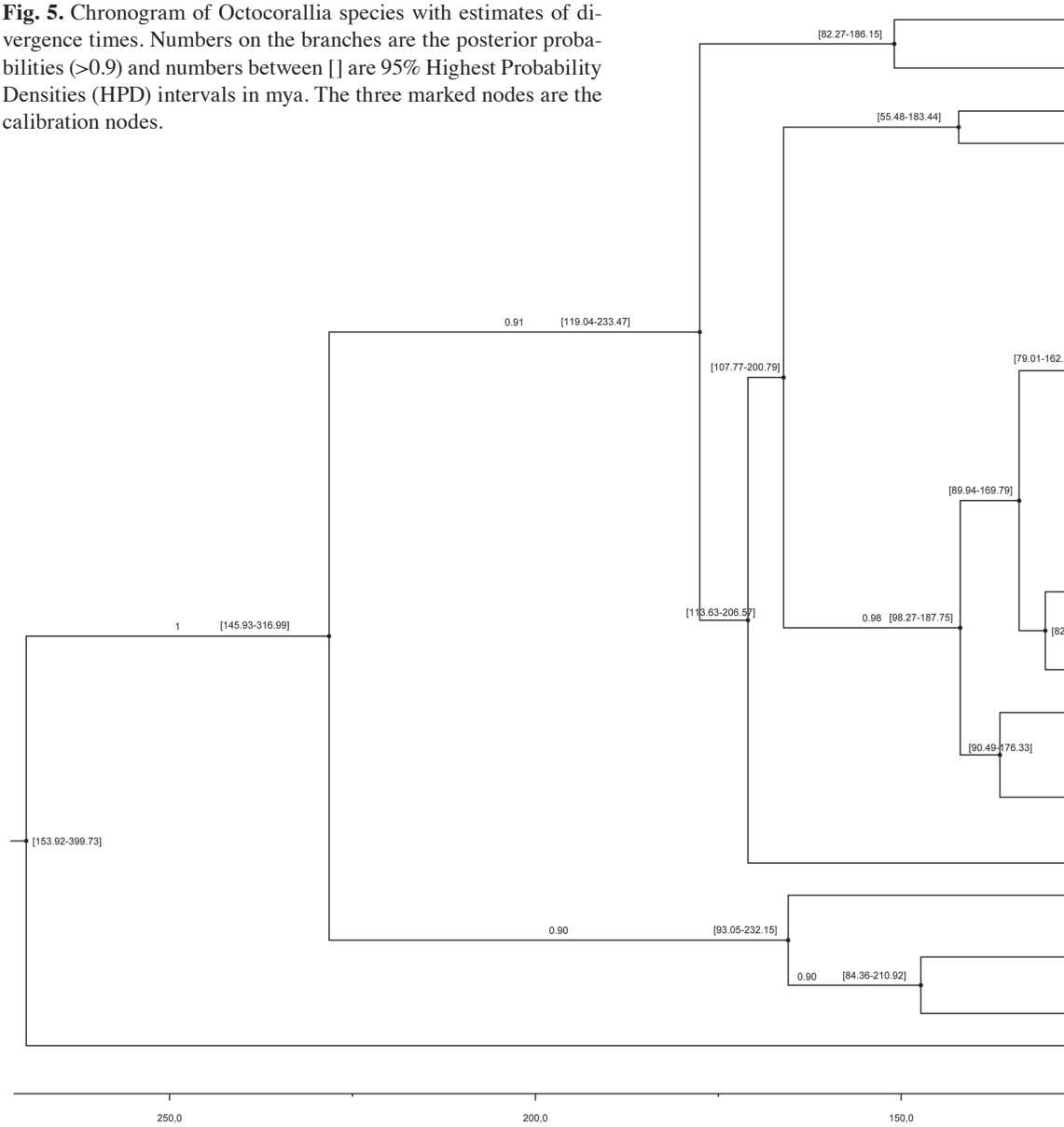


Fig. 4. Histogram produced by Jane 4 with randomly generated cospeciation tree costs versus the costs of the isomorphic solutions (red dotted line).

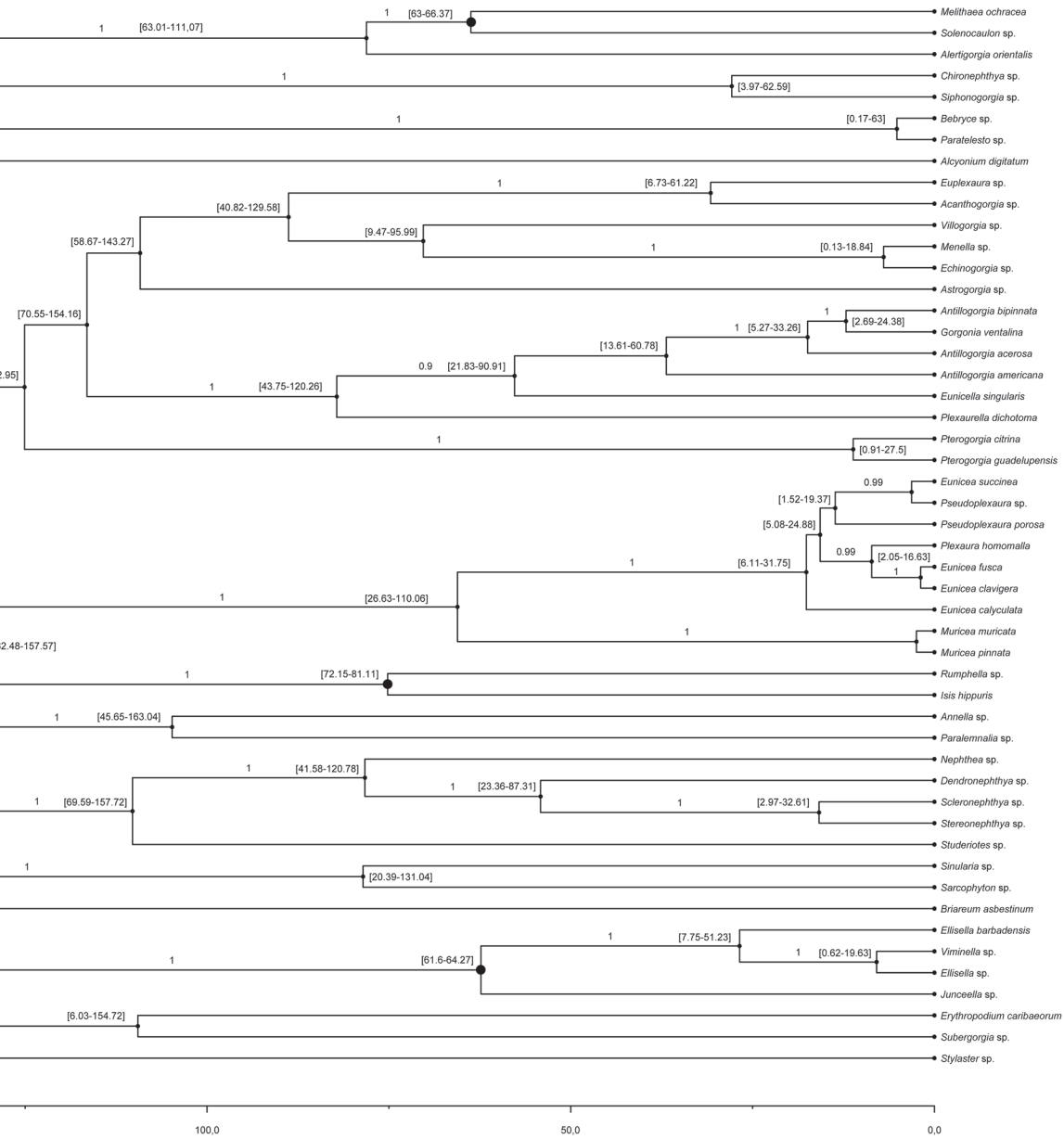
An identical tanglegram was created in CoRe-PA and tested for coevolutionary signals. CoRe-PA found 102 isomorphic trees. In contrast to the analysis in Jane, CoRe-PA found 19 cospeciation events, 75 sortings, 32 duplications and 11 host switches. CoRe-PA found more cospeciation events compared to Jane (19 vs 3 or 4), which could be the effect of different algorithms and definitions used by the programs.

Fig. 5. Chronogram of Octocorallia species with estimates of divergence times. Numbers on the branches are the posterior probabilities (>0.9) and numbers between [ ] are 95% Highest Probability Densities (HPD) intervals in mya. The three marked nodes are the calibration nodes.



Molecular clock analysis

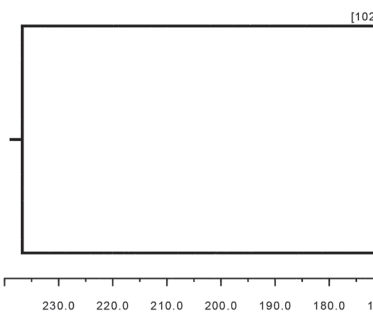
One of the requirements for divergence time estimates are calibration points that are based on the fossil record and can be assigned to specific nodes in the phylogenies. Therefore, two data sets were composed for Octocorallia (Table 3) and for Ovulidae (Table 4).



Octocorallia – Data on fossil Octocorallia are scarce and mostly based on axial remains and holdfasts, which lack most diagnostic characters to identify the fossil remains to genus level, let alone species level. However, Kocurko and Kocurko (1992) reported on actual sclerites found in the Red Bluff formation. These sclerites were well preserved and could be assigned to families or genera. In Table 3 eight records of octocoral fossils are mentioned, three of which were used to estimate divergence times in the octocoral phylogeny. The other records were neglected because of the lack of representatives in the phylogeny reconstruction or because of difficulties in assigning these dates to specific nodes in the cladogram.

The calibrated phylogeny reconstruction of the Octocorallia in BEAST2 shows that 95% Highest Probability Densities (HPD) are covering large time spans and most branches are not or only poorly supported (Fig. 5). Nevertheless the divergence estimates show that most octocoral genera originated probably between 100 mya and 50 mya. Most generic splits occur within this time period. One of the oldest families is probably the Ellisellidae, which was found together with *Subergorgia* and *Erythropodium* as the sistergroup of all other octocoral genera. According to the divergence time estimates by BEAST2 the family Ellisellidae (here represented by *Ellisella barbadensis*, *Ellisella* sp., *Viminella* sp. and *Junceella* sp.) probably originated 210 to 84 mya (Upper Triassic - Upper Cretaceous). Other families, e.g. Nephtheidae, Nidaliidae and Alcyoniidae originated later, i.e. 121-42 mya (Lower Cretaceous - Eocene), 62-4 mya (Palaeocene - Pliocene) and 131-20 mya (Lower Cretaceous - Miocene), respectively.

Ovulidae – Most ovulid fossils belong to the Eocypraeinae (Groves, 1994; Dolin and Lozouet, 2001), a subfamily that morphologically most closely resembles the Cypraeidae. In the extant ovulid fauna the Eocypraeinae is represented by only a single species, *Sphaerocypraea incomparabilis* (Briano, 1993), which is not included in the molecular dataset. Consequently, the data of the fossil Eocypraeinae can only provide little holdfast in dating the phylogeny of the Ovulidae. These records had to be ignored. A non-extensive overview of fossil, non-Eocypraeinae Ovulidae species (and one pediculariid) is provided in Table 4. Four records for fossil taxa were used to estimate the divergence times in the Ovulidae phylogeny. These four fossil records were selected because they are represented by extant conspecifics in the cladogram, are relatively easy identified compared to other ovulids and also represent three subfamilies (Ovulinae, Prionovolvinae and Simniinae) in the Ovulidae.



The divergence time estimation in BEAST2 resulted in a chronogram (Fig. 6) indicating that the Pediculariidae (clade E) diverged from the Ovulidae between 200 and 100

Fig. 6. Chronogram of Ovulidae species with estimates of divergence times. Numbers on the branches are the posterior probabilities (>0.9) and numbers between [ ] are 95% HPD intervals in mya. The four marked nodes are the calibration nodes. A-E refer to specific clades.

mya according to the 95% highest probability density (HPD). This divergence is very well supported (1.00). Other highly supported groups are the Atlantic ovulids (clade A), which diverged from the Indo-West Pacific (IWP) Ovulidae (clade B-D) 120-68 mya, i.e. during the Cretaceous. In the IWP Ovulidae (clade B-D), between 40 mya and 15 mya (middle Eocene- middle Miocene), branch lengths are shorter and most species divergences occur, these events could be indications of a large species radiation in that era.

The oldest split-off in the family Ovulidae are the Aclyvolvinae (clade D), which are endemic to the IWP, and diverged from the other IWP Ovulidae between the Lower Cretaceous and Upper Cretaceous (95% HPD = 137-76 mya). Therefore, the Aclyvolvinae might contain the oldest Ovulidae species.

Discussion

Octocorallia and Ovulidae phylogenies

The traditional morphological character states that are used to identify octocoral species and genera are mostly obtained from their sclerites. However, the different types of sclerites, the variation within a specific type, and the obvious interspecific overlap made it difficult to identify the specimens. Here, molecular analyses were used to investigate the value of these morphological features. This did not result in unambiguous results. Most gene regions turned out to be conserved and the 28S RNA gene region (which was found to contain pseudogenes) proved to result in phylogeny reconstructions that are incongruent with those of the mitochondrial data (McFadden *et al*., 2010, 2011). Nevertheless, a combination of genes was used to overcome the problems with incongruence and lack of phylogenetic signal (McFadden *et al*., 2006). For example in the Melithaeidae (Aguilar-Hurtado *et al*., 2012; Reijnen *et al*., 2014), a multiple-marker approach partially provided new insight in the relationships between genera and species, but some clades remained unresolved. The lack of phylogenetic resolution for the molecular markers is also represented in the tanglegram by the short and unsupported branches for Octocorallia genera and families. Due to the difficulties in identifying octocoral species and lack of resolution of the molecular markers at genus or family level the taxa in the tanglegram represent genera, unless accurate species identifications were possible (mostly for Caribbean species). The so-called species interdependencies that are indicated are therefore mostly not strict species-to-species associations but representatives of a host octocoral genus and their ovulid symbionts.

The phylogeny reconstruction of the Ovulidae (Fig. 6) is congruent with the topology of the only other large-scale molecular phylogeny reconstruction of Ovulidae, which was published by Schiaparelli *et al*. (2005). Their model is based on the mitochondrial marker 16S, and shows a polytomy for a large number of species currently placed in the subfamily Prionovolvinae. In the present article this clade was reconfirmed with even better support when compared with Schiaparelli *et al*. (2005). The subfamily classification published by Fehse (2007) is largely consistent with the one presented in the present study with the exception of some aberrant species and genera (*Hiatavolva depressa*, *Naviculavolva*, *Prosimnia*). Obviously, these subfamilies have to be redescribed, at least regarding shell morphology. The position of the *Pedicularia*

species has been under dispute. This genus has been placed in the Ovulidae (Schilder, 1931; Von Salvini-Plawen, 1972; Goud and Hoeksema, 2001), but has also been considered a separate family (Yamamoto, 1973; Fehse, 2007; Braga-Henriques *et al*., 2011). The molecular data support the interpretation of the *Pedicularia* species as a separate family, the Pediculariidae. This finding is supported by a difference in host species between the Pediculariidae and the Ovulidae. All *Pedicularia* species are affiliated with Stylasteridae from the class Hydrozoa, whilst all ovulid species are associated with species from the class Anthozoa. In their introduction to the Pediculariidae, Lorenz and Fehse (2009) indicate that there are differences in radula morphology between Ovulidae and the Pediculariidae, which could be an adaptation to the different hosts.

Host species associations can be relevant in identifying the status of symbiotic species. Gittenberger and Gittenberger (2011) for example found that for *Leptoconchus* spp. (Gastropoda) the rudimentary shell characters and the anatomical details do not allow identification. Molecular data, however, enabled the identification of a radiation of several morphologically cryptic species. When the phylogeny reconstruction for the snail species was linked to that of the host species, associations between fungiid coral species and *Leptoconchus* species were discovered.

Similarly, van der Meij *et al*. (2015) found that in the coral-associated crab family Cryptochiridae, species that morphologically looked almost identical belonged to an array of different coral genera. Later on, molecular analyses showed that these morphologically similar gall crabs actually represent a complex of cryptic species, each of which occurring in association with only one coral genus. Similar results were found when the associations between Ovulidae and Octocorallia were studied, as shown in the tanglegram (Fig. 2). Some genera are paraphyletic according to the molecular phylogeny, such as *Dentiovula* and *Prosimnia*. Two species in the genus *Dentiovula* (*D. eizoi* and *D. colobica*) are morphologically quite similar to their supposed conspecifics according to their shell morphology, but the two groups of *Dentiovula* species do not share a recent common ancestor. Moreover, species of these paraphyletic groups are found with different host genera, e.g. *D. eizoi* and *D. colobica* are associated with the octocoral genus *Acanthogorgia* (family Acanthogorgiidae) and the other *Dentiovula* species occur with the octocoral genera *Chironephthya* and *Siphonogorgia* from the family Nidaliidae. In this case, convergent morphological evolution has probably caused confusion, because both the molecular data and the host genera suggest that two different groups are involved. This also accounts for the *Prosimnia* species. All species in this genus except for one, i.e. *Prosimnia piriei*, are found on octocorals of the family Melithaeidae, whilst *P. piriei* is associated with the genus *Euplexaura* of the family Plexauridae. *Prosimnia piriei* is not closely related to the other so-called congeneric species, and its shell shape is unique among the Ovulidae.

Fossils and molecular clock analyses

For both the Ovulidae and the Octocorallia only few fossil records are available. Dating molecular phylogenies of either one of these groups therefore heavily relies on the small number of young (mostly Miocene) fossil remains or disputed old fossils. The lack of

fossils is probably the result of poor taphonomic conditions, such as the absence of strong endo- or exoskeletons and an environment that is unsuitable for fossilisation. Most ovulid species lack strong, big shells and even larger thick-shelled species such as Caribbean *Cyphoma* species, are hardly found as fossils. This could be because *Cyphoma* species are relatively young and are therefore not found in older deposits. Despite their recent common occurrence in the Caribbean, there are only eight records of fossil occurrences (Paleobiology Database; accessed October 2015), the oldest record being from the Aquitanien (23.0-20.4 mya; Woodring, 1973). Fossil remains of Octocorallia are also scarce. Most octocorals have fragmentary calcareous sclerites, which usually do not exceed 0.5 mm in length. Because of their small size, fossil sclerites are hardly found and if so, they are difficult to study while embedded in the concretion. Nevertheless, some fossilised sclerites have been found in the Caribbean and IWP (Kocurko and Kocurko, 1992; Jeng *et al*., 2011). Where Kocurko and Kocurko found a small number of sclerites, Jeng *et al*. (2011) found thick layers of them (also referred to as spiculite layers) in Taiwan, belonging to the genus *Sinularia*. These spiculite layers were found as young fossils as well as extant spiculite layers. Yet, the layers of fossilised sclerites were not correlated to a specific era, system or epoch and could therefore not be used in the molecular divergence estimates.

Instead of sclerites, more commonly the axis or holdfasts of gorgonians have been found as fossil remains (Kocurko, 1988), but these remains are often poorly conserved and can hardly be identified to family level, let alone to a lower taxonomical level. There is more fossil data reported, but the nature of these records is disputed (Taylor and Rogers, 2015) and they could not be related to the shallow-water species that are currently represented in the phylogeny reconstructions.

There are few scientific publications that deal with divergence time estimates for Octocorallia. Ardila *et al*. (2012) focussed on precious corals (Corallidae) and their species delimitations, Park *et al*. (2012) dealt with shallow-water Octocorallia mitogenomes amongst other cnidarian mitogenomes, Taylor and Rogers (2015) used a classical approach by combining molecular data for several gene regions of the family Primnoidae (deep-water, Antarctic), whilst Herrera and Shank (2015) use RAD sequencing on species from the deep-water octocoral genus *Paragorgia* to estimate the divergence splits. The divergence estimates calculated in these articles largely resemble the outcome presented here for the shallow-water, mostly tropical Octocorallia. According to the chronogram published by Park *et al*. (2012), modern Octocorallia have originated from tMRCA during the late Paleozoic and early Mesozoic (421-173 mya) and must therefore have survived the Permian-Triassic mass extinction 251 mya. The 95% HPD intervals from the chronogram (Fig. 5) fall within that range (400-153 mya). The generic diversification in the shallow water Octocorallia must have taken place between 100 and 50 million years ago, which is more or less in correspondence with the dates indicated by Park *et al*. (2012), but the 95% HPD confidence intervals can also exceed these times, up to more than 200 mya for *Sarcophyton glaucum*.

Herrera and Shank (2015) found that the split between the family Corallidae and Paragorgiidae must have taken place approximately 150-80 mya while Park *et al*. (2012) found that the MRCA of Corallidae is 50 my old, with a 95% confidence interval of

100-25 mya. Even though most divergences show overlap, there are also contradictions, which could result from differences in the data that were used (mitogenomes vs. RAD sequencing).

One of the oldest families in the Octocorallia chronogram (Fig. 5) is most probably the Ellisellidae (Bilewitch *et al*., 2014). This family has split-off from the group consisting of *Erythropodium caribaeorum* and *Subergorgia* sp. between 211 and 84 mya (95% HPD). According to a phylogenetic study by McFadden *et al*. (2006) and axial, morphological features by Bayer *et al*. (in Moore, 1956) Elliselllidae are the sister group of the pennatulaceans (Sea Pens), which are among the oldest known Octocorallia fossils, dating back to the Silurian (approximately 444-419 mya). These (trace) fossils are disputed however, and more reliable fossils belonging to the Pennatulacea are from the Cretaceous (Williams, 1999).

The Ovulidae species that are associated with Ellisellidae (Aclyvolvinae) are among the oldest ones. These could be hypothesised to be the first associations between ovulids and Octocorallia, although the fossil record for Ovulidae is poor and inconclusive and for the Aclyvolvinae there are no known fossil species. Conventionally, species of the genus *Archivolva* are considered to be the oldest species in the Ovulidae (hence the name *Archivolva*) because in contrast to all other Ovulidae species, the protoconch is not internalised and still visible (Lorenz and Fehse, 2009). This character was believed to represent an ancestral state, but instead of a basal position, *Archivolva* clusters within the Prionovolvinae and not as the sister species to all other ovulids (a position taken by the Aclyvolvinae).

The large radiation of octocoral genera in the IWP around 100-50 mya is not shared by Ovulidae genera. According to the chronogram (Fig. 6), most ovulid genera came into being 40-15 mya, which is much later than the octocorals. These divergence times are quite similar to the dates estimated by Williams and Duda (2008) for three gastropod species from the IWP. They reconstructed calibrated phylogenies for *Conus*, *Echinolittorina* and *Turbo* spp. and found that for each of these species groups an increased diversification in the IWP occurred between 23.7 and 21.0 mya. Williams and Duda (2008) matched the diversification of those species with the collision of the Australian and New Guinean plate with the southeast boundary of the Eurasian plate, approximately 25 mya. The collision of the tectonic plates is supposed to have resulted in an increase in shallow-water areas and, thereby, an increased number of different habitats triggering the diversification in IWP gastropods and zooxanthelate (octo)coral species. Maybe, most Ovulidae species and genera have originated around that same time in the IWP.

A small number of Atlantic ovulid representatives (*Cyphoma* spp., *Cymbovula acicularis*, *Neosimnia spelta* and *Simnia patula*) separated earlier than their IWP congeners according to the chronogram (Fig. 6). This Atlantic group diverged approximately 121-68 mya. There are fossil remains of *Neosimnia* spp. from the lower Eocene of Europe (Schilder, 1932), indicating that these species occurred in what is nowadays considered Europe 56-41 mya. Temperatures in Europe during the Eocene were higher and the climate could be considered tropical and might be comparable to the current climate in the Caribbean. Species in the Atlantic clade might therefore be relicts from the Eocene North Atlantic ovulid fauna.

Coevolution, cospeciation, or sequential evolution?

The chronograms for the Octocorallia (Fig. 5) and the Ovulidae (Fig. 6) indicate that the Octocorallia originated long before Ovulidae did. This, and given that both topologies of the Octocorallia and Ovulidae in the tanglegram (Fig. 2) are not mirror images, proves that coevolution and cospeciation did not govern the evolutionary history of the combined Octocorallia and Ovulidae. The evolutionary relationships between Octocorallia and Ovulidae are best described as sequential evolution. The changes in the host corals have influenced the symbionts (Ovulidae) without reciprocity. Van der Meij *et al*. (2015) found the same result for Cryptochiridae and scleractinian corals. The interactions between Octocorallia and Ovulidae that are reported in the present study are not based on strict coevolution and cospeciation. Sequential evolution is the most likely evolutionary model for the Octocorallia and Ovulidae.

Defence strategies in Ovulidae

As a result of the specific colouration, colour patters, and sometimes even the mimicry of ornaments resembling polyps, the relationships between Octocorallia and Ovulidae was thought to exemplify coevolution or cospeciation. The results show, however, that sequential evolution is most probably the evolutionary model here. This raises the question how ovulid species got morphologically adapted (mantle ornamentation and colour patterns) to their specific host corals for their own defence. Gastropods use wide-ranging defence strategies against potential predators such as shell thickness, spines, operculum, chemical defence, deep withdrawal and colouration. Shell colour as camouflage is already known for marine gastropods from the Mesozoic era (Vermeij, 2015). Especially in one of the sister groups of the Ovulidae, the Cypraeidae, shells can be vividly coloured whilst the pattern on the mantle that covers the shell can be highly contrasting, possibly confusing potential predators (Vermeij, 2015). Colour is also often used as a defensive strategy in Ovulidae but is more related to the mantle covering the shell rather than the shell itself. Most shells of Ovulidae are monochromous with only a limited number of species that exhibit patterns such as spots on the shells. In contrast, the mantles of Ovulidae can be vibrantly coloured with very abundant colour patterning. These patterns provide camouflage or communicate aposematic behaviour and in some rare occasions, can even mimic other species. The physiological origin of the colour pigments is under dispute but the colouration is believed to be obtained via alimentary homochromy (Salvini-Plawen, 1972; Rosenberg, 1994; Schiaperelli *et al*., 2005). For example, ovulid snails, which have been placed on a differently coloured host coral, changed their shell colour accordingly over time (Salvini-Plawen, 1972). Sacoglossans (*Elysia* spp.) do also change colour when they feed on differently coloured algae. These species are believed to take up the chlorophyll in their digestive tract for photosynthesis and are therefore also referred to as "solar-powered seaslugs" (Middlebrooks *et al*., 2012).

The majority of the Ovulidae species use camouflage as a defence strategy and these species occur in all clades in the phylogeny reconstruction. Some ovulid genera (*Aclyvolva*, *Dentiovula*) even take camouflage a step further and mimic host structures such as the retractable polyps on the mantle (Schiaparelli *et al*., 2005; Reijnen, 2010).

According to the chronogram (Fig. 6), Aclyvolvinae are among the oldest ovulids, so perhaps its MRCA were also camouflaged. The genera *Aclyvolva* and *Dentiovula* are not closely related; the mimicry of octocoral polyps on the mantle of these snails must therefore have arisen more than once in the Ovulidae. Aposematic species are more rare than camouflaged ones in the Ovulidae. Caribbean *Cyphoma* spp. are considered aposematic because they are very brightly coloured and easily observed. The unpalatability of *Cyphoma* is probably a consequence of their gregarious feeding behaviour on a large array of Caribbean octocorals (Reijnen *et al*., 2010; chapter 2) from which they acquire (precursors of) these unpalatable compounds. Its IWP equivalent is *Cuspivolva tigris* which has a conspicuous mantle pattern resembling tiger stripes. *Primovula rosewateri* / *P. beckeri*, *Calpurnus verrucosus* and *Ovula ovum* can be considered aposematic, but only for *O. ovum* there is data on toxicity or unpalatability (Coll *et al*., 1983). Aposematism has therefore arisen at least twice in ovulid evolution, once in the Atlantic (*C. gibbossum*) and once in the IWP (*O. ovum*). It is expected that more cases of aposematism will be discovered in the Ovulidae when the unpalatability of other candidate species will be investigated.

Acknowledgements

This study could not have been performed without the help of many people and organisations.

Dr Bert W. Hoeksema (Naturalis) organised the Raja Ampat, Ternate and Selat Lembeh expeditions together with Ir. Yosephine T. Hermanlimianto under the umbrella of E-win (Ekspedisi Widya Nusantara). Research permits were granted by LIPI and RISTEK and Papua Diving, Bunaken Village and the LIPI field stations at Ternate and Bitung accommodated the research. The Semporna Marine Ecological Expedition (SMEE2010) and Tun Mustapha Park expedition (TMP2012) were jointly organized by WWF-Malaysia (only SMEE2010), Universiti Malaysia Sabah's Borneo Marine Research Institute, Naturalis Biodiversity Center and Universiti Malaya's Institute of Biological Sciences, while research permission was granted by the Economic Planning Unit, Prime Minister's Department, Economic Planning Unit Sabah, Sabah Parks and Department of Fisheries Sabah. The MV Celebes Explorer and the schooner Raja Laut accommodated the research. Dr. Mike Berumen kindly accommodated and supported the research at the Red Sea. Dr. Simone Montano, Dr. Davide Seveso, Dr. Paolo Galli and Dr. Francesca Benzoni assisted, accommodated and helped with research at the Maldives. I would also like to thank John Slapcinsky and Gustav Paulay for some american ovulid specimens from the Florida Museum of Natural History. Kaveh Samimi is kindly acknowledged because he collected very rare ovulid species from the Persian Gulf. Camiel Doorenweerd is thanked for his help with setting up BEAUTi and BEAST2 analyses and Dr. Frank Wesselingh provided valuable information for the discussion on fossils and fossilisation processes. Dr. Leen van Ofwegen was essential for his help in identifying the Octocorallia. Dr. Bert Hoeksema, Prof. dr. Edmund Gittenberger and Dr. Leen van Ofwegen and Dr. Sancia van der Meij carefully read, commented and improved the manuscript. External funding for the various expeditions to Indonesia and Malaysia was provided by: the Van Tienhoven Foundation for International Nature Protection, Schure-Beijerinck-Popping Fund (KNAW), National Geographic Young Explorers Grant, Alida M. Buitendijkfonds, Jan-Joost ter Pelkwijkfonds, and Leiden University Funds.

Suppl. mat. 1. Provenance data for the Octocorallia.

Species (author)	Locality	Latitude	Longitude	Date	Depth (m)
Acanthogorgia sp.	Indonesia, Sulawesi, Lembeh, Tanjung Kuning, LEM.23	1.38633 N	125.17312 E	02/11/12	16
Alertigorgia orientalis (Ridley, 1884)	Malaysia, Borneo, Kudat, Pulau Banggi, TMP.38	7.13040 N	117.22832 E	09/20/12	23
Annella sp.	Indonesia, Halmahera, Hiri, Tanjung Ngafauda, TER.14	0.910639 N	127.317417 E	10/31/09	16
Antillogorgia acerosa (Pallas, 1766)	Curaçao, Marie Pampoen, CAO.21	12.090761 N	68.904956 W	11/05/13	10
Antillogorgia americana (Gmelin, 1791)	Curaçao, Waterfabriek I, CAO.02	12.108611 N	68.950333 W	10/19/13	-
Antillogorgia bipinnata (Verrill, 1864)	Curaçao, Grote Knip, CAO.22	12.351139 N	69.151917 W	11/06/13	37
Astrogorgia sp.	Indonesia, Halmahera, Ternate, Sulamadaha Beach, TER.04	0.863222 N	127.334472 E	10/26/09	8
Bebryce sp.	Malaysia, Semporna, Church Reef 2, SEM.46	4.686117 N	118.64855 E	12/13/10	10
Briareum asbestinum (Pallas, 1766)	Curaçao, Playa Jeremy, CAO.07	12.329028 N	69.079194 W	10/22/13	6
Chironephthya sp.	Malaysia, Sipadan Isl., Mid Reef, SEM.59	4.113333 N	118.636111 E	12/18/10	24
Dendronephthya sp.	Malaysia, Semporna, Pom Pom Isl., SEM.32	4.591944 N	118.861389 E	12/09/10	23
Echinogorgia sp.	Indonesia, Sulawesi, Bitung, Tanjung Nanas II, LEM.05	1.46213 N	125.22823 E	02/01/12	14
Ellisella barbadensis (Duchassaing & Michelotti, 1864)	Curaçao, Blauwbaai, CAO.04	12.134917 N	68.984306 W	11/02/13	37
Ellisella sp.	Malaysia, Semporna, Bohayen Isl., SEM.26	4.468333 N	118.9475 E	12/08/10	24
Erythropodium caribaeorum (Duchassaing & Michelotti, 1860)	Curaçao, Playa Forti, CAO.18	12.366139 N	69.15375 W	11/01/13	15
Eunicea calyculata (Ellis & Sollander, 1786)	Curaçao, Waterfabriek II, CAO.10	12.110278 N	68.954528 W	10/31/13	15
Eunicea clavigera Bayer, 1961	Curaçao, Slangenbaai, CAO.09	12.139722 N	68.997194 W	10/24/13	5
Eunicea fusca Duchassaing & Michelotti, 1860	Curaçao, Hilton Reef, CAO.01	12.121789 N	68.969508 W	10/31/13	25
Eunicea succinea (Pallas, 1766)	Curaçao, W Piscadera Baai, CAO.03	12.12275 N	68.970428 W	11/02/13	6
Euplexaura sp.	Indonesia, Sulawesi, Lembeh, N Sarena Kecil, LEM.32	1.45746 N	125.22711 E	02/16/12	28
Gorgonia ventalina Linnaeus, 1758	Curaçao, Marie Pampoen, CAO.21	12.090761 N	68.904956 W	11/05/13	8
Isis hippuris Linnaeus, 1758	Malaysia, Borneo, Kudat, Pulau Malawali, TMP.08	6.98313 N	117.50312 E	09/09/12	12
Junceella sp.	Republic of the Maldives, Maghoodoo Isl., Nilandhoo. MAD.17	3.049972 N	72.878778 E	02/24/15	25

Suppl. mat. 1. Cont.

Species (author)	Locality	Latitude	Longitude	Date	Depth (m)
Melithaea ochracea (Linnaeus, 1758)	Indonesia, Mollucas, Ambon, Hitu, N coast Mamala	3.537997 S	128.206414 E	11/21/90	10-15
Menella sp.	Malaysia, Johor, Pulau Sibu Tengah, Malang Acha	2.185033 N	104.105117 E	05/26/12	11
Muricea muricata (Pallas, 1766)	Curaçao, Waterfabriek II, CAO.10	12.110278 N	68.954528 W	10/31/13	8
Muricea pinnata Bayer, 1961	Curaçao, Slangenbaai, CAO.09	12.139722 N	68.997194 W	10/25/13	37
Nephthea sp.	Saudi Arabia, Thuwal, Al Dgiyg, THU.11	22.213861 N	38.983111 E	11/12/14	17
Paralemnalia sp.	Saudi Arabia, Thuwal, Um Albalam, THU.10	22.193556 N	38.9475 E	11/12/14	10
Paratelesto sp.	Malaysia, Semporna, Larapan Isl., SEM.47	4.574283 N	118.658017 E	12/13/10	23
Plexaura homomalla (Esper, 1792)	Curaçao, Playa Manzaliña, CAO.13	12.245611 N	69.105222 W	10/26/13	8
Plexaurella dichotoma (Esper, 1791)	Curaçao, Playa Lagun, CAO.16	12.318472 N	69.150167 W	10/29/13	8
Pseudoplexaura porosa (Houttuyn, 1772)	Curaçao, Playa Lagun, CAO.16	12.318472 N	69.150167 W	10/29/13	16
Pseudoplexaura sp.	Curaçao, Playa Manzaliña, CAO.13	12.245611 N	69.105222 W	10/26/13	14
Pterogorgia citrina (Esper, 1792)	Curaçao, Playa Kanoa, CAO.20	12.174722 N	68.865028 W	11/04/13	4
Pterogorgia guadelupensis Duchassaing & Michelin, 1846	Curaçao, Playa Lagun, CAO.16	12.318472 N	69.150167 W	10/29/13	5
Rumphella sp.	Saudi Arabia, Thuwal, Al Dgiyg, THU.11	22.213861 N	38.983111 E	11/12/14	29
Sarcophyton sp.	Saudi Arabia, Thuwal,Fsar+, THU.16	22.247639 N	39.003639 E	11/18/14	17
Scleronephthya sp.	Indonesia, Sulawesi, Bitung, Batu Kapal, LEM.35	1.54912 N	125.29218 E	02/18/12	23
Sinularia sp.	Saudi Arabia, Thuwal, Fsar+, THU.16	22.247639 N	39.003639 E	11/18/14	16
Siphonogorgia sp.	Malaysia, Semporna, Church Reef I, SEM.45	4.681667 N	118.658056 E	12/13/10	23
Solenocaulon sp.	Malaysia, Semporna, Boheydulang Isl. 2 outer reef, SEM.38	4.56755 N	118.757533 E	12/11/10	21
Stereonephthya sp.	Saudi Arabia, Thuwal, Al Bilut (Rose Reef), THU.20	22.31 N	38.886333 E	11/20/14	12
Subergorgia sp.	Republic of the Maldives, Maghoodoo Isl., Beyrufushi, MAD.07	3.108056 N	73.01875 E	02/26/15	31
Villogorgia sp.	Republic of the Maldives, Maghoodoo Isl., Free Climbing, MAD.18	3.065611 N	72.920972 E	02/24/15	23
Viminella sp.	Malaysia, Sipadan Isl., Mid Reef, SEM.59	4.113333 N	118.636111 E	12/18/10	32

Suppl. mat. 2. Provenance data for the Ovulidae.

Species (author)	Locality	Latitude	Longitude	Date	Depth (m)
Notadusta punctata (Linnaeus, 1771)	Malaysia, Borneo, Kapikan Reef, SEM.33	4.651367 N	118.821733 E	12/09/10	-
Pedicularia sp.	Indonesia, Sulawesi, Bitung, Tanjung Pandea, LEM.24	1.39797 N	125.16637 E	02/11/12	18
Pedicularia sp.	Malaysia, Semporna, Tabawan Island, SEM.51	4.78725 N	118.417033 E	12/16/10	12
Pedicularia vanderlandi Goud & Hoeksema, 2001	Indonesia, Bali, N side of Nusa Lembongan, Tanjung Taal	8.659167 S	115.443611 E	04/22/01	<35
Cymbovula acicularis (Lamarck, 1810)	Curacao, Barankí Karanito, CUR.15	12.037083 N	68.803944 W	05/19/05	25
Cyphoma gibbosum (Linnaeus, 1758)	Curacao, Barankí Karanito, CUR.15	12.037083 N	68.803944 W	05/19/05	9
Cyphoma signatum Pilsbry & McGinty, 1939	Curacao, Marie Pampoen, CUR.05	12.095028 N	68.911944 W	06/10/05	5
Cyphoma mcgintyi Pilsbry, 1939	USA, Florida, N of St. Petersburg	28.537806 N	84.272694 W	03/13/11	26
Neosimnia spelta (Linnaeus, 1758)	Spain, Begur, Aigua Blava	41.935542 N	3.218031 W	2008	<15
Simnia patula (Pennant, 1777)	North Sea, South side of Doggersbank	54.333333 N	2.333333 E	09/19/03	-
Primovula rosewateri (Cate, 1973)	Malaysia, Semporna, Sipadan Isl., Hanging Gardens, SEM.60	4.1112 N	118.624817 E	12/18/10	22
Primovula tadashigei (Cate, 1973)	Indonesia, W. Papua, Raja Ampat Isls., Yeffam Isl., NW Pulau Keruo, RAJ.65	0.5876 S	130.295183 E	12/13/07	18
Primovula beckeri (Sowerby III, 1900)	Republic of the Maldives, Maghoodoo Isl., Beyrufushi, MAD.07	3.108056 N	73.01875 E	02/26/15	31
Diminovula concinna (G.B. Sowerby II in Adams & Reeve, 1848)	Malaysia, Borneo, Kudat, NE Pulau Banggi, TMP.20	7.38158 N	117.37349 E	09/14/12	8
Hiatavolva depressa (G.B. Sowerby III, 1875)	Malaysia, Borneo, Kudat, NE Pulau Balambangan, TMP.30	7.33625 N	117.02342 E	09/23/12	12
Prosimnia draconis Cate, 1973	Malaysia, Borneo, Kudat, W of Pulau Tigaba, TMP.03	6.89584 N	117.35427 E	09/07/12	8
Prosimnia semperi (Weinkauff, 1881)	Indonesia, NW Lombok (mainland), Teluk Narat, underneath jetty	8.40833 S	116.07 E	08/04/11	5
Prosimnia korkosi Fehse, 2005	Saudi Arabia, Tiger Head Isl., SAU.23	16.47458 N	42.11919 E	03/10/13	10
Prosimnia hepcae Lorenz & Fehse, 2011	Saudi Arabia, Thuwal, Al Fahal S, THU.05	22.246528 N	38.959194 E	11/09/14	6
Dentiovula cf. *dorsuosa*	Malaysia, Semporna, Timba Timba Isl., SEM.27	4.560883 N	118.924817 E	12/08/10	17
Dentiovula masaoi Cate, 1973	Malaysia, Semporna, Kulapuan Isl. 2 N, SEM.31	4.5354 N	118.838383 E	12/09/10	20
Dentiovula dorsuosa (Hinds, 1844)	Malaysia, Semporna, Darby Bank, SEM.04	4.139722 N	118.170722 E	11/30/10	15
Dentiovula mariae (Schilder, 1941)	Malaysia, Borneo, Kudat, S Pulau Banggi, TMP.36	7.09993 N	117.0892 E	09/19/12	20

Suppl. mat. 2. Cont.

Species (author)	Locality	Latitude	Longitude	Date	Depth (m)
Dentiovula "*dorsuosa*"	Republic of the Maldives, Maghoodoo Isl., Free Climbing, MAD.18	3.065611 N	72.920972 E	02/24/15	23
Procalpurnus lacteus (Lamarck, 1810)	Indonesia, Sulawesi, Bitung, Batu Kapal, LEM.35	1.54912 N	125.29218 E	02/18/12	10
Cuspivolva cuspis (Cate, 1973)	Malaysia, Borneo, Kudat, S Pulau Banggi, TMP.39	7.12294 N	117.14334 E	09/19/12	14
Primovula astra Omi & Iino, 2005	Indonesia, N Sulawesi, Siladen Isl., Siladen Timur, MEN.08	1.635467 N	124.806717 E	11/30/08	30
Prosimnia piriei (Petuch, 1973)	Indonesia, N Sulawesi, Bunaken Isl., Lekuan II, MEN.04	1.6001 N	124.766217 E	11/27/08	10
Rotaovula septemmacula (Azuma, 1974)	Malaysia, Semporna, Larapan Isl. 2 S, SEM.57	4.547517 N	118.6087 E	12/17/10	12
Naviculavolva deflexa (G.B. Sowerby II, 1848)	Malaysia, Semporna, Boheydulang Isl. 1 S, SEM.37	4.58345 N	118.777917 E	12/11/10	21
Naviculavolva elegans Fehse, 2009	Indonesia, N Sulawesi, Manado, Tiwoho, MEN.19	1.596333 N	124.837633 E	12/06/08	10
Naviculavolva debelius Lorenz & Fehse, 2011	Saudi Arabia, Thuwal, Al Dgiyg, THU.11	22.213861 N	38.983111 E	11/12/14	23
Naviculavolva malaita (Cate, 1976)	Malaysia, Semporna, Horn Reef, SEM.08	4.242233 N	118.440033 E	12/01/10	6
Naviculavolva kurziana (Cate, 1976)	Indonesia, Halmahera, Maitara Isl., Maitara NW, TER.10	0.742222 N	127.364139 E	10/29/09	14
Crenavolva striatula (G.B. Sowerby I, 1828)	Malaysia, Borneo, Kudat, S Pulau Banggi, TMP.37	7.08533 N	117.05938 E	09/19/12	20
Crenavolva aureola (Fehse, 2002)	Malaysia, Semporna, Si Amil Isl., SEM.16	4.31725 N	118.875183 E	12/04/10	23
Crenavolva sp.	Oman, SE of Muscat, Bandar Al-Jissah	23.524983 N	58.739183 E	06/01/09	-
Crenavolva trailli (A. Adams, 1855)	Malaysia, Borneo, Kudat, NE of Kudat, TMP.41	6.9967 N	117.05372 E	09/18/12	10
Crenavolva tinctura (Garrard, 1963)	Indonesia, Halmahera, Tidore Isl., N of Desa Rum, TER.18	0.743278 N	127.385083 E	11/04/09	38
Crenavolva tinctura (Garrard, 1963)	Indonesia, Halmahera, Tidore Isl., N of Desa Rum, TER.18	0.743278 N	127.385083 E	11/04/09	38
Crenavolva cruenta Gowlett-Holmes & Holmes, 1989	Indonesia, Sulawesi, Bitung, Tanjung Nanas II, LEM.05	1.46213 N	125.22823 E	02/01/12	28
Dentiovula eizoi Cate & Azuma in Cate, 1973	Malaysia, Semporna, Pom Pom Isl., SEM.32	4.591944 N	118.861389 E	12/09/10	18
Dentiovula colobica (Azuma & Cate, 1971)	Indonesia, Halmahera, Pasir Lamo (W side), TER.26	0.889028 N	127.4595 E	11/08/09	15
Archivolva clava (Habe, 1991)	Indonesia, Halmahera, Tidore Isl., Desa Tahua, TER.07	0.75275 N	127.392028 E	10/28/09	11
Margovula bimaculata (Adams, 1854)	Malaysia, Semporna, Creach Reef, SEM.20	4.315933 N	118.605117 E	12/05/10	16
Diminovula margarita (G.B. Sowerby I, 1828)	Indonesia, Halmahera, Teluk Dodinga, Karang Galiasa Besar E, TER.38	0.846 N	127.585389 E	11/14/09	7

Suppl. mat. 2. Provenance data for the Ovulidae.

Species (author)	Locality	Latitude	Longitude	Date	Depth (m)
Diminovula sp.	Saudi Arabia, Thuwal, Al Asoul, THU.07	22.265361 N	39.002139 E	11/10/14	13
Diminovula aurantiomaculata (Cate & Azuma, 1973)	Malaysia, Borneo, Kudat, NE of Kudat, TMP.40	7.03377 N	117.07363 E	09/18/12	8
Diminovula alabaster (Reeve, 1865)	Malaysia, Borneo, Kudat, NE of Kudat, TMP.40	7.03377 N	117.07363 E	09/18/12	17
Diminovula sp.	Malaysia, Borneo, Kudat, NE of Pulau Bilang Bilangan, TMP.19	7.29537 N	117.40169 E	09/14/12	11
Prionovolva wilsoniana Cate, 1973	Indonesia, Sulawesi, Bitung, Tanjung Kubur, LEM.06	1.47908 N	125.24976 E	02/01/12	18
Diminovula culmen (Cate, 1973)	Malaysia, Semporna, Second Reef, SEM.02	4.175694 N	118.298472 E	11/29/10	3
Prionovolva ericae (Cossignani & Calo, 2002)	Indonesia, W. Papua, Raja Ampat Isls., S Friwin Isl., RAJ.55	0.481817 S	130.69835 E	12/07/07	20
Habuprionovolva aenigma (Azuma & Cate, 1971)	Indonesia, Halmahera, Tanjung Ratemu (S of river), TER.27	0.912361 N	127.486083 E	11/08/09	10
Calpurnus verrucosus (Linnaeus, 1758)	Malaysia, Semporna, Ligitan Isl. 1 SW, SEM.13	4.187056 N	118.79155 E	12/03/10	6
Pellasimnia annabelae Lorenz & Fehse, 2009	Malaysia, Semporna, Larapan Isl. 2 S, SEM.57	4.547517 N	118.6087 E	12/17/10	12
Pellasimnia angasi (Reeve, 1865)	Malaysia, Johor, Pulau (Babi) Besar, Teluk Bakau	2.451433 N	103.971017 E	05/30/12	3
Phenacovolva parvita Cate & Azuma in Cate, 1973	Malaysia, Semporna, Horn Reef, SEM.08	4.242233 N	118.440033 E	12/01/10	24
Ovula ovum (Linnaeus, 1758)	Malaysia, Semporna, Kapalai Isl., SEM.10	4.218 N	118.67225 E	12/02/10	3
Ovula costellata Lamarck, 1810	New Caledonia, Baie de Nakety	21.509467 S	166.097083 E	04/21/12	<30
Phenacovolva rosea (Adams, 1854)	Malaysia, Semporna, Horn Reef, SEM.08	4.242233 N	118.440033 E	12/01/10	20
Phenacovolva nectarea Iredale, 1930	Malaysia, Borneo, Kudat, S of Pulau Malawali, TMP.01	6.95247 N	117.28374 E	09/07/12	11
Phenacovolva morrisoni Lorenz & Fehse, 2009	Malaysia, Borneo, Kudat, E of Pulau Bilang Bilangan, TMP.17	7.24367 N	117.40119 E	09/12/12	6
Phenacovolva cf. *rosea*	Oman, SE of Muscat, Bandar Al-Jissah	23.524983 N	58.739183 E	06/01/09	-
Hiatavolva coarctata (G.B. Sowerby II in Adams & Reeve, 1848)	Malaysia, Semporna, Bohayen Isl., SEM.26	4.468333 N	118.9475 E	12/08/10	37
Aclyvolva lamyi (Schilder, 1932)	Malaysia, Borneo, Kudat, NE of Pulau Banggi, TMP.21	7.34737 N	117.35674 E	09/14/12	14
Aclyvolva lanceolata (G.B. Sowerby II, 1848)	Malaysia, Borneo, Kudat, SW of Pulau Tigaba, TMP.04	6.81093 N	117.36642 E	09/08/12	6