Phylogenetic ecology of octocoral - gastropod associations

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Chapter 4

Molecular data for *Crenavolva* species (Gastropoda, Ovulidae) reveals the synonymy of *C. chiapponii*

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Abstract

During fieldwork in Indonesia and Malaysia, eight lots containing 33 specimens belonging to the genus *Crenavolva* (Ovulidae) were collected. Species were initially identified as *C. aureola*, *C. chiapponii*, *C. striatula* and *C. trailli*, respectively. For *C. chiapponii* this is the second record. In contrast to the ecological data available from the original description of this species, it was found in shallow water on a gorgonian host coral, i.e. *Acanthogorgia* sp. A molecular analysis based on COI and 16S mtDNA markers, including sequence data obtained from GenBank, showed that *C. chiapponii* should be considered a junior synonym of *C. aureola* and that previously identified ovulid specimens are probably misidentified.

Introduction

The nominal taxon *Crenavolva* was introduced as a subgenus by Cate (1973), together with the subgenera *Crenavolva*, *Cuspivolva* and *Serratovolva*. In the most recent overview regarding Ovulidae these three taxa are considered genera (Lorenz and Fehse, 2009). At present 18 nominal species are recognized within *Crenavolva* (Rosenberg, 2014), most of which are considered rare (Lorenz and Fehse, 2009). These species are considered rare because few specimens have been collected, probably because they occur at depths greater than standard recreational diving depth of c. 30 m and/or are only known from a limited geographical area, usually just the type locality. This also accounts for *C. chiapponii* Lorenz and Fehse, 2009, which is only known from Balicasag Isl., Bohol, Philippines, where specimens were trawled from 70-120 m depth and, therefore, were considered rare and confined to deeper water (Lorenz and Fehse, 2009).

Table 1. Specimens used in the analyses, including locality, host, and GenBank accession data.

Collection number	Species (author)	Locality (Locality code)
RMNH.Mol.164072	*Crenavolva aureola* (Fehse, 2002)	Malaysia, Semporna, Si Amil Island (SEM.16)
RMNH.Mol.164085	*Crenavolva aureola* (Fehse, 2002)	Indonesia, Halmahera, Tidore, N of Desa Rum (TER.18)
RMNH.Mol.164209	*Crenavolva aureola* (Fehse, 2002)	Indonesia, Halmahera, Tanjung Ratemu (S of river) (TER.21)
RMNH.Mol.164211	*Crenavolva chiapponni* Lorenz and Fehse, 2009	Indonesia, Halmahera, Tanjung Ratemu (S of river) (TER.27)
RMNH.Mol.164217	*Crenavolva chiapponii* Lorenz and Fehse, 2009	Indonesia, Lembeh, Tanjung Kusukusu (LEM.31)
RMNH.Mol.164062	*Primovula rosewateri* (Cate, 1973)	Malaysia, Semporna, Kulapuan Island 2, N side (SEM.31)
RMNH.Mol.164186	*Crenavolva striatula* (Sowerby 1st, 1828)	Malaysia, Sabah, S Pulau Banggi, E Molleangan Besar Island, (TMP.37)
RMNH.Mol.164144	*Crenavolva trailli* (A. Adams, 1855)	Malaysia, Sabah, Kalang, (TMP.41)
RMNH.Mol.164189	*Crenavolva trailli* (A. Adams, 1855)	Malaysia, Sabah, Kalang, (TMP.41)
-	*Crenavolva* cf. *rosewateri* (Cate, 1973)	Philippines, Bohol, Balicasag Island
-	*Crenavolva tokuoi* Azuma, 1989	Philippines, Bohol, Balicasag Island
-	*Primovula beckeri* (Sowerby 3rd, 1900)	Indonesia, Sulawesi
-	*Ovula ovum* (Linnaeus, 1758)	Indonesia, Sulawesi, Spermonde Archipelago

Like almost all other ovulids, species of *Crenavolva* are associated with octocoral hosts (Schiaparelli *et al*., 2005; Reijnen, 2010) belonging to several families (e.g. Melithaeidae, Ellisellidae, Subergorgiidae and Plexauridae). However, the host species are usually not collected or are disregarded and therefore unknown, which is also the case for *C. chiapponii*.

Molecular data (16S and COI) obtained from *Crenavolva* was used by Meyer (2003) to root the phylogeny of the Cypraeidae. Later, the 16S sequence data were used by Schiaparelli *et al*., (2005) to produce the first molecular phylogenetic reconstruction of the Ovulidae, which included two *Crenavolva* species: *C.* cf. *rosewateri* (Cate, 1973) and *C. tokuoi* Azuma, 1989. In the present study, material of four additional nominal *Crenavolva* species, amongst other ovulids, have been used to reconstruct a phylogeny. The newly acquired molecular data are for *C. aureola* (Fehse, 2002), *C. chiapponii* Lorenz and Fehse, 2009, *C. striatula* (Sowerby I, 1828) (type species), and *C. trailli*

Coordinates	Date collected	Host species	GenBank Accession number (16S ; COI)	Reference
4°19’02.1”N; 118°52’30.7”E	4-12-2010	*Acanthogorgia* sp.	KP033143 ; KP033151	This publication
0°44’35.8”N; 127°23’06.3”E	4-11-2009	*Acanthogorgia* sp.	KP033144 ; KP033152	This publication
0°54’24.7”N; 127°29’17.7”E	5-11-2009	*Acanthogorgia* sp.	KP033148 ; KP033156	This publication
0°54’44.5”N; 127°29’09.9”E	8-11-2009	*Acanthogorgia* sp.	- ; KP033157	This publication
1°27’13.8”N; 125°14’13.0”E	16-2-2012	*Acanthogorgia* sp.	KP033149 ; KP033158	This publication
4°32’07.4”N; 118°50’18.2”E	9-12-2010	*Paratelesto* sp.	KP033142 ; KP033150	This publication
7°05’07.2”N; 117°03’33.8”E	19-9-2012	*Echinogorgia* sp.	KP033146 ; KP033154	This publication
6°59’48.1”N; 117°03’13.4”E	18-9-2012	*Subergorgia* sp.	KP033145 ; KP033153	This publication
6°59’48.1”N; 117°03’13.4”E	18-9-2012	*Paraplexaura* sp.	KP033147 ; KP033155	This publication
-	-	-	AY161394 ; AY161627	Meyer, 2003
-	-	-	AY161390 ; AY161623	Meyer, 2003
-	-	-	AJ868555 ; -	Schiaparelli *et al*., 2005
-	-	-	AY161399 ; AY161632	Meyer, 2003

(Adams, 1855). In addition to this phylogenetic reconstruction, data on host species and distributional records are given for this group of rarely recorded ovulid snails.

Material and methods

Collection and identification

During fieldwork in Indonesia (Halmahera, Ternate; Sulawesi, Lembeh Strait) and Malaysia (Borneo, Semporna and Kudat) specimens of *Crenavolva* species were collected by SCUBA diving (Table 1).

The snails and their octocoral hosts were photographed in situ (Fig. 1) whenever possible and subsequently fixed in 80% ethanol. The holotype of *C. chiapponii* was studied at the Muséum national d'Histoire naturelle (MNHN) in Paris. For the identification of the other ovulid species, Cate (1973), Fehse (2002b) and Lorenz and Fehse (2009) were used. For the identification of the host species, microscopy slides of their calcareous skeletal parts (sclerites) were made by dissolving the samples in a 4% solution of household bleach. The residual sclerites were rinsed with tap water followed by demineralised water before mounting on a slide or on a stub for Scanning Electron Microscopy (SEM). Stubs with sclerites were coated with Au/Pd before SEM images were made with a JEOL 6480 LV. Identification of the octocorals to genus level was based on Stiasny (1947) and Fabricius and Alderslade (2001).

Barcoding Ovulidae

Specimens were barcoded for the COI barcoding region and for additional phylogenetic research also for the 16S marker. Tissue samples obtained from the foot and/or mantle were extracted with the Machery-Nagel DNA extraction kit on a KingFisher Flex. The standard COI barcoding primers by Folmer *et al*. (1994) and the Palumbi (1996) 16S primers were used. PCR amplification was performed on a C1000 Touch Thermal Cycler (Bio-RAD). Sequencing of the PCR products was performed at Macrogen Europe on an ABI 3730xl Automated Sequencer. Sequences were edited in Sequencher 4.10.1

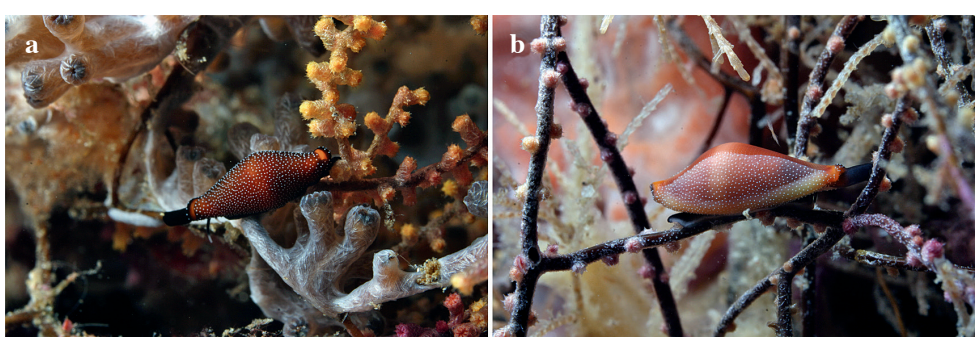

Fig. 1. a) In situ image of a) *Crenavolva aureola* (Fehse, 2002) (RMNH.Mol.164209) and b) *C. chiapponii* Lorenz and Fehse, 2009 (RMNH.Mol.164211) on *Acanthogorgia* sp. at Halmahera, Indonesia at 21 m and 17 m depth respectively.

and aligned with GUIDANCE (Penn *et al*., 2010) using the MAFFT algorithm (Katoh *et al*., 2005). Selecting an evolutionary model was done with jModeltest based on the Akaike Information Criterion score. MEGA 6.0.6 (Tamura *et al*., 2013) was used to perform Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses and to calculate p-distances. Bayesian analyses were performed in MrBayes 3.2.0 (Ronquist and Huelsenbeck, 2003). MrBayes was run for 4,000,000 generations with six chains. Data were sampled every 100 generations. Sequence data for *Ovula ovum* (Linnaeus, 1758) from GenBank was used as an outgroup. GenBank data for *Crenavolva* cf. *rosewateri* (Cate, 1973), *C. tokuoi* Azuma, 1989 and *Primovula beckeri* (Sowerby III, 1900) was also included in the phylogenetic analyses.

Results

Collecting and morphology

Eight lots, containing 33 specimens representing four nominal *Crenavolva* species (*C. aureola*, *C. chiapponii*, *C. striatula* and *C. trailli*) were collected in Indonesia and Malaysia (Table 1; Fig. 2). For *C. chiapponii* this is the first record from shallow water. The specimens were assigned to these nominal species based on shell shape (rhomboid, inflated or slender) and the colour bands on the dorsum, which in case of *C. striatula* were also present on the labrum. For *C. aureola* and *C. chiapponii* the absence or presence of a white dorsal band on the shell is allegedly the most obvious character to distinguish the species.

After examination of the illustrations presented by Lorenz and Fehse (2009) and the newly collected material, minor morphological differences (strongly or weakly pronounced dentation, keeling angle, strongly or weakly produced funiculum, position of the widest part of the shell) do not clearly separate between *C. aureola* and *C. chiapponii* and can be considered morphological variation in a single species. The soft tissue colouration of both *C. aureola* and *C. chiapponii* is very similar (e.g. Fig. 1; Lorenz and Fehse 2009: A106, A107, p. 527). Both have a semi-transparent mantle which is entirely covered with small, irregularly placed, white dots, and both have a completely black or white foot, black tentacles with white tips, and a black siphon.

Molecular data

Nine specimens representing five species were sequenced for COI and 16S. For one sample of *C. chiapponii* (RMNH.Mol.164211) the 16S marker could not be amplified. Sequences were concatenated and aligned (GUIDANCE alignment score: 0.965034) which resulted in an alignment length of 1,080 base pairs per specimen including indels. Sequences obtained from GenBank are slightly shorter (~40 base pairs), these missing base pairs were coded as 'missing data'. The program jModeltest yielded in HKY+G as most optimal evolutionary model. This evolutionary model was implemented in the Bayesian and ML analysis. The results from the different phylogenetic reconstructions were congruent, therefore only the ML tree is shown (Fig. 3).

In the phylogenetic reconstructions, specimens of *Crenavolva striatula* and *C. tokuoi* form an unresolved trichotomy with the other *Crenavolva* specimens. The two

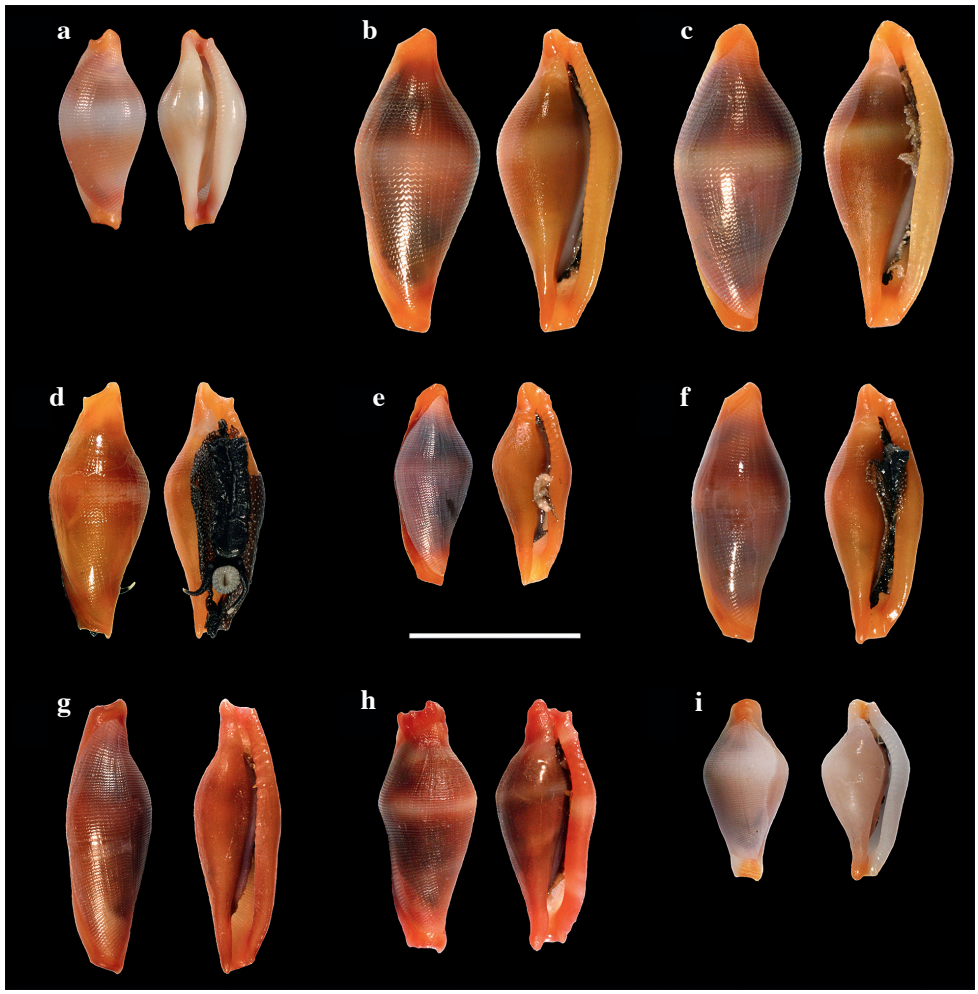

Fig. 2. Dorsal and ventral views of shells. a) Holotype of *Crenavolva chiapponii* Lorenz and Fehse, 2009 (MNHN 21244) b) *C. chiapponii* Lorenz and Fehse, 2009 (RMNH.Mol.164211) c) *C. chiapponii* Lorenz and Fehse, 2009 (RMNH.Mol.164217) d) *C. aureola* (Fehse, 2002) (RMNH.Mol.164085) e) *C. aureola* (Fehse, 2002) (RMNH.Mol.164072) f) *C. aureola* (Fehse, 2002) (RMNH.Mol.164209) g) *C. trailli* (Adams, 1855) (RMNH.Mol.164144) h) *C. striatula* (Sowerby I, 1828) (RMNH.Mol.164186) i) *Primovula rosewateri* (Cate, 1973) (RMNH.Mol.164062). Scale bars: 5 mm.

Primovula species cluster together and are well-supported sister species to all the *Crenavolva* species (with *C. striatula* as type species for the genus). This implies that the *Crenavolva* species used herein form a monophyletic group. The clustering of two *C. trailli* specimens is highly supported. Another well-supported clade holds three nominal species: *Crenavolva aureola*, *C. chiapponii* and *C.* cf. *rosewateri*. The pairwise p-distances between these three species are very low (16S: 0.2%; COI: 0.7%; concate-

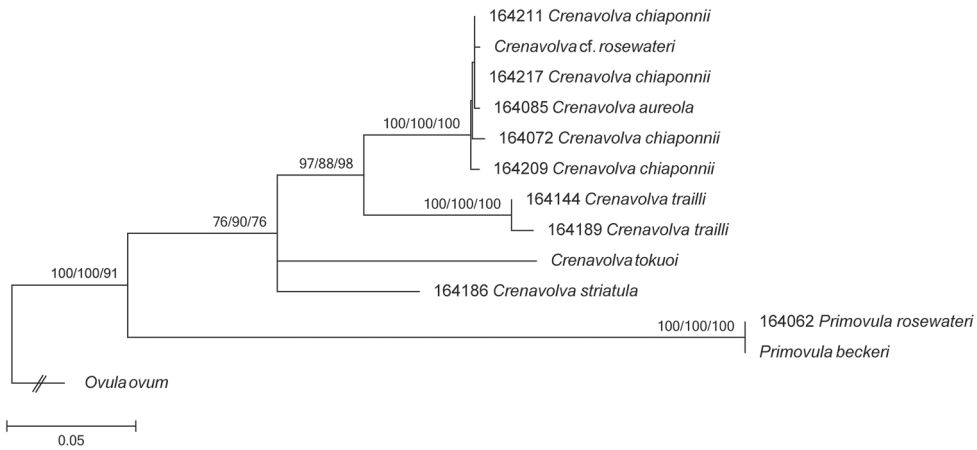

Fig. 3. Maximum Likelihood cladogram with support values for the ML/MP/BP analyses. Numbers preceding the species names represent RMNH.Mol. collection numbers of Naturalis Biodiversity Center; species names without numbers are obtained from GenBank for which additional data can be found in Table 1.

nated: 0.9%). In contrast, the sequence divergence between *C. trailli* and the *C. chiapponii* / *C. aureola* clade is almost ten times larger (16S: 5.2%; COI: 8.7%; concatenated: 8.2%). The sequence divergence between the two *C. trailli* specimens (16S: 0.6%; COI: 0.8%; concatenated: 0.8%) is almost equal to that between *C. aureola* and *C. chiapponii*. With the help of the Automatic Barcode Gap Discovery tool (ABGD) (Puillandre *et al*., 2011), the data were analysed to identify the MOTU's within the dataset. The results of this analysis showed that the barcode gap to identify the different species is 5-6% sequence divergence. This resulted in five groups containing the following species: 1, *C. aureola*, *C. chiapponii*, *C.* cf. *rosewateri*; 2, *C. trailli*; 3, *C. tokuoi*; 4, *C. striatula*; 5, *P. rosewateri*. One of the samples obtained from GenBank, viz. *Crenavolva* cf. *rosewateri* (= *Primovula* cf. *rosewateri*), clusters surprisingly within the clade containing *C. aureola* and *C. chiapponii* and not with the other *Primovula rosewateri* specimen. Instead, *Primovula beckeri* proves to be identical to the newly sequenced specimen of *Primovula rosewateri* from Malaysia.

Octocoral hosts

Almost all Ovulidae species are associated with Octocorallia hosts. By examining the sclerites and the habitus of the host corals, several new host species for ovulids of the genus *Crenavolva* could be identified. An overview of previously identified host species and new records is provided in Table 2. Some of the former host identifications were published with obsolete generic names, and therefore their names in the current literature are also provided.

Before *C. chiapponii* was synonymised, *Acanthogorgia* would have been a new host record. Yet, Reijnen (2010) already recorded *Acanthogorgia* sp. as a host for *C. aureola* and therefore it is not a new host record. Morphologically at least two different species

Table 2. Literature overview of the octocoral hosts of selected *Crenavolva* species including new records. Updated names of the octocoral hosts are provided between parentheses.

Ovulid species	Host genera	Reference
Crenavolva aureola	*Euplexaura*; *Astromuricea* (= *Echinogorgia*); *Acanthogorgia*	Lorenz and Fehse, 2009; Reijnen, 2010
Crenavolva chiapponii (= *C. aureola)*	*Acanthogorgia*	this publication; Reijnen, 2010
Crenavolva striatula	*Ellisella*; *Euplexaura*; *Echinogorgia*	Lorenz and Fehse, 2009; Yamamoto, 1973; Cumming, 1997; Mase 1989;
Crenavolva trailli	*Echinogorgia*; *Anthoplexaura* (= *Astrogorgia*); *Plexauroides* (= *Echinogorgia*); *Euplexaura*; *Subergorgia*	Goh *et al*., 1999; Mase, 1989
Primovula rosewateri	*Subergorgia*; *Dendronephthya*; *Stereonephthya; Paratelesto*	Goh *et al*., 1999; Lorenz and Fehse, 2009; this publication
Primovula beckeri	*Acanthogorgia*; *Acabaria* (= *Melithaea*);*Unicella* [sic] (= *Eunicella*); *Lophogorgia* (= *Leptogorgia*)	Schiaparelli *et al*., 2005; Lorenz and Fehse, 2009

of *Acanthogorgia* could be distinguished but these could not be identified since a revision of the family Acanthogorgiidae is lacking.

Furthermore, examination of the ovulid species and their octocoral hosts revealed that in two instances individuals formerly identified as *C. chiapponii* and *C. aureola* would have co-occurred on the same host coral, in both cases *Acanthogorgia* sp.

Discussion

Based on the molecular data and morphological observations listed above, *C. chiapponii* is considered a junior synonym of *C. aureola*. The systematic account is therefore as follows:

Systematic part

Family Ovulidae Fleming, 1822
Genus *Crenavolva* Cate, 1973

Crenavolva aureola (Fehse, 2002)

Primovula aureola Fehse 2002: 37, pl. 1, fig. 1.
Delonovolva formosa. — Gosliner *et al*. 1996: 136, fig. 469. Not *Delonovolva formosa* (Sowerby II in Adams and Reeve 1848). [= *Cuspivolva formosa* (Sowerby II in Adams and Reeve 1848)]
Primovula sp. — Coleman 2003: 51, fig. (Ovul: 121).
Crenavolva chiapponii Lorenz and Fehse 2009: 69, pl. 74, fig. 7-11.

The occurrence of *C. chiapponii* (= *C. aureola*) on Indonesian shallow water coral reefs would have represented new distribution records, both geographically and bathymetrically, before it was synonymised. However *C. chiapponii* proved to be a junior synonym of *C. aureola* and the new distribution records fall within the distribution range already known for *C. aureola*. Apparently, the dorsal white band and the minor morphological differences in shell shape are not indicative of species-level differences between *C. aureola* and *C. chiapponii*.

Molecular data

The species *Primovula rosewateri* was previously placed in the genus *Crenavolva* by Cate (1973) but Fehse (2002a) moved it to *Primovula*, primarily based on the triangular shape of the funiculum. The results of the molecular analyses (Fig. 3) support this decision. There is great genetic similarity between *C.* cf. *rosewateri* (= *Primovula* cf. *rosewateri*) obtained from GenBank, and *C. aureola*. However, the specimen from GenBank was collected from Balicasag Island, near Bohol, Philippines, which is the type locality of *C. chiapponii*. This location is approximately 85 km from Mactan Island of Cebu, Philippines which is the type locality of *C. aureola*. It is not unlikely that the so-called *C.* cf. *rosewateri* from GenBank (AY161394 (16S), AY161627 (COI)) was misidentified and actually represents *C. aureola*. Moreover, the newly sequenced specimen of *P. rosewateri* from Malaysia convincingly clusters with *Primovula beckeri*. According to Lorenz and Fehse 2009, *P. beckeri* has an E African distribution and was originally described from South Africa. The specimen obtained from GenBank is from Sulawesi, Indonesia (Schiaparelli *et al*., 2005). It is therefore unlikely that this sequence represents *P. beckeri* but instead is the quite similar species from the central Indo-Pacific, *P. rosewateri*.

Host species and distribution records

The ranges of the presently discussed species all fit within the Coral Triangle (see Hoeksema, 2007) and depend on the ranges of their host species. Species of the genus *Acanthogorgia* are not unique hosts for just *Crenavolva* spp. Reijnen (2010) already mentioned *Acanthogorgia* spp. as a host for *Dentiovula eizoi* Cate and Azuma, 1973 (in Cate, 1973) and *D. colobica* (Azuma and Cate, 1971). *Acanthogorgia* species and their ovulid associates are both known to occur from shallow to deep water in the Coral Triangle. In an overview of the Acanthogorgiidae by Stiasny (1947) the deepest record for an *Acanthogorgia* species is 4,239 m, collected SE of Seram, Indonesia (*Acalycigorgia densiflora* = *Acanthogorgia densiflora* (Kükenthal and Gorzawsky, 1908). Nevertheless, Stiasny (1947) doubts the identification and compared it to congeneric species which are found in waters not exceeding 400 m depth. As a result Stiasny (1947) doubts the entire record. Therefore the deepest reliable record for an *Acanthogorgia* species in the Malayan Archipelago is 1,254 m for *Acanthogorgia multispina* (Kükenthal and Gorzawsky, 1908). The deepest record for *Crenavolva* species is from approximately 1,000 m, which is the deepest record for any ovulid species found to date (Lorenz and Fehse, 2009).

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