Zoological Journal of the Linnean Society, 2020, **190**, 1077–1094. With 10 figures.

A stranger among us: the occurrence of *Cantellius* (Balnoidea: Pyrgomatidae) an epibiont of scleractinias in stylasterids (Hydrozoa)

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Received 22 August 2019; revised 11 February 2020; accepted for publication 16 February 2020

Barnacles that fit morphologically into the description of the pyrgomatid genus *Cantellius* were retrieved from hydrozoan Stylasteridae. The use of molecular markers also confirmed the assignment of these barnacles to the genus *Cantellius*. Hitherto, stylasterids have not been recorded as hosts of pyrgomatids. This finding conflicts with and refutes the statement that scleractinans (Hexacorallia) are obligatory hosts of pyrgomatids. These are the first unequivocal records of living pyrgomatids in stylasterids, thus documenting a new type of habitat for this group of barnacles. Further inspections of stylasterids will probably reveal more new host records and, possibly, new pyrgomatids.

ADDITIONAL KEYWORDS: barnacles – coral reef – homoplasy – hydrozoan – lace coral – new species – phylogeny – Stylasteridae – symbiosis – taxonomy.

INTRODUCTION

The family Pyrgomatidae includes highly modified epibiotic barnacles, traditionally considered to live in association with three host taxa, i.e. scleractinians, milleporids and sponges (Ross & Newman, 1973). Ross & Newman (2000) noted 24 genera of pyrgonatids that encompass 73 living species inhabiting 70 coral genera (Ogawa & Matsuzaki, 1992). Since then, more species of pyrgomatids have been described, and the number of the existing pyrgomatids is currently > 100. Some pyrgomatid genera are restricted to a single coral host genus or family, such as *Hoekia* Ross and Newman, 1973 found only on *Hydnophora* Fischer von Waldheim, 1807, whereas others are widely distributed, such as species of *Trevathana* Anderson, 1992, *Galkinius* Perreault, 2014 and *Cantellius* Ross and Newman, 1973 found on a variety of hosts. However, among these genera, there are also species that are found on a single host genus. For example, *Cantellius septimus* (Hiro, 1938) is found only on *Montipora* Blainville, 1830.

Exploiting three concatenated molecular markers [12S ribosomal DNA(rDNA), 16S rDNA and 18S rDNA], Simon-Blecher *et al.* (2007) have narrowed down the pyrgomatid hosts to a sole subclass, Hexacorallia. They found that *Wanella milleporae* (Darwin, 1854), regarded as a pyrgomatid inhabiting the hydrozoan *Millepora* Linnaeus, 1758 (Darwin, 1854; Ross & Newman, 1973), did not cluster with the other pyrgomatids, but with free-living balanids. Their finding was supported by Malay & Michonneau (2014), who used two additional markers (*COI* and *H3*), by Perez-Losada *et al.* (2014) based on molecular analyses of five markers (18S rDNA, 28S rDNA, 12S rDNA, 16S rDNA and *COI*) from acorn barnacles, and

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[Version of record, published online 9 April 2020; http://zoobank.org/ urn:lsid:zoobank.org:pub:96954D17-136F-4D68-9D59-64C5EAFB6053]

the topology found by Tsang *et al.* (2014) using five other markers (12S rRNA, 16SrRNA, *EF1*, *H3* and *RP* gene sequences). In addition, in all four analyses, the archaeobalanid *Armatobalanus allium* (Darwin, 1854) is nested in Pyrgomatidae, suggesting that Pyrgomatidae is a paraphyletic taxon. Furthermore, Achituv & Simon-Blecher (2006, 2014) showed that *Pyrgopsella* Zullo, 1967 is associated with hexacorals and not with sponges as previously suggested by Rosell (1975). They also pointed out that morphological traits, such as the fused shell plates and elongated scuta, found in the '*Savignium–Pyrgopsella*' clade and in *Wanella*, are homoplasious traits, an adaptation to symbiotic life within the calcareous skeleton of scleractinians and hydrozoans. Taken together, these findings have led to the conclusion that pyrgomatids are restricted to Scleractinia and that the taxonomic position of *Wanella* should be re-evaluated.

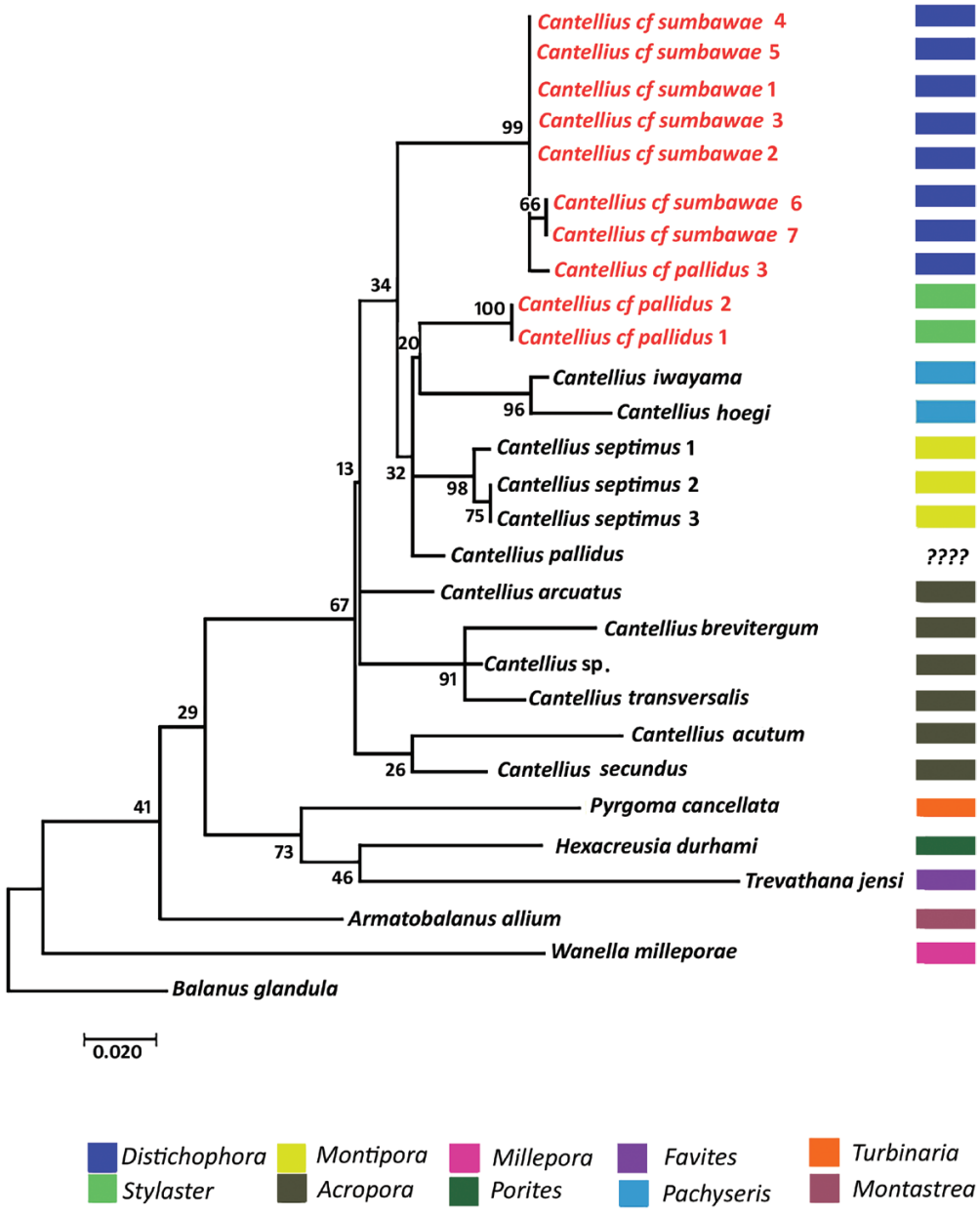

Figure 1. Maximum likelihood phylogenetic tree of *Cantellius* from stylasterids and scleleractinians and representative pyrgomatids based on 12S rRNA. The outgroup was *Balanus glandula* Darwin, 185. The analysis involved 26 nucleotide sequences, of which 22 sequences were of *Cantellius* and ten were extracted from stylasterids (in red). Bootstrap support of nodes is shown next to the branches. The tree is drawn to scale, with branch lengths presenting the number of substitutions per site. Hosts are indicated by colour.

Stylasteridae, commonly known as 'lace corals', is a family of colonial cnidarians of the class Hydrozoa characterized by having a hard, calcareous skeleton. Owing to their three-dimensionally branching skeleton, they are considered habitat-forming species that are able to enhance the complexity of the habitat (Roberts *et al.*, 2006). Like many other sessile organisms, their skeleton serves as a substratum for other organisms establishing symbioses (Zibrowius, 1981; Pica *et al.*, 2012, 2015, 2016; Tribollet *et al.*, 2018). The epibiotic fauna of the stylasterids exhibits relatively poor documentation, probably owing to their cryptic nature or to the great depth at which most Stylasteridae occur. There is an equally poor understanding of barnacles as epibionts of stylasterids. Until recently, *Armatobalanus nefrens* (Zullo, 1963) was reported as an epibiont of *Errinopora pourtalesi* (Dall, 1884) and *Stylaster californicus* (Verrill, 1866) in northern California (Newman, 2007). Pica *et al.* (2015) reported the presence of eight different symbiotic scalpellid species in five deep-water stylasterid corals. In the literature, the presence of pyrgomatids on Stylasteridae was recorded in two *Stylaster* species from the tropical shallow waters in the Indian Ocean (Broch, 1935, 1947). The specimens were identified as *Pyrgoma* sp., but this identification remains doubtful (Pica *et al.*, 2015). The comprehensive list of cnidarians hosting pyrgomatids compiled by Ogawa & Matsuzaki (1992) does not include stylasterids as hosts of pyrgomatids.

Preliminary examinations of barnacles found on several stylasterids revealed that they fit morphologically into the description of the pyrgomatid genus *Cantellius*. This result led us to hypothesize

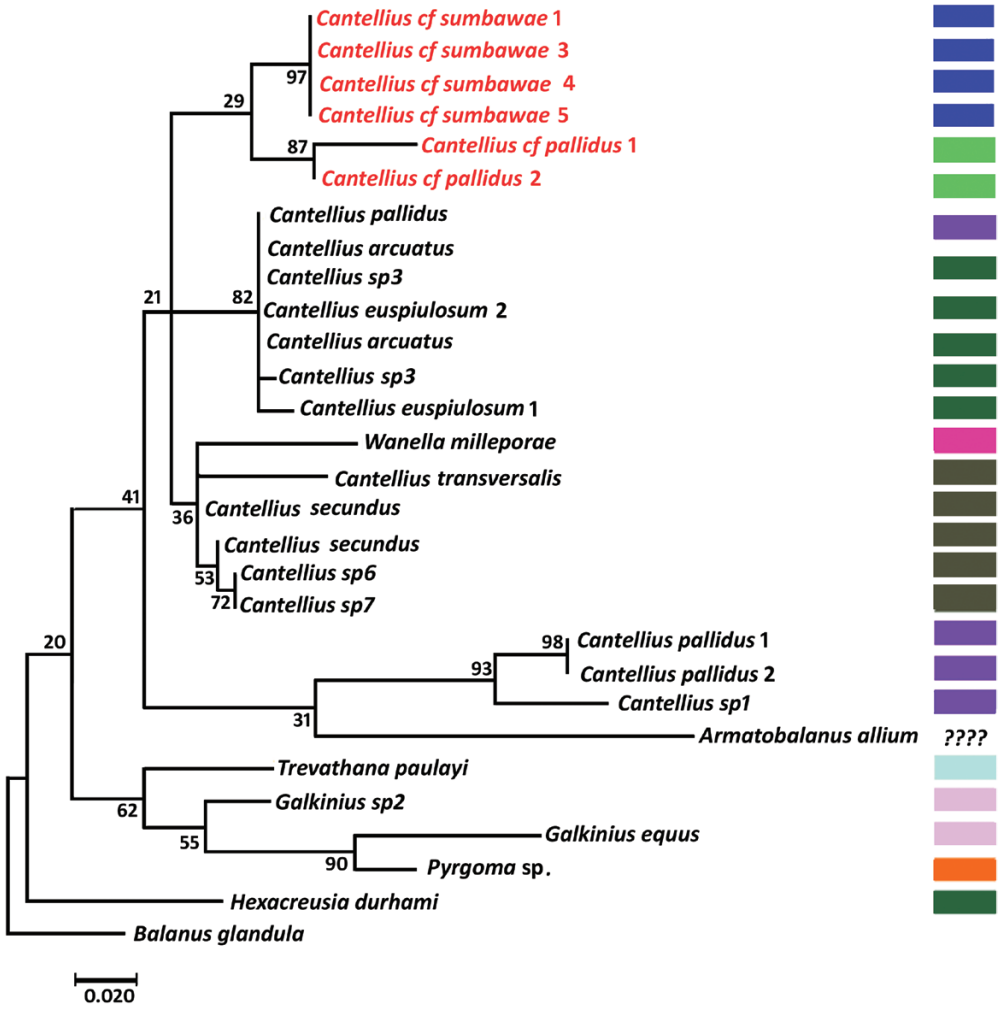

Figure 2. Maximum likelihood phylogenetic tree of *Cantellius* from stylasterids and scleractinians and representative pyrgomatids based on the nuclear marker histone 3 (*H3*). The outgroup is *Balanus glandula*. The analysis involved 25 nucleotide sequences, of which 19 sequences were of *Cantellius* and six were extracted from stylasterids. Bootstrap support of nodes is shown next to the branches. The tree is drawn to scale, with branch lengths presenting the number of substitutions per site. For key to host colour, see Figure 1.

that this association could be similar to the case of *Wanella* hosted by the hydrocoral *Millepora*, and therefore another case of convergent evolution, evolving independently in a similar habitat. However, the similarity of molecular markers indicates that in barnacles from stylasterids the morphological characters are homologous and casts doubt on our previous statement that pyrgomatids are obligatory epibionts of scleractinans (Hexacorallia) and on our previous concept of the taxonomy of the Pyrgomatidae.

The taxonomy of coral-inhabiting barnacles is based mainly on the morphology of hard parts, opercular valves and the shell. This is because, in many cases, the barnacles are retrieved from dried skeletons of corals, such as Darwin's (1854) eight varieties of *Creusia spinulosa* Leach, 1818 that are presently assigned to the genus *Cantellius*. The use of hard parts for description of species was followed by others (Borradaile, 1903; Hoek, 1913; Broch, 1931; Hiro, 1935, 1938; Kolosvary, 1947, 1948; Achituv, 2001) and also in the present study.

Based on the morphology of the shell and opercular valve, barnacles from stylasterids can be assigned to *Cantellius*, but owing to the small size and brittleness of the opercular valves, it is not always possible to assign all samples to known species of *Cantellius*. In addition, the absence of the type specimens of previously described species and the incomplete description of the type specimens do not enable the barnacles extracted from the stylasterids to be assigned with certainty to a known species of *Cantellius*. As a result of these uncertainties, we refer to these specimens as 'cf.'. We recognize three morphological forms, two of which, based on the number of shell plates and the morphology of scutum and tergum, are similar to known species of *Cantellius*, *Cantellius sumbawae* (Hoek, 1913) and *Cantellius pallidus* (Hiro, 1935), and a third one described below as a new species.

Table 1. Estimates of evolutionary distance between sequences (number of base substitutions per site) of 12S rRNA

			1	2	3	4	5	6	7	8	9
1	*Cantellius* cf. *sumbawae* 3	*Distichopora* sp.									
2	*Cantellius* cf. *sumbawae* 2	*Distichopora* sp.	0.000								
3	*Cantellius* cf. *sumbawae* 4	*Distichopora* sp.	0.000	0.000							
4	*Cantellius* cf. *sumbawae* 7	*Distichopora* sp.	0.004	0.004	0.004						
5	*Cantellius* cf. *sumbawae* 6	*Distichopora* sp.	0.004	0.004	0.004	0.000					
6	*Cantellius* cf. *sumbawae* 1	*Distichopora* sp.	0.000	0.000	0.000	0.004	0.004				
7	*Cantellius* cf. *sumbawae* 5	*Distichopora* sp.	0.000	0.000	0.000	0.004	0.004	0.000			
8	*Cantellius* cf. *pallidus* 3	*Distichopora* cf. *violacea*	0.000	0.000	0.000	0.004	0.004	0.000	0.000		
9	*Cantellius* cf. *pallidus* 4	*Stylaster tenisowoodi*	0.048	0.048	0.048	0.052	0.052	0.048	0.048	0.048	
10	*Cantellius* cf. *pallidus* 2	*Stylaster* cf. *eximus*	0.048	0.048	0.048	0.052	0.052	0.048	0.048	0.048	0.000
11	*Cantellius hoegi*	*Pachyseris speciosa*	0.056	0.056	0.056	0.059	0.059	0.056	0.056	0.056	0.056
12	*Cantellius iwayama*	*Pachyseris* sp.	0.056	0.056	0.056	0.059	0.059	0.056	0.056	0.056	0.044
13	*Cantellius pallidus*	Unknown	0.052	0.052	0.052	0.056	0.056	0.052	0.052	0.052	0.048
14	*Cantellius septimus* 1	*Montipora* sp.	0.041	0.041	0.041	0.044	0.044	0.041	0.041	0.041	0.041
15	*Cantellius septimus* 2	*Montipora* sp.	0.041	0.041	0.041	0.044	0.044	0.041	0.041	0.041	0.041
16	*Cantellius septimus* 3	*Montipora* sp.	0.041	0.041	0.041	0.044	0.044	0.041	0.041	0.041	0.041
17	*Cantellius arcuatum*	*Porites* sp.	0.037	0.037	0.037	0.041	0.041	0.037	0.037	0.037	0.044
18	*Cantellius brevitergum*	*Acropora* sp.	0.052	0.052	0.052	0.056	0.056	0.052	0.052	0.052	0.048
19	*Cantellius* sp.	*Acropora* sp.	0.052	0.052	0.052	0.056	0.056	0.052	0.052	0.052	0.048
20	*Cantellius transversalis*	*Acropora* sp.	0.044	0.044	0.044	0.041	0.041	0.044	0.044	0.044	0.044
21	*Cantellius secundus*	Unknown	0.056	0.056	0.056	0.059	0.059	0.056	0.056	0.056	0.063
22	*Cantellius acutum*	Unknown	0.067	0.067	0.067	0.071	0.071	0.067	0.067	0.067	0.059
23	*Wanella milleporae*	*Millepora dichotoma*	0.127	0.127	0.127	0.131	0.131	0.127	0.127	0.127	0.111
24	Pyrgoma cancellatum	*Turbinaria* sp.	0.103	0.103	0.103	0.107	0.107	0.103	0.103	0.103	0.099
25	*Hexacreusia durhami*	*Porites* sp.	0.103	0.103	0.103	0.107	0.107	0.103	0.103	0.103	0.091
26	*Armatobalanus allium*	*Montastrea* sp	0.079	0.079	0.079	0.083	0.083	0.079	0.079	0.079	0.063

The analysis involved 22 nucleotide sequences of *Cantellius* and three other pyrgomatids and the archobalanid *Armatobalanus allium*. Analyses were conducted using the maximum composite likelihood model.

MATERIAL AND METHODS

Material from three scientific collections is studied: The Steinhardt Museum of Natural History, Tel Aviv University, Tel Aviv, Isreal (TAU); the Università Politecnica delle Marche-DiSVA (UNIVPM DiSVA), Ancona, Italy; and the Museum National d'Histoire Naturelle, Paris, France (MNHN). Details on the samples used are presented in the Supporting Information (Appendix S1).

For the morphological study, the wall plates and opercular valves were removed from the hydroid, immersed for ~2 h in household bleach, rinsed in tap water followed by distilled water and then dried on a hotplate at 80 °C. The specimens were examined under a dissecting microscope, and the adherent chitin was removed using needles and a fine paintbrush. The dried parts were mounted on brass stubs, coated with gold and examined with a JEOL scanning electron microscope at 25 kV. Images were stored using the Autobeam software.

Only the material attached to the colony of *Distichopora* sp. from The Steinhardt Museum of Natural History stored in ethanol was suitable for studying the soft parts, the trophi and cirri, and could be used for molecular analysis. Material from other hosts was either dried or too small to use for the morphological study of soft parts or molecular work.

For DNA extraction, barnacles were dissected, and muscles and cirri fixed in ethanol were used. DNA was extracted using a genomic DNA isolation kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), according to the manufacturer's protocol. The DNA concentration was determined by NanoDrop ND1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 260 nm.

The DNA from small specimens was extracted at the forensic biology laboratory of Israel Police HQ, Jerusalem and transferred into clean tubes containing ethanol. Each sample was dried on filter paper and moved to a new clean tube. Two-step DNA extraction was performed. Samples were first extracted at 56 °C for 2 h using a Chelex extraction (Walsh *et al.*, 1991) and then by using the AutoMate

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
0.056															
0.044	0.022														
0.048	0.063	0.052													
0.041	0.052	0.048	0.037												
0.041	0.052	0.048	0.041	0.015											
0.041	0.052	0.048	0.037	0.007	0.007										
0.044	0.048	0.041	0.041	0.037	0.037	0.037									
0.048	0.075	0.063	0.052	0.041	0.044	0.041	0.044								
0.048	0.063	0.052	0.056	0.044	0.044	0.044	0.029	0.029							
0.044	0.067	0.056	0.052	0.048	0.048	0.048	0.029	0.029	0.022						
0.063	0.075	0.071	0.067	0.059	0.059	0.059	0.041	0.059	0.048	0.048					
0.059	0.063	0.067	0.083	0.067	0.067	0.067	0.052	0.059	0.059	0.063	0.067				
0.111	0.103	0.099	0.111	0.107	0.103	0.103	0.111	0.127	0.123	0.131	0.115	0.135			
0.099	0.127	0.127	0.111	0.107	0.107	0.107	0.095	0.103	0.111	0.107	0.123	0.115	0.135		
0.091	0.091	0.091	0.107	0.091	0.091	0.091	0.087	0.099	0.103	0.107	0.099	0.095	0.103	0.095	
0.063	0.083	0.075	0.087	0.083	0.079	0.079	0.071	0.071	0.075	0.075	0.067	0.079	0.099	0.107	0.095

Express DNA Extraction System in conjugation with the PrepFiler Express Forensic DNA Forensic Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol (Feine *et al.*, 2016).

For amplification and sequencing of the 12S subunit of mitochondrial rRNA, we used the primer set of Kocher *et al.* (1989) as modified by Mokady *et al.* (1999). For histone 3 (*H3*), we used the primers of Colgan *et al.* (1998). Amplification was carried out in a personal combi-thermocycler (Biometra, Göttingen, Germany), following the protocols of Tsang *et al.* (2012). The PCR products were purified and sequenced by MCLAB (San Francisco, CA, USA). Both strands were sequenced using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Additional DNA sequences were retrieved from GenBank. Details about hosts, when available, and accession numbers are provided in the Supporting Information (Table S1), which contains sequences from *Cantellius* marked by numbers, i.e. sp. 1, etc., by their submitter rather than being identified to the species level. We adhered to the naming and numbering of each sample as originally submitted to GenBank.

Sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994). The length of the aligned sequences of *12S* is 297 bp with 87 variable sites, of which 60 are parsimony informative. The aligned *H3* contigs are shorter, i.e. 197 bp with 56 variable sites, of which 46 are parsimony informative. Phylogenetic analyses were performed based on maximum likelihood (ML) analysis, and 1000 bootstrap replicates were conducted using MEGA7 (Kumar *et al.*, 2016). A matrix of pairwise distances within and among the species was calculated in MEGA7 using Kimura's 2-parameter (K2P; Kumar *et al.*, 2016).

Table 2. Estimates of evolutionary distance between sequences (number of base substitutions per site) of histone 3 (*H3*)

		Host	1	2	3	4	5	6	7	8	9	10	11
1	*Cantellius* cf. *sumbawae* 1	*Distichopora* sp.											
2	*Cantellius* cf. *sumbawae* 2	*Distichopora* sp.	0.000										
3	*Cantellius* cf. *sumbawae* 3	*Distichopora* sp.	0.000	0.000									
4	*Cantellius* cf. *sumbawae* 4	*Distichopora* sp.	0.000	0.000	0.000								
5	*Cantellius* cf. *pallidus*	*Distichopora* cf. *vervoorti*	0.029	0.029	0.029	0.029							
6	*Cantellius arcuatum*	*Porites*	0.042	0.042	0.042	0.042	0.036						
7	*Cantellius euspinulosum*	*Porites*	0.042	0.042	0.042	0.042	0.036	0.012					
8	*Cantellius transversalis* 1	*Acropora* sp.	0.055	0.055	0.055	0.055	0.054	0.048	0.061				
9	*Cantellius* sp. 7	*Acropora* sp.	0.042	0.042	0.042	0.042	0.041	0.041	0.054	0.036			
10	*Cantellius* sp. 6	*Acropora* sp.	0.042	0.042	0.042	0.042	0.041	0.041	0.054	0.036	0.000		
11	*Cantellius euspinulosum*	Unknown	0.017	0.017	0.017	0.017	0.023	0.041	0.042	0.061	0.048	0.048	
12	*Cantellius secundus* 2	Unknown	0.036	0.036	0.036	0.036	0.035	0.035	0.048	0.042	0.006	0.006	0.042
13	*Cantellius secundus* 1	*Acropora* sp.	0.030	0.030	0.030	0.030	0.029	0.029	0.042	0.036	0.011	0.011	0.036
14	*Cantellius pallidus*	*Pocillopora damicornis*	0.042	0.042	0.042	0.042	0.036	0.000	0.012	0.048	0.041	0.041	0.041
15	*Cantellius* sp. 3	*Porites* sp.	0.042	0.042	0.042	0.042	0.036	0.000	0.012	0.048	0.041	0.041	0.041
17	*Cantellius euspinulosum*	*Porites* sp.	0.042	0.042	0.042	0.042	0.036	0.000	0.012	0.048	0.041	0.041	0.041
18	*Cantellius* sp. 3	*Porites rus*	0.036	0.036	0.036	0.036	0.042	0.006	0.017	0.055	0.048	0.048	0.048
19	*Cantellius euspinulosum*	*Porites*	0.048	0.048	0.048	0.048	0.042	0.006	0.006	0.055	0.048	0.048	0.048
20	*Cantellius pallidus*	Unknown	0.066	0.066	0.066	0.066	0.066	0.093	0.093	0.121	0.106	0.106	0.060
21	*Cantellius iwayama* 1	*Pachyseris*	0.073	0.073	0.073	0.073	0.087	0.109	0.110	0.101	0.101	0.101	0.081
22	*Cantellius pallidus* 1	*Pocillopora damicornis*	0.066	0.066	0.066	0.066	0.066	0.093	0.093	0.121	0.106	0.106	0.060
23	*Cantellius* sp. 1	*Pachyseris rugosa*?	0.073	0.073	0.073	0.073	0.087	0.109	0.110	0.101	0.101	0.101	0.081
24	*Trevathana paulayi*	*Acanthastrea echinata*	0.068	0.068	0.068	0.068	0.074	0.079	0.080	0.086	0.066	0.066	0.081
25	*Pyrgoma cancellata*	*Turbinaria* sp.	0.113	0.113	0.113	0.113	0.112	0.127	0.142	0.108	0.092	0.092	0.113
26	*Galkinia* sp. 2	*Hydnophora exesa*	0.081	0.081	0.081	0.081	0.074	0.086	0.087	0.107	0.066	0.066	0.093
27	*Galkinia* sp. 1	*Goniastrea pectinata*	0.092	0.092	0.092	0.092	0.079	0.093	0.106	0.100	0.084	0.084	0.105
28	*Galkinia equus*	*Favites abdita*	0.126	0.126	0.126	0.126	0.132	0.132	0.146	0.120	0.097	0.097	0.126
29	*Hexacreusia durhami*	*Porites* sp.	0.109	0.109	0.109	0.109	0.102	0.102	0.108	0.088	0.067	0.067	0.123
30	*Armatobalanus allium*	Unknown	0.121	0.121	0.121	0.121	0.118	0.107	0.113	0.123	0.093	0.093	0.106

The analysis involved 22 nucleotide sequences of *Cantellius* and six other pyrgomatids and *Armatobalanus allium*. Analyses were conducted using the maximum composite likelihood model.

RESULTS

PHYLOGENY

Figures 1 and 2 present ML trees based on the mitochondrial marker 12S rRNA and the nuclear marker *H3*, respectively. Both markers show that the specimens extracted from stylastarids cluster with *Cantellius*.

In the ML trees based on 12S rRNA, all sequences of barnacles extracted from *Distichopora* are grouped. The sequences that we obtained from *Distichopora violacea* (Pallas, 1766) and identified morphologically as *Cantellius* cf. *pallidus* (Broch, 1931) cluster with those from the unidentified colony of *Distichopora*. The two sequences extracted from *Stylaster* cf. *eximius* (Hickson & England, 1905) and *Stylaster tenisonwoodsi* Cairns, 1988 form a separate clade within the *Cantellius* clade, but the bootstrap support values of the nodes within this clade are low. The phylogenetic pattern based on *H3* agrees with the 12S analyses with regard to the grouping of the barnacles from *Distichopora*. In the *H3* tree, the two sequences of *Cantellius* cf. *pallidus* extracted from *Stylaster* form a sister group to the sequences of *Cantellius* cf. *sumbawae* extracted from *Distichopora*. However, the bootstrap support of the node that separates this clade from the other taxa of *Cantellius* is low.

Of interest is the position of *Wanella* in the two phylogenetic trees. The position of *Wanella* on the tree based on 12S rDNA sequences is similar to what was found previously, i.e. as a monogeneric clade sister to the pyrgomatids (Tsang *et al.*, 2012; Simon-Blecher *et al.*, 2007), or it clustered with other balanids (Malay & Michonneau, 2014; Pérez-Losada *et al.*, 2014). The position of *Wanella* in the tree based on *H3* is different. In this analysis, *Wanella* clusters with the pyrgomatids. This does not agree with the analyses of Malay & Michonneau (2014) and Tsang *et al.* (2012),

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
0.006																			
0.035	0.029																		
0.035	0.029	0.000																	
0.035	0.029	0.000	0.000																
0.042	0.036	0.006	0.006	0.006															
0.042	0.036	0.006	0.006	0.006	0.012														
0.099	0.093	0.093	0.093	0.093	0.086	0.100													
0.107	0.101	0.109	0.109	0.109	0.102	0.117	0.048												
0.099	0.093	0.093	0.093	0.093	0.086	0.100	0.000	0.048											
0.107	0.101	0.109	0.109	0.109	0.102	0.117	0.048	0.000	0.048										
0.060	0.054	0.079	0.079	0.079	0.080	0.086	0.108	0.124	0.108	0.124									
0.098	0.092	0.127	0.127	0.127	0.128	0.135	0.142	0.144	0.142	0.144	0.086								
0.060	0.066	0.086	0.086	0.086	0.087	0.093	0.122	0.124	0.122	0.124	0.060	0.073							
0.078	0.072	0.093	0.093	0.093	0.093	0.100	0.106	0.135	0.106	0.135	0.066	0.041	0.041						
0.103	0.097	0.132	0.132	0.132	0.133	0.140	0.113	0.136	0.113	0.136	0.091	0.061	0.091	0.066					
0.074	0.080	0.102	0.102	0.102	0.102	0.102	0.138	0.111	0.138	0.111	0.087	0.093	0.074	0.087	0.105				
0.099	0.106	0.107	0.107	0.107	0.114	0.107	0.133	0.129	0.133	0.129	0.133	0.133	0.142	0.153	0.141	0.114			

in which one of their markers was *H3*. The inclusion of *Wanella* in the *Cantellius* clade supports the hypothesis that they share a common ancestor. The *H3* gene is more conserved, and its evolution is slower than that of the rDNAs and mitochondrial genes used in the concatenated markers. Hence, its weight in the phylogeny is 'diluted' by the other genes.

The pairwise divergence values of 12S rRNA and *H3* between specimens of our material and those of other species of *Cantellius* and representative pyrgomatids enable us to set boundaries between evolutionary significant units (ESUs). These values are presented in Tables 1 and 2, respectively. The within-group pairwise distances of 12S rRNA of the seven specimens extracted from the single colony of *Distichopora* collected in Bali, Indonesia do not exceed 0.004. The distance between the 12S sequence of the barnacles extracted from *Distichopora violacea* from the Siladen Islands and those from Bali are in the same range. The pairwise 12S rRNA of two specimens, one extracted from *Stylaster tenisonwoodsi* and the other from *S.* cf. *eximius*, is 0.000. The distance between these and those from *Distichopora* is 0.048 and 0.052, respectively. In 12S rRNA sequences, the range of pairwise distances between these specimens and other species of *Cantellius* lies between 0.041 in *C. septimus* and 0.071 in *Cantellius acutum* (Hiro, 1938) (Table 1). These values are within the range found among other species of *Cantellius*, with the highest being 0.083 between *C. acutum* and *C. pallidus* and the lowest being 0.022 between *Cantellius brevitergum* (Hiro, 1938) and an unidentified *Cantellius*.

In the *H3* sequences, the within-group pairwise distances of the four specimens extracted from *Distichopora* is 0.000, and the one between those and the specimen from *Stylaster* is 0.048. The maximal divergence between the specimens from stylasterids and the two unidentified species *Cantellius* sp. 1 and *Cantellius* sp. 6 extracted from the scleractinians is 0.086. These values are within the range found among different morphologically identified species of *Cantellius*; the highest 0.120 between *Cantellius*

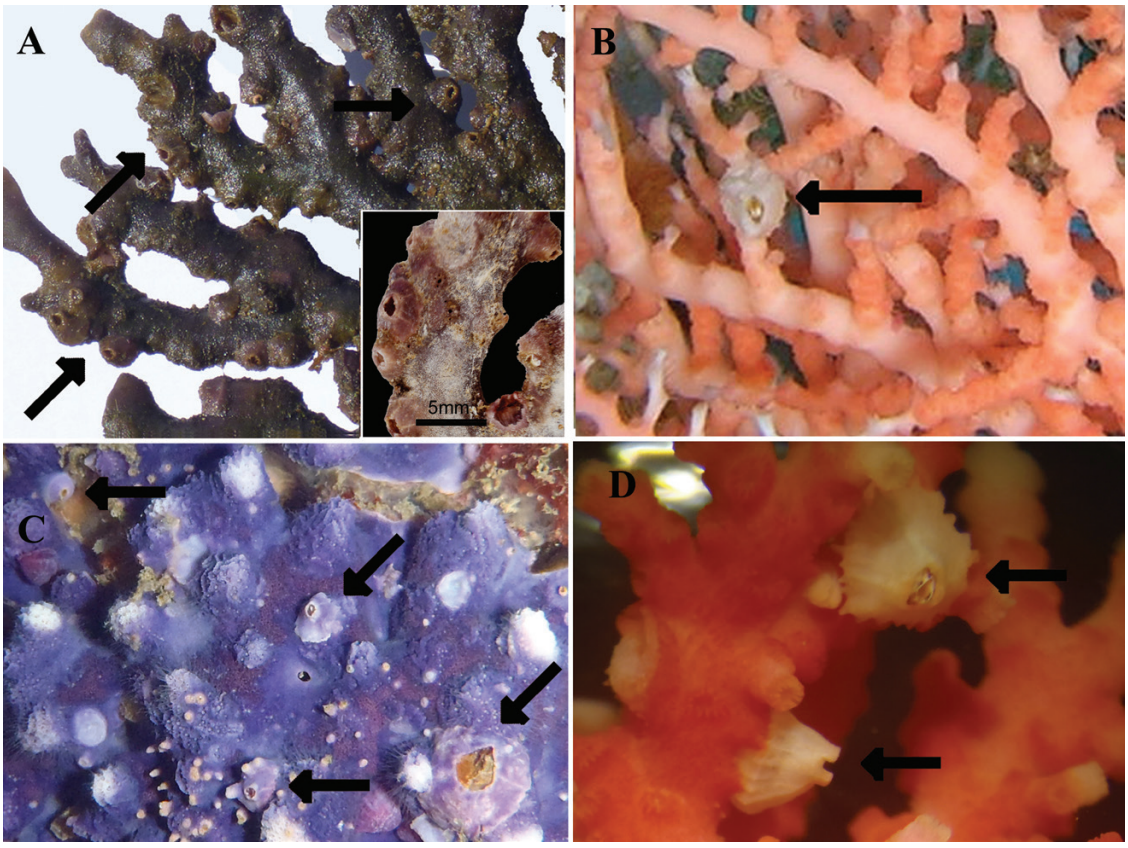

Figure 3. *In situ* pictures of colonies of Stylasteridae with barnacles. A, colony of *Distichopora* sp. from Bali, Indonesia, with *Cantellius* cf. *sumbawae*; inset, enlargement of a branch. B, *Stylaster* cf. *eximius* from Siladen Island, Indonesia, colony carrying barnacles. C, *Distichopora* cf. *vervoorti* colony carrying barnacles. D, colony of *Stylaster tenisonwoodsi* from Bangka Island, Indonesia, carrying barnacles. Barnacles are indicated by arrows.

transversalis (Nilsson-Cantell, 1938) and *C. pallidus*. Therefore, we propose that taxa with pairwise distances of sequences that are within the range of these divergence levels should be assigned to the same clade as *Cantellius*.

TAXONOMY

CANTELLIUS CF. *SUMBAWAE* (HOEK, 1913)

Examined material: Host *Distichopora* sp., TAU AR29843, Bali, Indonesia (Figs 3A, 4–7), 5 November 2017.

Description: Barnacles are scattered along the branches of the hydrozoan colony (Fig. 3A), mostly on the lateral side of the branches (Fig. 3A, inset). Shell conical, four plated (rostrum, carina and paired latera; Fig. 4A, C), externally covered by the hydroid skeleton and tissue (Fig. 4A, B). Carinorostral diameter, 4.35 ± 1.36 mm (N = 11), sheath forming inner lamina. Basis, shallow cup shape with radiating ridges and furrows (Fig. 4D, E) reaching the centre of the basis. Orifice central, small. Scutum and tergum (Fig. 5A) separated, white. Scutum triangular; basal margin sinusoidal; length approximately equal to tergal margin. Both the occludent margin and the tergal margin straight. Externally, growth ridges parallel to basal margin outline, forming teeth on occludent margin. Shallow cavity for lateral depressor. Inner side with prominent adductor ridge; articular ridge on tergal margin occupying nearly the entire length of the margin. Tergum elongated; carinal margin about two-thirds of scutal margin, with blunt rounded spur; scutal margins slightly curved, apical angle ~60°; small beak at the apex; basal margins sinusoidal; external surface with growth ridges parallel to basal margins; shallow median furrow from apex to spur base. Inner side with articular ridge along the scutal margin. Small pits scattered on the inner side. Maxilla rounded (Fig. 6A), with simple setae along interior margin and distal part. Maxillule (Fig. 6C) cutting edge straight, without notch, with row of seven large setae. Surface of maxillule close to cutting edge, with short, simple type of setae. Simple setae on upper and lower margins. Mandibule (Fig. 6E) with five teeth; gap between first tooth and second tooth; second tooth located in middle of cutting edge; gap between second and third tooth. Second to fourth teeth bidentate. Surface of mandible close to cutting edge from upper to third tooth, with short simple setae. Mandibular palp elongated (Fig. 6D); setae on inferior margin; lower

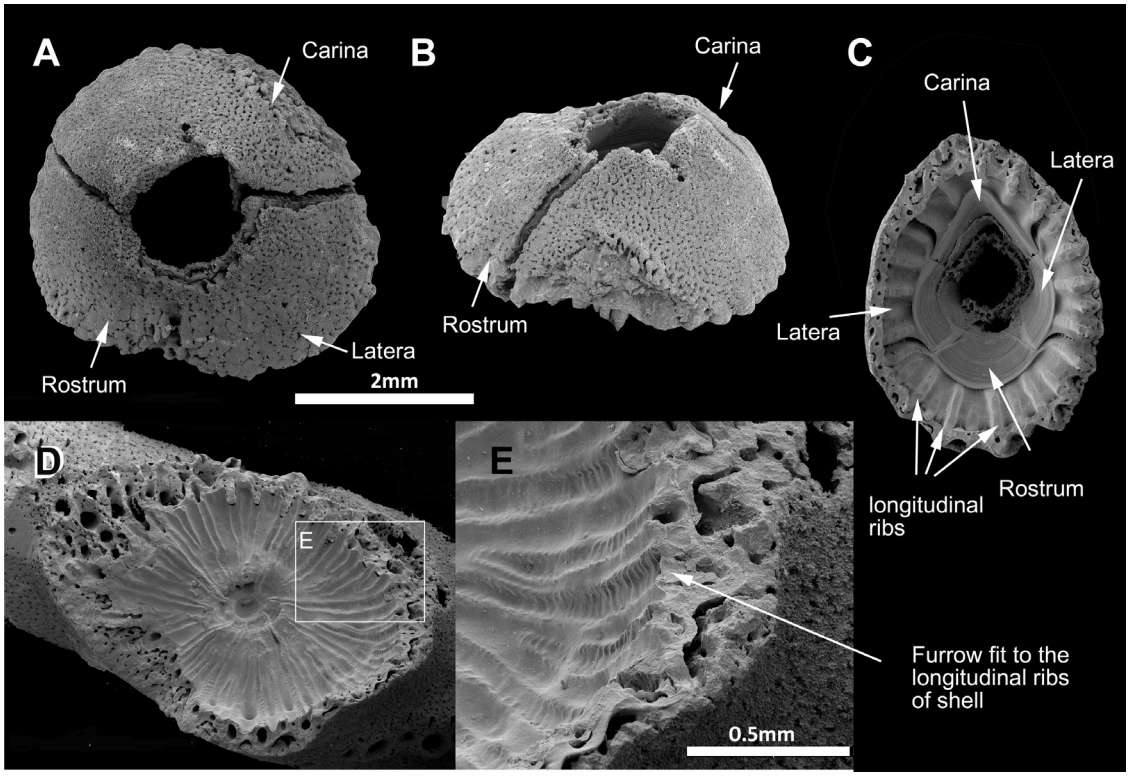

Figure 4. *Cantellius* cf. *sumbawae*. A, external view of shell, upper view. B, external view of shell, side view. C, inner view of shell. D, basis. E, enlargement of basis.

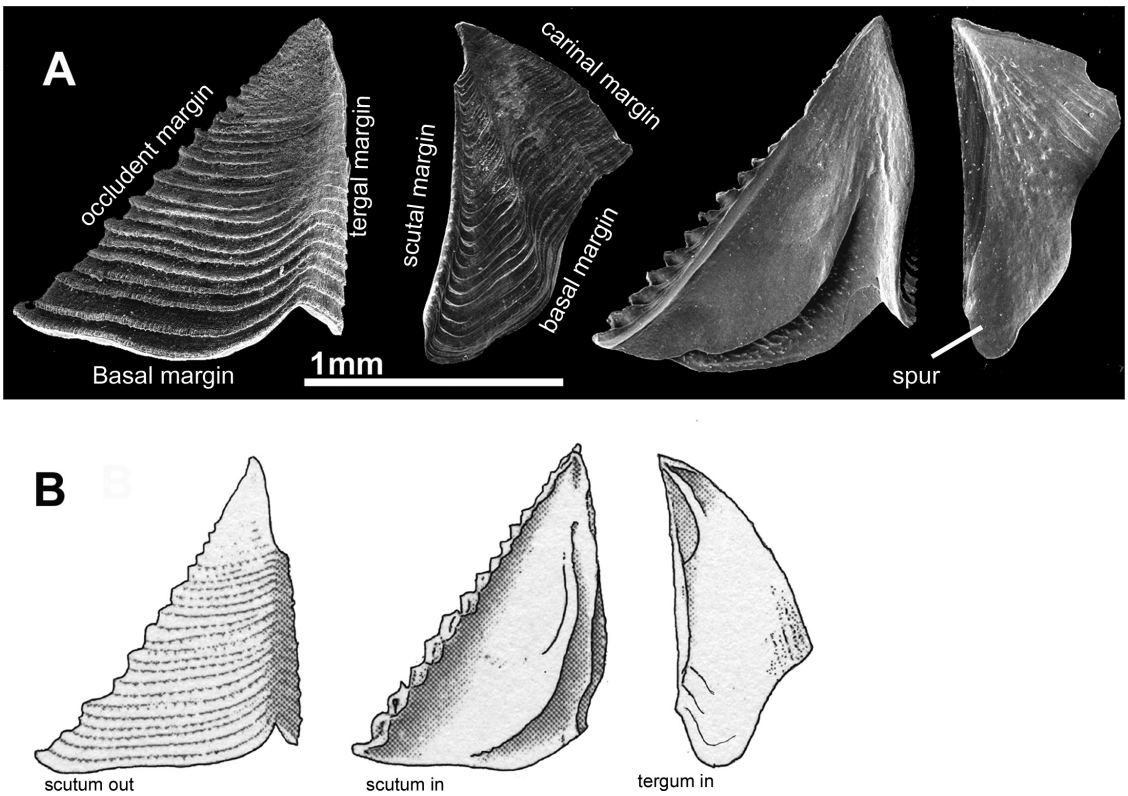

Figure 5. A, opercular valves of *Cantellius* cf. *sumbawae* from *Distichopora* sp. Outer and inner sides are shown. B, *Cantellius sumbawae* opercular valves from the small, solitary, free-living coral *Heteropsammia*, redrawn from plate XXVII, Hoek (1913).

and upper margins with simple setae. Labrum bilobed (Fig. 4B) with V-shaped notch between lobes; two or three sharp teeth on each lobe (Fig. 6B). Cirrus I (Fig. 7A) rami unequal; anterior ramus ten articles longer; posterior ramus five articles; distal article with mixed setae. Cirrus II with equal rami; anterior with seven articles and posterior with five articles. Terminal setae (Fig. 7G) simple. Cirrus III (Fig. 7C) anterior ramus with eight to nine articles, with short sharp teeth on front of articles (Fig. 7F); posterior ramus with six to seven articles. Cirri IV–VI long (Fig. 7D, E), slender, with both rami of similar length. Penis long, annulated with scattered short simple setae; pedicel (Fig. 7E) with short basidorsal point.

Remarks: Based on the morphology of the opercular valves, the barnacles found on *Distichopora* fit into the description of *C. sumbawae* (Fig. 5B). *Cantellius sumbawae* was based on a single specimen attached to the small, solitary, free-living coral, *Heteropsammia*. The coral and its barnacle were dredged during the Siboga expedition in February 1900 at ~36 m depth on a sandy or muddy bottom in Saleh Bay anchorage, east of Dangar Besar on the Indonesian island of Sumbawa. The comprehensive list of barnacles and their host corals (Ogawa & Matsuzaki, 1992) indicates that since *C. sumbawae* was described, it has not been recorded from any other coral. The Hoek specimen could not be traced in the collection of the Naturalis Biodiversity Centre, Leiden, The Netherlands, where the Siboga expedition material is stored. There are differences between Hoek's specimen and those found on *Distichopora*. The stylasterid skeleton completely encrusts the barnacle shell, whereas this was not reported by Hoek (1913). Brickner *et al.* (2010) suggested that the overgrowth of the coral skeleton and coverage of the barnacle shell is a result of the coral growth and should be regarded as a coral character rather than a barnacle character. However, without examination and comparison of barnacles on *Heteropsammia*, it is uncertain whether the barnacles from both hosts belong to the same species. Owing to this uncertainty, we prefer to identify our specimens as *Cantellius* cf. *sumbawae*.

CANTELLIUS CF. *PALLIDUS* (BROCH, 1931) (FIGS 8, 9)

Examined material: MNHN-IU-5863, host *Stylaster flabelliformis* (Lamarck, 1816). MNHN-IK-2015-658, 12°34.6′S, 45°05.2′E, 21 March 1977. MNHN-IU-2014-5873, host *Distichopora violacea*

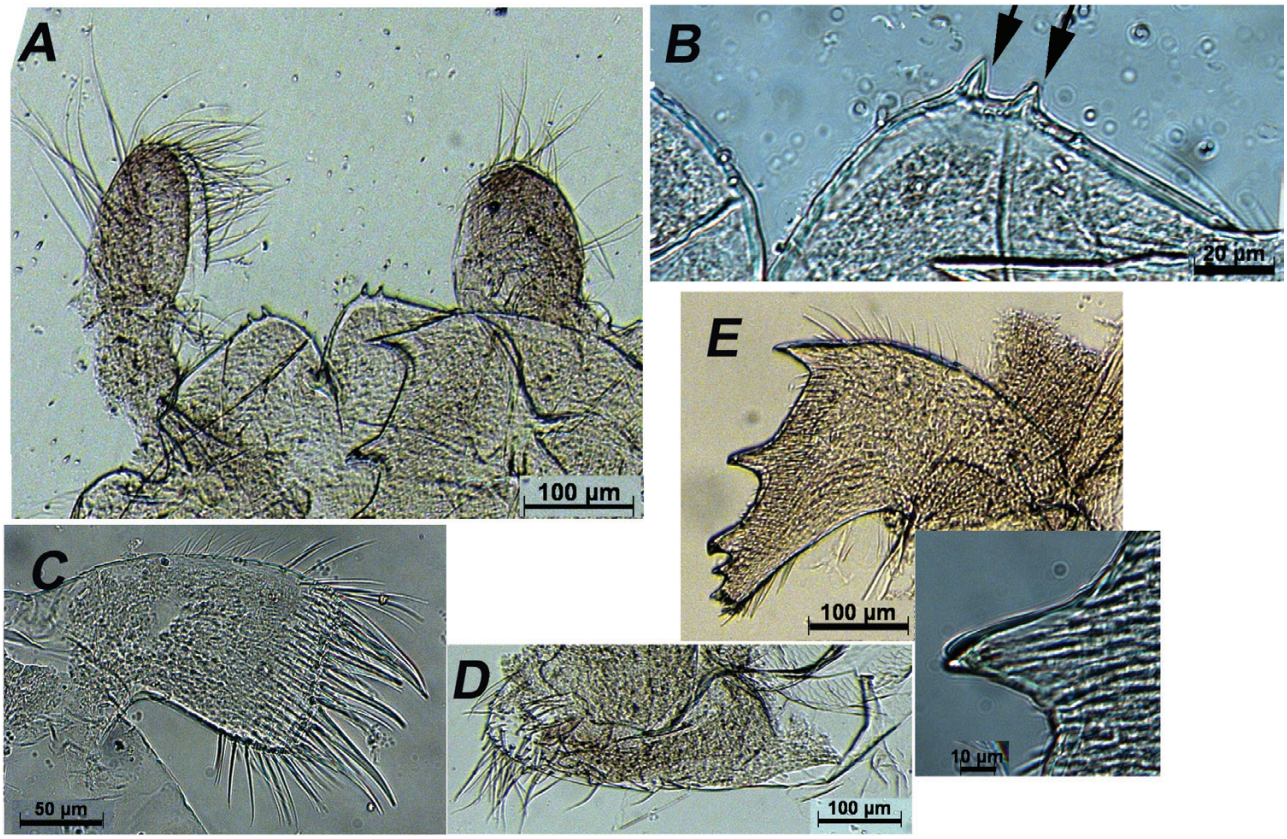

Figure 6. *Cantellius* cf. *sumbawae*, trophi. A, labrum and two maxillae. B, enlargement of labrum, with arrows indicating teeth on labrum. C, maxillule. D, mandibular palp. E, mandible; inset, median tooth of mandible.

MNHN-IK-2015-660, 11°34.6′S, 45°05.2′E, 12 April 1977. TAU AR29859, host *Stylaster tenisonwoodsi* PC190371, Bangka Island, 5 m, Indonesia, Università Politecnica delle Marche-DiSVA, Ancona, Italy, 16 December 2011. TAU AR29860, host *Stylaster* cf. *eximius*, BALA1, Siladen Islands Indonesia, 15 m, Università Politecnica delle Marche-DiSVA, Ancona, Italy, 13 December 2011.

Description: Shell conical, four plated, externally covered by the hydroid skeleton and tissue. Sheath forming inner lamina, basis, shallow cup shape with radiating ridges and furrows reaching the centre of the basis. Orifice central, small. Scutum and tergum separated, white. Scutum triangular, basal margins curved; pit at basitergal angle; occludent margin length approximately equal to tergal margin. Occludent margin and tergal margin straight. Externally, growth ridges parallel to basal margin outline, forming teeth on occludent margin. Cavity for lateral depressor wide, shallow. Inner side with adductor ridge that varies in different specimens; articular ridge on tergal margin curved and occupying nearly entire length of margin. Tergum elongated; carinal margin about two-thirds of scutal margin with blunt rounded spur; scutal margins slightly curved, with small beak at the apex; basal margins sinusoidal; external surface with growth ridges parallel to basal margins; shallow median furrow from apex to spur base.

Remarks: The identification of MNHN-IU-5863 is based only on the morphology of scuta, because the terga are broken and cannot be used as a morphological character. In the specimens from the two species of *Stylaster*, there is a prominent pit at the basitergal angle and a cavity for the lateral depressor, as in *C. pallidus*. The apices of the terga are broken, and the wide spur might fit also to *Cantellius arcuatus* (Hiro, 1938). However, it is more likely that it belongs to *C. pallidus*. *Cantellius pallidus* is the most abundant species of *Cantellius* and has been recorded from 37 species of corals, whereas *C. arcuatus* has been recorded from only two species of corals (Ogawa & Matsuzaki, 1992).

CANTELLIUS CORNUTERGUM ACHITUV **SP. NOV.**

(FIG. 10)

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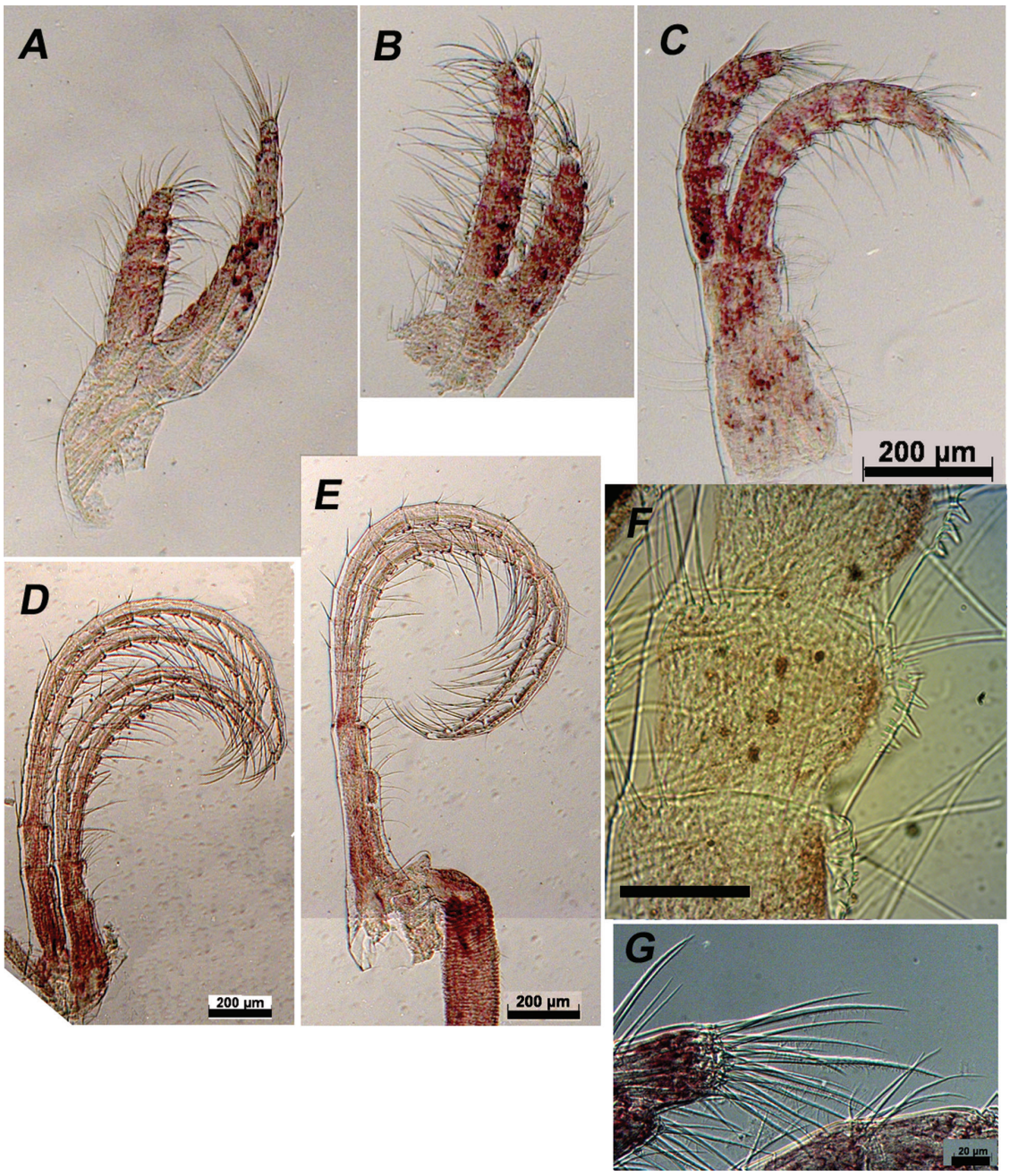

Figure 7. *Cantellius* cf. *sumbawae*. A–C, cirri I–III, respectively. D, cirri IV and V. E, cirrus VI with basidorsal point and proximal part of penis. F, Cirrus III spines on front of articles. G, Cirrus II, terminal setae.

Holotype: MNHN-IU-5872, host *Distichopora violacea* MNHN-IK-2015-660, 11°34.6′S, 45°05.2′E, 12 April 1977.

Paratype: MNHN-IU-5869, host *Distichopora violacea* MNHN-IK-2015-660, same data as holotype.

Diagnosis: Pyromatid with four shell plates. Scutum triangular, with occludent margin longer than basal margin. Small crests for the depressor muscle in the scutum. External median furrow along tergum with external median furrow. Carinal margin of tergum strongly curved.

Description: Shell conical, four plated (rostrum, carina and paired latera), externally covered by the hydroid skeleton and tissue (Fig. 10A). Orifice central, rhomboid. Scutum and tergum separated,

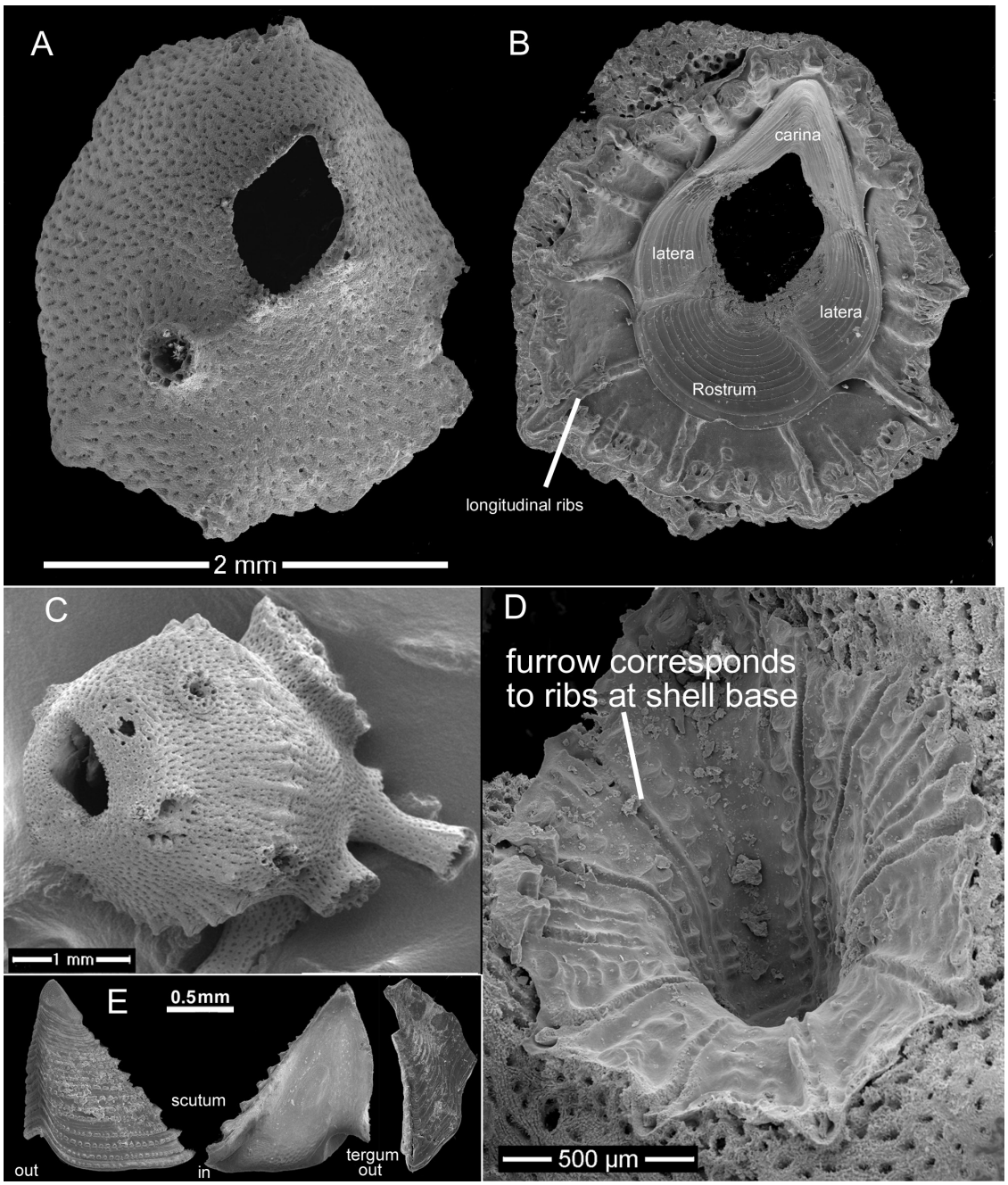

Figure 8. *Cantellius* cf. *pallidus* from *Stylaster tenisonwoodsi*. A, shell outer view. B, shell inner view. C, specimen on host. D, basis. E, opercular valves.

white. Scutum triangular; basal margin straight, curving at the basitergal angle; four to five parallel small pits for the scutal depressor muscle at basioccludent angle; four parallel small pits for the lateral depressor muscle at basitergal angle (Fig. 10B). Occludent margin length approximately equal to tergal margin length. Occludent margin and tergal margin straight. Deep, round pit for adductor muscle. Adductor ridge prominent; articular ridge occupies about four-fifths of tergal margins. Externally, growth ridges parallel to basal margin outline, forming teeth on occludent margin. Tergum elongated; carinal margin about half of scutal margin, with blunt rounded spur; scutal margins strongly curved, forming prominent beak at the apex; basal margins slightly concave;

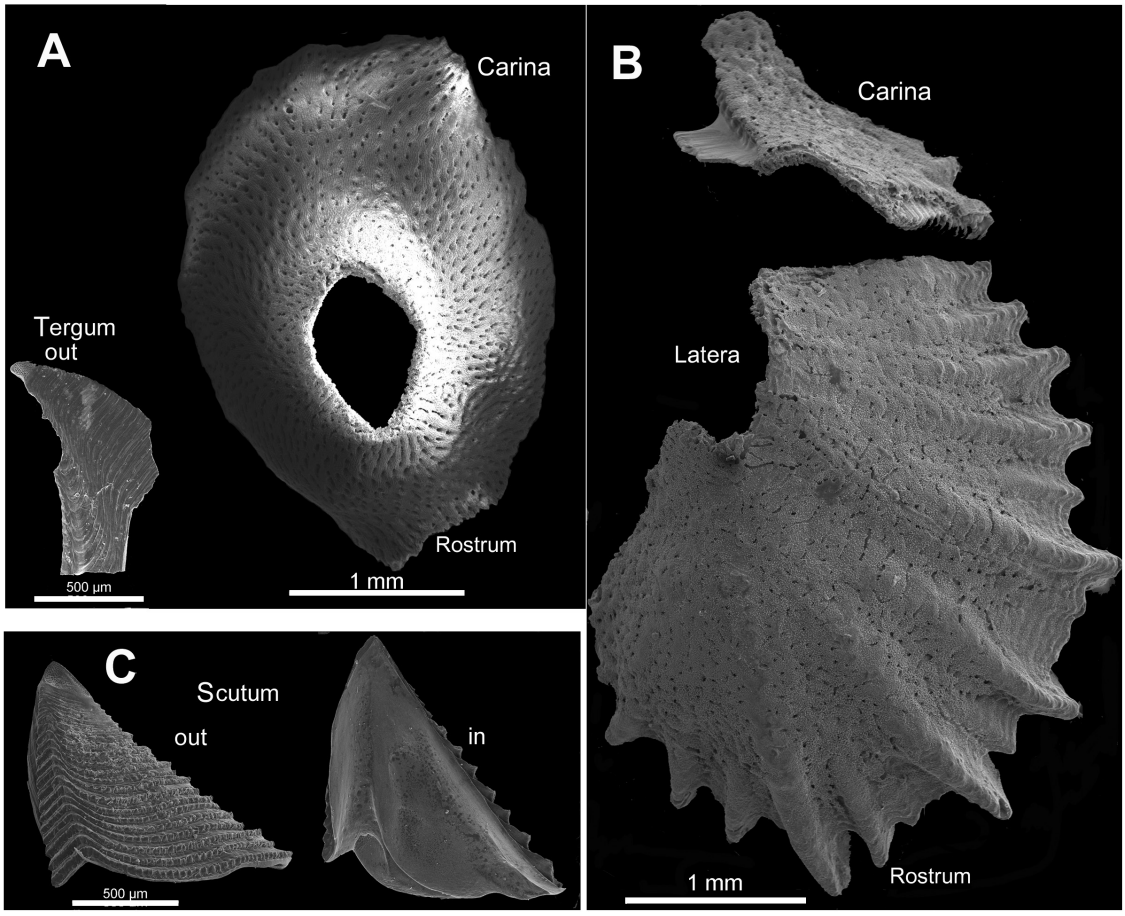

Figure 9. *Cantellius* cf. *pallidus* from *Stylaster* cf. *eximius*. A, shell and tergum (broken). B, shell: rostrum, laterum and carina. Scutum outer and inner side.

external surface with growth ridges parallel to basal margins; shallow median furrow from apex to spur base. Internally, shallow crests for depressor muscle.

Etymology: From Latin *cornu*, horn, indicating the presence of prominent beak at the apex of the *tergum*, meaning 'back' or 'rear'.

Remarks: The opercular valves of this species are different from all known species of *Cantellius*. The noticeable features are the small crests for the two depressor muscles. The shape of the scutum is most similar to that of *Cantellius tredecimus* (Kolosvary, 1947), which has neither adductor ridges nor adductor pits. The tergum may resemble that of *C. arcuatus*; the lower part toward the basis is straight, whereasa in *C. arcuatus* the upper part next to the carinal margin is strongly curved. In the original description of *C. arcuatus*, Hiro (1938) does not mention the presence of an external median furrow and internal crests for the depressor muscle of the tergum. On the basis of these differences, we think this is a new species.

DISCUSSION

Based on the criteria presented above, the barnacles from the hydrozoans *Stylaster* and *Distichopora* should be classified as *Cantellius* owing to the presence of four-plate shells and balanoid-type opercular plates. The determination of species of this genus is mainly, and in some cases exclusively, based on the morphology of the opercular valves (Ross & Newman, 1973). Using this character, the barnacles described in the present study fit different species of *Cantellius*, including a new species.

Many samples of *Cantellius* could not be identified with full confidence to the species level. Malay & Michonneau (2014) did not use nominal species for

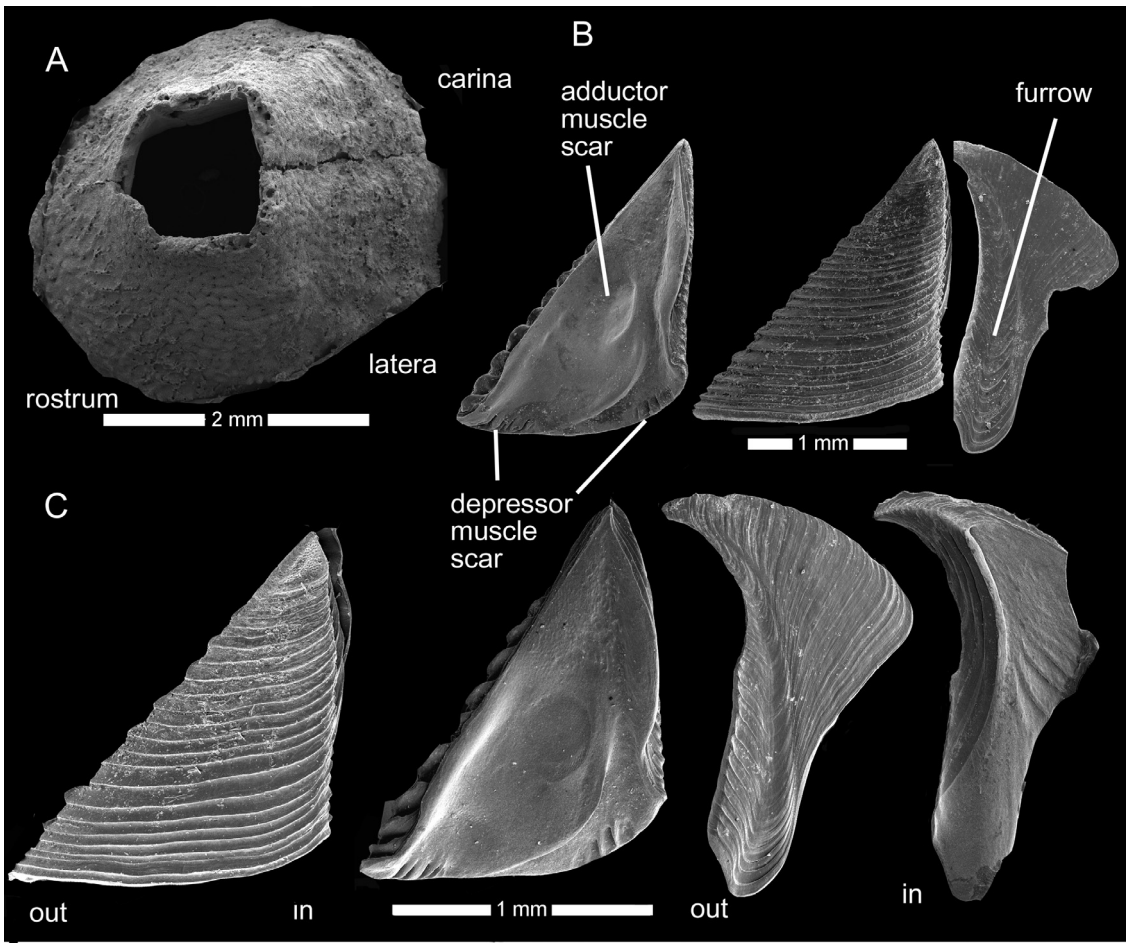

Figure 10. ***Cantellius cornutergum*** from *Distichopora violacea*. A, shell. B, scutum inner and outer view, and terga outer view. C, scutum inner and outer view, and terga outer and inner view.

most of their samples, but showed that barnacles of the same genus originating from different hosts belong to different ESUs. These authors argued that the species-level taxonomy of coral-dwelling barnacles is problematic and that some genera included many yet undescribed species. Using molecular markers, they noted that even a monotypic genus, such as *Neotrevathana*, is found to be a complex of three different ESUs, all fitting the morphological description for *Neotrevathana*. Brickner *et al.* (2010) showed that what was regarded as a single species of *Trevathana* encompasses four species. Tsang *et al.* (2009) revealed that the barnacle *Wanella milleporae*, which inhabits the fire coral, is a complex of cryptic species inhabiting different species of *Millepora*. Also, our results reflect the existence of cryptic species within the *Cantellius* complex. Our material contains sequences of morphologically defined species of *Cantellius*, e.g. *H3* of *C. pallidus*, extracted from different coral hosts and found on different clades of the phylogenetic tree. Therefore, it is tempting to speculate that, although morphologically *Cantellius* from *Heteropsammia* and the population from *Distichopora* are assigned to *C.* cf. *sumbawae*, they are, in fact, two different ESUs. However, without appropriate material, this assumption cannot be validated.

The genus *Cantellius* is the most species-rich genus of Pyrgomatidae, with 22 nominal reported species (Ross & Newman, 2000), and more have been added during the last two decades (Achituv, 2001; Achituv & Hoeksema, 2003; Achituv *et al.*, 2009; present study). This genus occupies the largest number of scleractinians (Ogawa & Matsuzaki, 1992), with no record from other taxonomic units. Ross & Newman (1973) stated that highly modified forms are highly host specific, all the more so for monotypic genera. However, within the genus *Cantellius*, some species were recorded from a single host, whereas others were from several hosts, with *C. pallidus* being

recorded from nearly 40 coral species (Ogawa & Matsuzaki, 1992).

In the phylogenetic trees based on molecular markers, *Armatobalanus allium* and *Cantellius* are located at the base of Pyrgomatidae (Simon-Blecher *et al.*, 2007; Malay & Michonneau, 2014; Tsang *et al.*, 2014). Morphologically, *Cantellius* shows the most plesiomorphic characteristics within the Indo-Pacific coral-inhabiting barnacles, with four shell-wall plates and unmodified balanoid-type opercular valves. It was Darwin (1854) who first pointed out that *Armatobalanus allium* 'shows the affinity and passage to the coral-inhabiting genus *Creusia*', with the reduction of the carino lateral plate that is absent in the Pyrgomatidae. Ross & Newman (1973) suggested that Pyrgomatinae and perhaps Megatrematinae evolved independently from an *Armatobalanus* ancestor. It appears that *Cantellius* inhabiting the hydrozoan *Distichopora* also evolved from an *Armatobalanus* or a common ancestor of the genus *Cantellius*. Speciation in Pyrgomatidae led to the inhabitation of a large variety of scleactinians. Moreover, this speciation is not limited to scleactinians, but also encompasses hydrozoans.

We show here that the barnacles extracted from the hydrozoan *Distichopora* cluster with the pyrgomatid *Cantellius* that usually inhabits a different class of Cnidaria, the Scleractinia. The inclusion of the barnacles extracted from the hydrozoan within the Pyrgomatidae refutes our previous hypothesis that the symbiosis of pyrgomatids involved only Scleractinia. These barnacles are found in the same clade as *Cantellius* and *Armatobalanus allium*. It is of special interest to study the relationship of these barnacles with *Armatobalanus nefrens* inhabiting the stylasterids *Errinopora pourtalesi* and *Stylaster californicus* from northern California.

ACKNOWLEDGEMENTS

The study was supported by Israel Science Foundation grant 308/14. Material used in this study was collected during Y.A.'s visit to the Museum National d'Histoire Naturelle, Paris (MNHN), supported by the SYNTHESYS Project (http://www.synthesys.info), financed by the European Community Research Infrastructure Action under the Integrating Activities Programmes (FR-TAF-3382). The specimens collected in Indonesia were imported under CITES permit code 04291/IV/SATS-LN/2011. The help of Dr Laure Corbari and Ms Paula A. Rodriguez-Moreno of MNHN Paris is greatly appreciated. Dr Yaakov Langzam of the Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, helped with the scanning electron microscopy work. Finally, we thank Ms Yael Laure for helping in editing and improving this paper. We thank the anonymous reviewers for their comments and the editor for comments that helped improve this paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Information about the specimens used for the phylogenetic and morphological studies.
Table S1. GenBank accession numbers used in for maximum likelihood phylogenetic trees and for estimation of pairwise distances between specimens of different species of *Cantellius* and other pyrgomatids.