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Biodiversity and phylogeny of Cocculinidae (Gastropoda: Cocculinida) in the Indo-West Pacific

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Keywords:	classification < Phylogenetics, deep-sea < Geography, species delineation < Taxonomy, Gastropoda < Taxa
Abstract:	<p>The family Cocculinidae (Gastropoda: Cocculinida) consists of small, usually colorless benthic limpets living primarily at depth below 100 m and on decaying plant or animal remains. These habitats are difficult to sample and the knowledge about their species diversity, biogeography, ecology, and evolution is poor. To explore the species diversity of the Cocculinidae we examined 499 specimens collected from 196 sites, mainly explored during expeditions of the Tropical Deep-Sea Benthos program in the Indo-West Pacific (IWP). To propose species-hypotheses, we used an integrated approach to taxonomy in which we combined DNA-based methods, with morphological, geographical, and ecological considerations. To classify the species-hypotheses into genera, we used a combination of one mitochondrial and two nuclear genes fragments to reconstruct a phylogenetic tree. We then used six morphological characters to diagnose the identified genera. Our results revealed an exceptionally high diversity of the IWP Cocculinidae, with 51 species-hypotheses that were mostly not assigned to available species names. We also discovered a previously unknown type of copulatory structure in the group. At higher taxonomic level, we identified ten main clades within the family. While six of them match to existing genera, four others should be regarded as new genera awaiting formal description</p>

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4 **1 Biodiversity and phylogeny of Cocculinidae (Gastropoda: Cocculinida) in**
5 **2 the Indo-West Pacific**
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8 4 HSIN LEE^{1,2}, NICOLAS PUILLANDRE¹, YASUNORI KANO³, WEI-JEN CHEN² and
9 5 SARAH SAMADI^{1*}
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11 7 ¹*Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS,*

12 8 *Sorbonne Université, EPHE, Université des Antilles, CP 51, 57 rue Cuvier, 75005 Paris, France*

13 9 ²*Institute of Oceanography, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan*

14 10 ³*Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5, Kashiwanoha, Kashiwa-shi, Chiba,*

15 11 *Japan*
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32 14 Running title: Biodiversity and phylogeny of Cocculinidae
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37 17 ABSTRACT
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40 19 The family Cocculinidae (Gastropoda: Cocculinida) consists of small, usually colourless
41 20 benthic limpets living primarily at depth below 100 m and on decaying plant or animal
42 21 remains. These habitats are difficult to sample and the knowledge about their species
43 22 diversity, biogeography, ecology and evolution is therefore poor. To explore the species
44 23 diversity of the Cocculinidae we examined 499 specimens collected from 196 sites, mainly
45 24 explored during expeditions of the *Tropical Deep-Sea Benthos* program in the Indo-West
46 25 Pacific (IWP). To propose species-hypotheses, we used an integrated approach to taxonomy
47 26 in which we combined DNA-based methods, with morphological, geographical and
48 27 ecological considerations. To classify the species-hypotheses into genera, we used a
49 28 combination of one mitochondrial and two nuclear gene fragments to reconstruct a
50 29 phylogenetic tree. We then used six morphological characters to diagnose the identified
51 30 genera. Our results revealed an exceptionally high diversity of the IWP Cocculinidae, with 51
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4 31 species-hypotheses that were mostly not assigned to available species names. We also
5 32 discovered a previously unknown type of copulatory structure in the group. At higher
6 33 taxonomic level, we identified ten main clades in the family. While six of them matched
7 34 existing genera, four others should be regarded as new genera awaiting formal description.
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12 36 ADDITIONAL KEYWORDS: classification – deep sea – organic fall – species delimitation –
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14 37 sunken wood – wooden-steps hypothesis
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INTRODUCTION

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20 41 The diversity of the metazoans inhabiting deep-sea habitats remains poorly known with biases
21 42 towards larger organisms (Costello *et al.*, 2010; Lee *et al.*, 2019; Danovaro *et al.*, 2020) and
22 43 of some spectacular habitats (notably hydrothermal vents). The organic remains decaying on
23 44 the deep-sea floor are one of the poorly-studied deep-sea habitats where small and discreet
24 45 organisms are living (Saeedi *et al.*, 2019; Harbour *et al.*, 2021; Souza *et al.*, 2021). Sunken
25 46 pieces of wood are carried along rivers to the ocean, drift with ocean currents and then sink to
26 47 the ocean floor. Seagrass leaves, algal holdfast, bones of marine vertebrates and hardened
27 48 parts of invertebrates also remain for an extended period of time as sunken organic material
28 49 (Cunha *et al.*, 2013; Amon *et al.*, 2017; Plum *et al.*, 2017; Soltwedel *et al.*, 2018). They are
29 50 commonly colonized by invertebrate communities (Samadi *et al.*, 2010; Kano *et al.*, 2016:
30 51 table 1). In terms of diversity, mollusks (gastropods, bivalves and chitons) constitute the main
31 52 component of these communities (Turner, 1977; Wolff, 1979; Kiel & Goedert, 2006; Warén,
32 53 2011). Among the wood-associated mollusks, some groups have relatives in hydrothermal
33 54 vents and cold seeps, which are habitats that have been the focus of much of the deep-sea
34 55 research in the last few decades. Thanks to these relatives, often having large body sizes, they
35 56 have been more frequently studied. For instance, Distel *et al.* (2000) suggested that the giant
36 57 bathymodioline mussels living in vents and seeps have originated from tiny ancestors that
37 58 were living on sunken woods and other organic falls (the “wooden-steps” hypothesis). This
38 59 and many subsequent studies (e.g. Samadi *et al.*, 2007; Lorion *et al.*, 2009, 2010, 2013;
39 60 Fujiwara *et al.*, 2010; Thubaut *et al.*, 2013; Souza *et al.*, 2021; Zhang *et al.*, 2021) suggested
40 61 that exploratory efforts still mostly focus on vents and seeps and that this bias had hindered
41 62 the understanding of evolutionary history of deep-sea mussels.

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58 63 Among the numerous and small cryptic taxa inhabiting the deep sea, Cocculinidae Dall,
59 64 1882 (Gastropoda: Cocculinida) encompasses limpet-shaped gastropods that live attached to

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4 65 primarily organic substrates laying on the ocean floor such as sunken woods, cephalopod
5 66 beaks and fish and whale bones. They are grazers with a rhipidoglossan radula to feed most
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7 67 probably on co-habiting micro-organisms that decompose these organic remains (Marshall,
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9 68 ["1985"]1986; Lesicki, 1998). Their unique habitats, small sizes and simple shell shapes make
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11 69 Cocculinidae one of the most taxonomically puzzling groups of gastropods. Presently, the
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13 70 *World Register of Marine Species*, lists 52 valid species classified under seven recognized
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15 71 genera (Moskalev, 1976; Marshall, [1985] 1986; Haszprunar, 1987; McLean, 1987; McLean
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17 72 & Harasewych, 1995; Warén, 1996; Hasegawa, 1997; Leal & Harasewych, 1999; Ardila &
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19 73 Harasewych, 2005; Zhang & Zhang, 2018; Chen & Linse, 2020).

19 74 Although relatively stable for the past 35 years, the taxonomy of the family
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21 75 Cocculinidae, named by Dall in 1882, has undergone significant changes as the exploration of
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23 76 deep-sea habitats progressed. For example, the monotypic genus *Fedikovella* Moskalev, 1976
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25 77 was described to classify the newly sampled species *Fedikovella caymanensis* Moskalev,
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27 78 1976 sampled from hadal depth of Cayman Trench in the Caribbean Sea. In the meantime, he
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29 79 proposed five other new genera to revise the classification of the family. Similarly, the
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31 80 monotypic genus *Macleaniella* Leal & Harasewych, 1999 was described to classify a new
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33 81 species collected from 8595 m at the bottom of Puerto Rico Trench, the deepest point in the
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35 82 Atlantic Ocean. This new species, named *Macleaniella moskalevi* Leal & Harasewych, 1999
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37 83 differentiated from other cocculinids in having a unique inner septum in its shell. New data
38
39 84 and samples also allowed the revision of the family as well as the superfamily Cocculinoidea
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41 85 (Thiele, 1909). For example, the previously recognized cocculinoid genera *Addisonia* Dall,
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43 86 1882, *Cocculinella* Thiele, 1909, *Lepetella* Verrill, 1880 and *Pseudococculina* Schepman,
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45 87 1908 are now placed in another superfamily, Lepetelloidea (Bouchet *et al.*, 2017).

43 88 Despite the establishment of the classification, the monophyly of the defined genera and
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45 89 the phylogenetic hypotheses were not steadily tested, especially not with molecular tools.
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47 90 Strong *et al.* (2003) conducted the first and only phylogenetic study for the Cocculinidae.
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49 91 They examined 31 morphological characters for 15 cocculinoid species, resulting in a single
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51 92 most parsimonious tree. The monophyly of superfamily Cocculinoidea (Cocculinidae +
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53 93 Bathysciadiidae), family Cocculinida, and the genera *Cocculina* Dall, 1882 and *Coccapigya*
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55 94 B. A. Marshall, 1986 were all supported. In contrast, *Paracocculina* Haszprunar, 1987 and
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57 95 *Cococrater* Haszprunar, 1987 were recovered as paraphyletic. *Fedikovella* and *Teuthirostria*
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59 96 Moskalev, 1976 collectively formed a monophyletic group sister to all other cocculinids.

58 97 Members of Cocculinidae have been recorded from all oceans of the world. Their
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3 98 lecithotrophic larvae (Young *et al.*, 2013) suggests limited dispersal ability and many
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5 99 geographically restricted species have been defined. In the Indo-West Pacific (IWP), the
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7 100 focused area of the present study, 30 species in four genera are currently recognized.
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9 101 *Cocculina*, the most studied genus of the family, contains 20 IWP species (e.g. Watson, 1886;
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11 102 Schepman, 1908; Thiele, 1925; Kuroda & Habe, 1949; Hasegawa, 1997; Zhang & Zhang,
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13 103 2018). *Coccopygia* includes seven IWP species with five possible endemics in the waters
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15 104 around New Zealand (Marshall, [1985] 1986) and two others distributed in the north-western
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17 105 Pacific including the seas around Japan, Korea and Taiwan (Kuroda & Habe, 1949;
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19 106 Hasegawa, 1997). *Cococrater* contains the type species *C. radiatus* (Thiele, 1903) from off
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21 107 Sumatra as the only IWP member of the genus. *Paracocculina* is composed only of two IWP
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23 108 species, namely *P. cervae* (C. A. Fleming, 1948) from off New Zealand and *P. laevis* (Thiele,
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25 109 1903) from off Sumatra, Indonesia.

24 110 In this study, we aim at exploring the species diversity and phylogeny of the
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26 111 Cocculinidae by broadly sampling the upper bathyal habitats in the tropical IWP. As it is clear
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28 112 that the systematic investigations cannot rely solely, or even primarily, on morphological
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30 113 examination (e.g. Puillandre *et al.*, 2017; Razkin *et al.*, 2017; Nantararat *et al.*, 2019; Horsáková
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32 114 *et al.*, 2020), we here use an integrated approach by combining evidence from morphology,
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34 115 geography, ecology and DNA-based species delimitation. We then assigned the inferred IWP
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36 116 species into monophyletic groups or genera based on the molecular phylogenetic
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38 117 reconstruction of the Cocculinidae. We also examine how useful are the six morphological
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40 118 characters in the redefinition of the existing genera and the definition of new genus-level
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42 119 clades.

43 121 MATERIAL AND METHODS

44 122 SAMPLE COLLECTION AND SELECTION

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47 125 The specimens examined were mostly collected during 21 IWP expeditions of the *Tropical*
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49 126 *Deep-Sea Benthos* (TDSB) program led by the Muséum national d'Histoire naturelle
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51 127 (MNHN) and the Institut de Recherche pour le Développement (IRD), in collaboration with
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53 128 the National Taiwan University (NTU) and the National Taiwan Ocean University (NTOU),
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55 129 between 2004 and 2018: AURORA 2007, BIOPAPUA, BOA1, CONCALIS, DONGSHA
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57 130 2014, EBISCO, EXBODI, KAVALAN 2018, KAVIENG 2014, MADEEP, MAINBAZA,
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3 131 MIRIKY, NANHAI 2014, NORFOLK 2, PANGLAO 2004, PANGLAO 2005, PAPUA
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5 132 NIUGINI, SALOMON 2, SALOMONBOA 3, SANTO 2006 and TAIWAN 2013. During the
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7 133 listed expeditions (see for more details <https://expeditions.mnhn.fr>) specific efforts were made
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9 134 by the on-board scientific teams to collect the fauna associated with plant remains (reviewed
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11 135 notably in Samadi *et al.*, 2010 and Pante *et al.*, 2012). The sampling covered the areas
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13 136 surrounding Taiwan (including the South China Sea and East China Sea), the Philippines
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15 137 (Bohol and Sulu Seas and the Pacific coast), Papua New Guinea (Bismarck Sea and Solomon
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17 138 Sea), Solomon Islands (Solomon Sea), New Caledonia (Coral Sea), Vanuatu (Coral Sea) and
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19 139 Madagascar (in the Mozambique channel) (Fig. 1). Most specimens were found attached on
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21 140 organic substrates collected by dredging or trawling at depths ranging from 100 to 1500
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23 141 meters. Some specimens were collected from organic substrates that were deployed on the
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25 142 deep-sea floor as traps to attract the recruitment of larvae (Samadi *et al.*, 2010 for more
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27 143 details). Additional specimens were collected from areas surrounding Japan and from East
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29 144 China Sea (Fig. 1; Table S1). We also included several Caribbean specimens from the
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31 145 expedition KARUBENTHOS 2 from TDSB program available in the collection of Muséum
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33 146 national d'Histoire naturelle (MNHN) to increase sample diversity and to assign generic
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35 147 names to clades; the type species of *Cocculina* and *Macleaniella* were described from this
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37 148 area.

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39 149 From the samples collected at 250 sampling stations during these 21 IWP expeditions of
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41 150 the TDSB program, we gathered thousands of “cocculiniform” limpets. Cocculiniforms refer
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43 151 to both of the phylogenetically distant cocculinoids and lepetelloids (Ponder & Lindberg,
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45 152 1997; Bouchet *et al.*, 2017; Lee *et al.*, 2019), which are barely differentiable by examining the
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47 153 shape and ornamentation of the teleoconch (post-metamorphic shell) alone. Scientific teams
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49 154 on board of research vessels therefore sorted all cocculiniform limpets and preserved them in
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51 155 95% ethanol. The number of specimens per sampling station was uneven, and these
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53 156 specimens often contained multiple morphotypes with different relative abundances. To cover
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55 157 at best the species diversity, we first sorted the specimens from each station into morphotypes
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57 158 and then selected one to three specimens from each morph (Fig. 2). This resulted in our
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59 159 selection of 709 cocculiniform specimens for genetic and morphological analyses.

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161 DNA SEQUENCING

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163 We extracted total genomic DNA from the muscle tissue of 710 specimens using either the

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4 164 automated extractor LabTurbo 48 Compact System and LGD 480-220 kits (Taigene
5 165 BioSciences Corp., Taiwan) or the NucleoSpin 96 Tissue kit (Macherey-Nagel, France) with
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7 166 the automated pipetting system epMotion 5075 according to the manufacturers' instruction.

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9 167 We amplified for all or a subset (see below) of these specimens a barcode fragment of
10 168 the mitochondrial *cytochrome c oxidase subunit 1* (*cox1*), a fragment of the nuclear *Histone*
11 169 *H3* (*H3*) gene and a C1–D2 region of the *28S ribosomal RNA* gene (*28S*). We amplified the
12 170 *cox1* fragment for all specimens mainly using the Folmer *et al.*'s (1994) primers LCO-1490
13 171 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO-2198 (5'-TAA ACT TCA
14 172 GGG TGA CCA AAA AAT CA-3'). However, these universal primers did not always work
15 173 and an alternative primer set was designed for the amplification and sequencing: CoccCOI-
16 174 43F (5'- GGA ACA CTY TAT ATT YTA TTA GG - 3') and CoccCOI-631R (5'- GTN GTA
17 175 TTR AAA TTT CGA TC - 3'). For a subset of the specimens, we also amplified a fragment
18 176 of the *28S* gene using the C1 (5'- ACC CGC TGA ATT TAA GCA T-3') and D2 (5'-TCC
19 177 GTG TTT CAA GAC GGG-3') primer set (Chisholm *et al.*, 2001) and a fragment of the *H3*
20 178 gene using the primers H3F1 (5'-ATG GCT CGT ACC AAG CAG ACV GC-3') and H3R1
21 179 (5'-ATA TCC TTR GGC ATR ATR GTG AC-3') (Colgan *et al.*, 1998).

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24 180 We performed the PCR reactions in 20 μ l, using 1–3 μ l of DNA, 1 \times reaction buffer, 1.25
25 181 mM MgCl₂, 0.26 mM dNTP, 0.3 μ M of each primer and 1.5 unit of Q-Bio Taq
26 182 (MPBiomedicals, LLC., USA). We started the PCR with a cycle of 94°C for 5 min, followed
27 183 by 35 cycles of denaturation step (94°C, 30 s), annealing step (51°C for *cox1*, 56°C for *28S* and
28 184 53°C for *H3*, 30 s) and elongation step (72°C, 45 s for *cox1*, 1 min for *28S* and 30 s for *H3*)
29 185 and a final elongation step for 5 min (72°C). The purification and sequencing for PCR
30 186 products were mainly carried out by Eurofins Scientific (France). For others the purification
31 187 was conducted with the AMPure magnetic bead clean-up protocol (Agencourt Bioscience
32 188 Corp.) and the sequencing was performed at the Center of Biotechnology, National Taiwan
33 189 University.

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36 190 The obtained DNA sequence chromatograms were visualized, edited and assembled
37 191 using CodonCode Aligner v.6.0.2 (Codoncode Corporation, Dedham, MA, USA). The edited
38 192 sequences were manually aligned and compiled using the software Se-AL v. 2.0 (Rambaut,
39 193 1996). However, some *H3* sequences (n = 39) presented more than one peak at multiple
40 194 nucleotide positions of the sequences. We thus suspected a presence of paralogs or
41 195 contaminant sequences. To resolve this, we pooled the PCR products of those 39 problematic
42 196 *H3* samples to construct a library using the NEB next Library preparation kit (New England
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3 197 Biolabs, MA, USA) with a single *H3* PCR per tag (Hinsinger *et al.*, 2015) for next generation
4 198 sequencing (NGS) under the Ion Torrent system (Life technologies, France). Template
5 199 amplifications of the library were performed by emulsion PCR on an Ion OneTouch robotic
6 200 system, and the subsequent sequencing was performed by an Ion Torrent PGM sequencer
7 201 using Hi-Q chemistry (Life technologies, France). The sequences were demultiplexed *a*
8 202 *posteriori* (Hinsinger *et al.*, 2015) and assembled using Geneious R9 (Biomatters Ltd.,
9 203 Auckland, New Zealand). To rule out the possibility of contamination, we compared the
10 204 obtained sequences using the BLAST search as implemented in NCBI GenBank and
11 205 topologies of phylogenetic trees inferred from individual gene fragments.
12 206

207 PRIMARY SPECIES HYPOTHESES (PSHs)

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209 Following Castelin *et al.* (2012) and Puillandre *et al.* (2012b), we defined an integrative
210 workflow for species delimitation of the sampled cocculinids (Fig. 2).

211 We first examined the 589 *cox1* sequences that we successfully obtained from 709
212 specimens. We compared these sequences to each other by reconstructing preliminary
213 maximum likelihood (ML) trees. The data matrix for the ML reconstruction also included
214 GenBank sequences of seven cocculinoids, 12 vetigastropods including nine lepetelloids and
215 one cephalopod (data not shown). We considered that the new sequences represent true
216 cocculinids when they form a well-supported clade with sequences from GenBank attributed
217 to this family. Conversely, we considered that we should probably attribute those grouping
218 with sequences attributed to other vetigastropods to lepetelloids. We also used the same
219 sequences in a BLAST-search to see if there was any cocculinoid sequence in the top five hits
220 (sorted by Max Score). Consequently, we concluded that 498 specimens from 187 of our
221 sampling stations were true cocculinoids (Table S1); the collection sites of these specimens
222 are shown in Figure 1.

223 We analysed 517 *cox1* sequences including the 498 newly obtained and 19 GenBank
224 sequences with two methods of species delimitation: Automatic Barcode Gap Discovery
225 (ABGD) (Puillandre *et al.*, 2012a) and Bayesian Poisson Tree Processes (bPTP) model
226 (Zhang *et al.*, 2013). We collected from GenBank (listed in Table S1 with available
227 information on the origin of the sequences) sequences attributed to *Cocculina messingi*
228 McLean & Harasewych, 1995 (AY377731, AY923910 and EU530108), *Cocculina*
229 *enigmadonta* C. Chen & Linse, 2020 (MN539277–MN539281), *Coccapigya punctoradiata*

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3 230 (Kuroda & Habe, 1949) (AB365259 and AB238590), *Cocculina subcompressa* Schepman,
4 231 1908 (GQ160744), *Coccopigya hispida* B. A. Marshall, 1986 (AY296823 from the voucher
5 232 NMNZ M075188, which is a paratype of the species) and seven sequences unidentified at the
6 233 species level: Cocculinidae sp. (HG942540), *Cocculina* spp. (AB238591, AB238592,
7 234 GQ160743 and GQ160745) and *Coccopigya* spp. (FM212785 and FM212786). ABGD, an
8 235 exploratory tool based on pairwise genetic distances, detects if there is a significant gap
9 236 between inter- and intraspecific variation (the so-called barcode gap). We used the online
10 237 version of ABGD (bioinfo.mnhn.fr/abi/public/abgd/) with K2P distance (Kimura, 1980) and
11 238 other default parameters. We also delineated the cocculinids into species with bPTP on the
12 239 bPTP webserver (<http://species.h-its.org/ptp/>) with 500000 MCMC generations and the
13 240 default parameters. We used bPTP, rather than the Generalized Mixed Yule Coalescent
14 241 method (Pons *et al.*, 2006), by following Tang *et al.* (2014) who suggested its robustness with
15 242 a user-specified tree (here a rooted ML tree inferred from a *cox1* dataset with all cocculinid
16 243 haplotypes and two bathysciadiid sequences as outgroup taxa). We then proposed PSHs for
17 244 clades delimited by both ABGD and bPTP. Finally, we evaluated different lines of evidence
18 245 to define secondary species hypotheses (Fig. 2).
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33 247 SECONDARY SPECIES HYPOTHESES (SSHs)

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36 249 To decide if we turn the detected PSH into secondary species hypotheses (SSHs) we used
37 250 additional criteria as described in Figure 2. The main additional sources of evidences were
38 251 firstly the nuclear genetic data that are unlinked to the mitochondrial data used to establish the
39 252 PSH, and then the comparisons of the geographic range and the ecological data (depth and
40 253 habitat) with the sister PSH.
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42
43 254 The recovery of a given PSH as a clade in both mitochondrial- and nuclear-gene trees strongly
44 255 supports the distinctiveness of the species (Pante *et al.*, 2015). Consequently, we
45 256 reconstructed phylogenetic trees using 28S sequences (for more details notably on alignment
46 257 methods see “Phylogenetic inference”). We tried to sequence a fragment of the 28S gene for
47 258 up to five representatives of each PSH defined with the *cox1* gene and for another specimen
48 259 from the Caribbean Sea for which we failed to obtain a *cox1* sequence (MNHN-IM-2013-
49 260 60187; Table S1). The first 415 base pairs of the 28S fragment were too conserved at the
50 261 species level, but useful at a deeper phylogenetic scale. Consequently, we reconstructed a tree
51 262 based only on this conserved part (hereafter referred to as the 28S-gene “master” tree) to
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3 263 define main lineages. We then reconstructed a subtree for each main lineage by aligning the
4 264 entire length of the *28S* fragment, which were partly too variable to be aligned with other
5 265 lineages (hereafter referred to as the *28S*-gene “sub” trees). We regarded a PSH as a potential
6 266 SSH when its component individuals constituted a supported phylogenetic clade in the *28S*
7 267 subtree or if they shared an identical *28S* sequence.

8 268 The paucispiral protoconch of cocculinids indicates a lecithotrophic larval development
9 269 and thus a limited dispersal capability (Young *et al.*, 2013). Closely related PSHs separated by
10 270 a geographic barrier, such as a landmass, an oceanic threshold or current, might have diverged
11 271 under an allopatric process of genetic differentiation, and we thus combined them into the
12 272 same SSH. On the contrary, the presence of sister PSHs in the same geographic region
13 273 suggests the presence of an effective reproductive isolation mechanism. In this case, we
14 274 maintained the sister PSHs as separate SSHs. We here defined 11 geographic regions based
15 275 on the distributions of landmasses, boundaries of sea basins and major ocean currents: seas
16 276 surrounding Japan (JP), East China Sea (ECS), seas surrounding Taiwan plus the South China
17 277 Sea (TW), seas surrounding the Philippines (PHI), Bismarck Sea (BIS), Solomon Sea (SOL),
18 278 seas surrounding Vanuatu (VAN), seas surrounding New Caledonia (NC), Coral Sea (COR),
19 279 sea off North Madagascar (MAD) and Mozambique Channel (AFR) (Fig. 1).

20 280 Depth is a major element of the ecological niche of benthic species (e.g. Stuart *et al.*,
21 281 2017). In a given geographic area, two closely related PSHs displaying distinct depth ranges
22 282 have probably distinct ecological niches and were therefore considered as separate SSHs.
23 283 However, the exact depth of the occurrence of the sampled specimens is difficult to determine
24 284 because a trawling or dredging operation starts and ends at different depths. To estimate the
25 285 bathymetric range of each proposed PSH, we followed the method used by Bouchet *et al.*
26 286 (2008:15), taking the inner values of the deepest and shallowest stations. For those PSHs that
27 287 contained only single specimen, we kept the original operating depth range of the station.

28 288 In taxonomic literature, cocculinid species are often described as specifically associated
29 289 to a given type of organic substrate (e.g. Zhang & Zhang, 2018). The field data allowed us to
30 290 determine on which organic substrates the specimens were collected and thus to highlight a
31 291 potential association of a PSH to a specific substrate. We considered that sister PSHs
32 292 associated to distinct substrates may be considered as different SSHs.

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BETA DIVERSITY ANALYSIS

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3 296 To estimate the dissimilarity of cocculinid species diversity among stations from the 11
4 297 geographic regions defined above, we used non-metric multidimensional scaling (NMDS)
5 298 plots based on the Jaccard similarity coefficient with the presence/absence data matrix,
6 299 implemented in the R packages “vegan” (Oksanen *et al.*, 2019).
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11 301 PHYLOGENETIC INFERENCE WITHIN THE COCCULINIDAE

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15 303 To examine the relationships among the delimited species (SSHs) and to revise the genus-
16 304 level classification of the Cocculinidae, we inferred phylogenetic trees based on three genes.
17 305 To complement the *cox1* and *28S* datasets we employed a *H3* dataset obtained using both
18 306 Sanger and NGS technics. This *H3* dataset contained representatives from each SSH,
19 307 including sequences extracted from GenBank (Table S1). We aligned the *cox1* and *H3*
20 308 sequences by eye and the *28S* using the automatic multiple alignment tool implemented in
21 309 MAFFT ver. 7 with default parameters (Kato & Standley, 2013).
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27 310 We compiled a combined dataset with the *cox1*, *28S* and *H3* sequences for a common set
28 311 of 140 taxa, including two distant outgroups of Neomphalida (*Melanodrymia aurantiaca*
29 312 Hickman, 1984 and *Peltospira smaragdina* Warén & Bouchet, 2001) and the two
30 313 bathysciadiids used for species delimitation. The dataset included at least one representative
31 314 of each SSH (Table S1).
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36 315 We reconstructed phylogenetic trees with the partitioned ML method using the *cox1*
37 316 dataset, reduced *cox1* dataset for bPTP, *28S* master dataset, *28S* sub datasets, *H3* dataset and
38 317 the combined dataset implemented in RAxML v.8.0 (Stamatakis, 2014). The GTR+G
39 318 substitution model was employed for the analyses because RAxML only provides GTR-
40 319 related models of rate heterogeneity. Partitions were set by genes and for *cox1* and *H3* by
41 320 codon positions. Nodal support was assessed by bootstrapping (Felsenstein, 1985) with 1000
42 321 pseudoreplicates.
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48 322 For the combined dataset, we also performed a Bayesian inference (BI) using MrBayes
49 323 v.3.2.6 (Ronquist *et al.*, 2012) on the CIPRES Science Gateway (Miller *et al.*, 2010), with eight
50 324 Markov chains in two parallel runs for 30000000 generations, a sampling frequency of one tree
51 325 per thousand generations and a heating temperature of 0.02. The convergence of the likelihood
52 326 scores for parameters was further evaluated using Tracer v.1.6 (Rambaut *et al.*, 2014) to make
53 327 sure that all ESS values were over 200. To be consistent with the ML analysis, we used the
54 328 same partitions and the same GTR+G substitution model for the BI method.
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MORPHOLOGICAL CHARACTERIZATION

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332 We used four morphological characters to complete the morphological diagnosis of the SSHs
333 and to guide the required final revision of the cocculinids. The shell shape, teleoconch
334 structure, position of the copulatory organ and presence or absence of epipodial tentacles, are
335 characters commonly used in the literature to describe cocculinid species. We thus divided the
336 examined specimens into morphogroups by identifying the character states as follows (Table
337 S2).

338 *Shell shape.* We classified the shapes of the examined cocculinid shells into round (oval
339 to conical) or spindle-shaped.

340 *Teleoconch sculpture.* Most cocculinid species have a patelliform teleoconch with simple
341 radial ribs and fine concentric growth lines (Fig. 3A), radial ribs with pits and periostracal
342 spines (Fig. 3B), plainly raised radial ribs (Fig. 3C), a clathrate sculpture with concentric lines
343 more prominent than axial ribs (Fig. 3D) or a strong concentric sculpture (Fig. 3E). Other
344 cocculinids have a smooth teleoconch with indistinct growth lines only (Fig. 3F).

345 *Copulatory organ.* The copulatory organ of cocculinids is highly variable and has been
346 considered a useful character for generic diagnoses (Haszprunar, 1987). We observed four
347 types of copulatory organs at different positions. These were associated with or branched
348 from the right cephalic tentacle (Fig. 4A), the anterior right corner of the foot (Fig. 4B), the
349 right neck under the oral lappet (Fig. 4C) or the right mantle margin (Fig. 4D).

350 *Epipodial tentacle.* All cocculinids except *Coccopigyra* species have a pair of epipodial
351 tentacles on the posterior foot (Marshall, [1985] 1986; Strong *et al.*, 2003).

352 We then assigned these groups to the existing genera according to the taxonomic
353 literature wherever possible (Marshall, [1985] 1986; Haszprunar, 1987; McLean, 1987;
354 McLean & Harasewych, 1995; Leal & Haszprunar, 1999).

355 To complement the description of the new genera identified by our molecular analysis,
356 we also coded the variability of the protoconch and the radula. These characters can only be
357 observed under the scanning electron microscopy (SEM). For the preparation of the radula,
358 we pulled out the entire radular ribbon by hand dissection and treated it with diluted bleach
359 until surrounding tissue was completely dissolved. After rinsed several times with distilled
360 water, the radula was unfolded and then mounted on a stub. We conducted the SEM
361 observation mainly at MNHN and some at the Atmosphere and Ocean Research Institute

362 (AORI), the University of Tokyo.

363 *Protoconch*. A reticulate sculpture is dominant in the protoconchs of the Cocculinidae
364 (Fig. 5A). However, in some cocculinids the protoconch shows concentric lines in the first
365 half and smooth in the last half (Fig. 5B).

366 *Radula*. The rachidian tooth of the cocculinoid radula is characterized by overhanging
367 cusps, which may be present or obsolete (Strong *et al.*, 2003). Then, we distinguished the
368 radula based on two features, the shape of the rachidian and the number of cusps on the
369 rachidian. We defined six distinct types of the cocculinid rachidian tooth: (a) “obsolete”, no
370 rachidian observed (Fig. 5C); (b) “acuspate-flat”, tip flat without a cusp (Fig. 5D); (c)
371 “unicuspid narrow”, tip narrow with a pointed cusp (Fig. 5E); (d) “unicuspid broad”, tip
372 broad and pointed (Fig. 5F); (e) “multicuspid narrow”, tip narrow with more than one cusp
373 (Fig. 5G); and (f) “multicuspid broad”, tip broad with more than one cusp (Fig. 5H).

374 375 RESULTS

376 377 DATASETS FOR SPECIES DELIMITATION

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379 The final alignment of the *cox1* dataset contained 657-bp long sequences from 498 sampled
380 cocculinids and 19 GenBank entries, plus two bathysciadiids for rooting. The inferred
381 phylogenetic tree is presented in Figure S1. The terminal taxon labels are composed of two
382 letters, corresponding to eight morphogroups as defined with the four primary morphological
383 characters (shell shape, teleoconch sculpture, conditions of copulatory and epipodial tentacles;
384 Tables 1, S3), followed by a number, corresponding to PSHs (see below), namely MA1–
385 MA12, MB1–MB10, MC1–MC19, MD1–MD8, ME1–ME3, MF1–MF9, MG1–MG18 and
386 MH1–MH2.

387 We aligned 415 bp of the sequences from the 147 samples successfully sequenced
388 (including one outgroup) in the 28S “master” dataset. The inferred ML tree allowed us to
389 reveal seven genetically distinct clades or groups (Fig. S2). Based on this phylogenetic result,
390 we compiled seven subdatasets, including 28S-a (404 bp, 14 samples), 28S-b (816 bp, 39
391 samples), 28S-c (800 bp, 18 samples), 28S-d (408 bp, 13 samples), 28S-e (731 bp, 7 samples),
392 28S-f (791 bp, 20 samples) and 28S-g (811 bp, 35 samples). We then added an additional
393 sample as outgroup taxon to each subdataset. However, 18 sequences in 28S-b were too
394 different and we were not able to correctly align them with others. We thus removed them

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3 395 (809 bp, 21 samples left) and put them aside in a new dataset, 28S-h (816 bp, 18 taxa).
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5 396 Finally, the inferred tree shows eight groups (Fig. S2). Phylogenetic trees inferred from the
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7 397 28S subdatasets are shown in Figure S3A–H.
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10 399 SPECIES DELIMITATION ANALYSES

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12 401 The ABGD and bPTP analyses delimited 89 and 92 PSHs, respectively, 78 of which were
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14 402 recovered in both analyses. Figure 6 summarizes results from ABGD, bPTP and other criteria
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16 403 used for species delimitation. Also shown in this figure are the numbers of shell morphotypes,
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18 404 which were classified on the basis of global shell morphology, within each morphogroup
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20 405 (MA–MH). We considered PSHs as separate species when they were reciprocally
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22 406 monophyletic in a 28S subtree. Some pairs or triplets of PSHs (MG11–MG13; MD2 and
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24 407 MD3; MA6 and MA7; MA11 and MA12; MC16 and MC17; MF3 and MF4; Fig. 6) shared
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26 408 the same 28S sequence, and we conservatively considered such PSHs as a single species.
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28 409 Similarly, if two allopatric PSHs were gathered in a same 28S clade we considered them as
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30 410 geographic populations of single species (i.e. MG2–MG4; MG6–MG10; MD4 and MD5;
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32 411 MB9 and MB10; Fig. 6A, B). Figure 6 further illustrates how we integrated the different lines
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34 412 of evidence to make our decisions. Note that in all cases, our final decision was conservative,
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36 413 i.e. if there was no clear evidence to choose between one single species or two different
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38 414 species, we decided to choose the single species option. Examples include MC13, MC14 and
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40 415 MC15 that lacked 28S data (Fig. 6C). By taking all lines of evidence into account, we inferred
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42 416 60 SSHs (including one without *cox1* data but with 28S, morphological and geographic data).
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44 417 Of these, we recorded 51 from the 187 stations sampled in the IWP. Morphological characters
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46 418 of each SSH are shown in Table S2.
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49 420 REGIONAL SPECIES DIVERSITY

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51 422 We included the 187 IWP stations in our NMDS analysis. The stress value reached close to 0
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53 423 (around 0.0007) after 200 NMDS runs, suggesting the presence of outlier stations. This result
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55 424 was not surprising since some stations contained a single, unique species. For a better
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57 425 recognition of underlying patterns, we removed 25 dissimilar stations that showed
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59 426 NMDS1/NMDS2 over 20 or below -20. The re-analysis with 162 stations resulted in the final
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427 stress value of 0.022. NMDS plots showed two main groups. One contained most stations in

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3 428 the south-western Pacific and Indian Ocean, and part of the stations in the north-western
4 429 Pacific; the other contained many of the stations in the north-western Pacific and one station
5 430 in the south-western Pacific (Fig. 7).
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10 432 PHYLOGENETIC RELATIONSHIPS AMONG SPECIES AND MORPHOGROUPS

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12 434 The combined dataset of three genes (*cox1*, *H3* and *28S*) consisted of 1,421 aligned sites and
13 435 140 terminals, including 136 cocculinids, two bathysciadiids and two neomphaloids. The
14 436 topologies of the inferred ML and BI trees were similar to each other but with a noticeable
15 437 difference in the position of the clade MH (Figs S4, S5). The ML tree is shown in Figure 8
16 438 with reference to PSH names. The Cocculinidae consisted of ten major clades and a single
17 439 species (*Cocculina messingi*) that did not fall into any one of the major clades. The latter may
18 440 be due to the quantity of missing data. Seven out of the ten clades were strongly supported
19 441 with a bootstrap value (BS) of $\geq 80\%$ and posterior probability (PP) of ≥ 0.95 . Six of the
20 442 seven well-supported clades corresponded to the morphogroups MD, MH, MB, ME, MG and
21 443 MF. Conversely, the morphogroups MA and MC appeared to be non-monophyletic (Fig. 8).
22 444 Morphogroup MC consisted of two distantly related clades that were denoted as MCI and
23 445 MCII; the former was recovered as a sister clade to MB and the latter to MF. One species of
24 446 MA was distinct from others in the same morphogroup and instead sister to the clade ME.
25 447 However, this relationship was not supported with a significant BS value (73%) and we
26 448 cannot exclude that MA is monophyletic. Overall, relationships among the ten major clades
27 449 were not resolved expect the sister group relationships between MB and MCI (BS = 92%, PP
28 450 = 1.00), MF and MCII (86%, 1.00) and MG and MF + MCII (90%, 1.00) (Figs 8, S4, S5).
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45 452 PROTOCONCH AND RADULA

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47 454 To further diagnose the cocculinid clades we used two morphological characters: the
48 455 protoconch and radula. However, the protoconch of adult cocculinids are often eroded,
49 456 potentially due to high dissolution rates of calcium carbonate in the deep sea. Cocculinid
50 457 radulae are also known to be similar within a genus (e.g. Haszprunar, 1987; Marshall, [1985]
51 458 1986; McLean & Harasewych, 1995). Thus, we selected only 21 and 24 representative
52 459 specimens for the examination of the protoconch (Figs 9, 10) and radula (Fig. 11),
53 460 respectively.
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3 461 Most protoconchs had a reticulated sculpture, whereas the individuals of clade MD
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5 462 displayed a concentric sculpture in the initial part of shell formation (Fig. 10A–D). The radula
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7 463 showed more variation between and within the clades (Fig. 8). Clade MA had two different
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9 464 types of the rachidian tooth: “unicuspid broad” and “multicuspid broad” (Fig. 11A–D).
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11 465 Among the species assigned to the polyphyletic morphogroup MC, those of clade MCI had
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13 466 two types of “unicuspid broad” and “obsolete” (Fig. 11G–J), whereas two species from MCII
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15 467 shared the same “acuspate-flat” rachidian (Fig. 11K, L). One species examined for clade ME
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17 468 had the rachidian tooth of the “acuspate flat” (Fig. 11O). On the other hand, the clades MB,
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19 469 MD, MF and MG were diagnosed by their “unicuspid narrow” (Fig. 11E, F), “multicuspid
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21 470 narrow” (Fig. 11M, N), “acuspate-flat” (Fig. 11P–R) and “obsolete” (Fig. 11S–W) types of
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23 471 the rachidian, respectively. Finally, the only specimen examined for the clade MH had a
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25 472 rachidian tooth that can be classified into the “unicuspid broad” type (Fig. 11X). This
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27 473 specimen was also unique in having unicuspid first lateral teeth; all other radulae examined
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29 474 here had multiple cusps in the first lateral tooth.
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32 DISCUSSION

33 SPECIES DIVERSITY IN THE INDO-WEST PACIFIC

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36 480 The integrative taxonomy as implemented in this study revealed a remarkable species richness
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38 481 of cocculinids with 51 delimited species occurring in the IWP. However, only 29 extant
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40 482 species have been described from the IWP and specimens examined in previous studies
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42 483 originated from a few restricted areas. These include five *Coccopigya* and one *Paracocculina*
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44 484 species around New Zealand and off the east coast of Australia (Fleming, 1948; Marshall,
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46 485 [1985] 1986), six *Cocculina* and two *Coccopigya* species around Japan (Kuroda & Habe,
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48 486 1949; Hasegawa, 1997, 2009; Zhang & Zhang, 2018), one *Cocculina* species around the
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50 487 Philippines (Watson, 1886), one *Cococrater*, eight *Cocculina*, one *Paracocculina* species
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52 488 around Indonesia (Thiele, 1903; Schepman, 1908) and five *Cocculina* species off West Africa
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54 489 (Thiele, 1925). Our specimens were collected mainly in the vicinity of Papua New Guinea,
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56 490 the Solomon Islands, New Caledonia and Vanuatu, but also from around Madagascar, in the
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58 491 South China Sea, near Taiwan, the East China Sea and Japan. There is thus a relatively small
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60 492 overlap between our sampling areas and the known distribution ranges of the described
493 cocculinid species. This suggests that many, if not most, of the species we delimited in this

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4 494 study are new to science as discussed below.

5 495 Based on our sampling, 21 inferred species seemed to have limited, regional distributions
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7 496 (see Figure 1 for 11 geographic regions defined in this study). Some other species showed
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9 497 wider ranges, but they generally occurred only in adjacent regions. Only a few had wide
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11 498 ranges. For instance, *Coccapigya* sp. 3 ranged from Madagascar (MAD) in the West Indian
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13 499 Ocean to Taiwan (TW), the Philippines (PHI) and Solomon Sea (SOL) in the West Pacific;
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15 500 nGen4 sp. 2 was collected from the Mozambique Channel (AFR) and the Bismarck Sea (BIS).
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17 501 *Cococrater* sp. 7 is distributed in the East China Sea, Philippine waters, Bismarck Sea,
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19 502 Solomon Sea and Vanuatu waters. We found *Paracocculina subcompressa* in Philippine
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21 503 waters, Bismarck Sea, Solomon Sea, Vanuatu and New Caledonian waters (Fig. 6C). This
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23 504 pattern of limited geographic range for most of the identified species is cohesive with the
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25 505 lecithotrophic larvae and the low instantaneous fecundity (less than 40 oocytes) in the life
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27 506 history (Young *et al.*, 2013). Indeed, low connectivity among populations are more frequently
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29 507 observed in deep-sea species with lecithotrophic or non-planktotrophic larvae (e.g. Plouviez *et*
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31 508 *al.*, 2009; Coykendall *et al.*, 2011; Young *et al.*, 2012; Chen *et al.*, 2015) than for those with
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33 509 planktotrophic larvae (e.g. Castelin *et al.*, 2012; Arellano *et al.*, 2014; Zaharias *et al.*, 2020).
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35 510 However, for such poorly dispersive organisms, interpretation should be made with caution as
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37 511 the sampling bias may also explain the apparent endemism (Castelin *et al.*, 2012).

38 512 Regional endemism was observed for most of the IWP cocculinid species we identified.
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40 513 This general trend contrasts with the case of nGen4 sp2 that gathered specimens collected in
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42 514 two distant regions: the Mozambique Channel and the Bismarck Sea (Fig. 6B). The genetic
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44 515 divergence between the samples from these regions was low (i.e. *cox1* distance = 0.64%)
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46 516 despite a geographic distance of more than 5000 km between them. Strikingly, we collected
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48 517 this species always on chondrichthyan egg cases and sister to nGen4 sp1 that was collected
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50 518 exclusively on the skeleton of deep-sea corals. We should stress that plant remains are highly
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52 519 abundant around tropical islands, and these were thus well represented in the catches available
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54 520 in the MNHN's collection. Animal remains seem to be much more unevenly distributed on
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56 521 the deep-sea floor and were much rarer in our samples. The two species were never present in
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58 522 the abundant wood material and we can therefore regard them as truly specialized to either
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60 523 egg cases or coral skeletons. Their robust sister relationship (Figs 6B, 8) and vast geographic
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525 524 range of nGen4 sp1 suggest that habitat shift from the plesiomorphic wood to other biogenic
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527 525 substrates has been uncommon in the evolutionary history of the Cocculinidae. We found few
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529 526 specimens of *Coccapigya hispida* from the specimens collected during the experimentally

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3 527 deployed leaves and bones in New Caledonia (MG13, Fig. 6A; Samadi *et al.*, 2010), but those
4 528 cases might represent rare, opportunistic use of substrates by an essentially wood-dwelling
5 529 species. Additional efforts for sampling species associated to animal remains are still required
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7 530 to better understand the ecological radiation of cocculinid limpets.

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9 531 At the community scale, the sampling stations were divided into two main groups based
10 532 on cocculinid species composition: one included most stations in the Indian Ocean and south-
11 533 west Pacific and some stations in the north-west Pacific; the other one contained stations
12 534 mostly from the north-west Pacific, but also one in the south-west Pacific (experimentally
13 535 deployed organic materials; Samadi *et al.*, 2010) (Fig. 7). Actually, we found only 13 out of
14 536 the 51 cocculinid species in both the north-west and south-west Pacific. Physical factors such
15 537 as ocean currents might play an important role in shaping the community pattern of deep-sea
16 538 animals (McClain & Hardy, 2010). Deep-sea planktotrophic larvae often migrate from the
17 539 deep seabed to the photic layer and are then transported across thousands of kilometres across
18 540 large geographic areas via surface currents like the Equatorial and Kuroshio Currents (e.g.
19 541 Arellano *et al.*, 2014; Hilário *et al.*, 2015; Yahagi *et al.*, 2017, 2019, 2020). Conversely, most
20 542 lecithotrophic developers such as cocculinids are probably transported by bottom currents.
21 543 Although bottom currents generally move slowly across ocean floors (Stow *et al.*, 2002), such
22 544 currents in the Southern Hemisphere may play an important role to transport lecithotrophic
23 545 larvae of cocculinid species crossing the oceans. This might explain the homogeneous species
24 546 composition and wide-ranging distributions of some species in the Indian and south-west
25 547 Pacific Oceans (Figs 6, 7).

548 549 CLASSIFICATION OF COCCULINID GENERA

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551 We here provide the first DNA-based phylogeny of Cocculinidae, where ten major clades or
552 lineages are recognized (Fig. 8). Among the ten clades five contain the type species of a
553 named genus, or a species similar to the type, and can therefore be attributed to the following
554 generic names: *Cococrater* (MA), *Coccopigya* (MG), *Cocculina* (MCI), *Fedikovella* (MD)
555 and *Paracocculina* (MF). The clades ME and MH each constitutes an unnamed genus without
556 doubt. The clades MB, MCII and MA1 as independent genera are also plausible, but less
557 certain, partly due to insufficient support values of the tree topology (Fig. 8). We propose the
558 following generic classification for the IWP cocculinids based on the phylogenetic
559 reconstruction and morphological examination in this study (Table 2). These putative new
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genera and relevant species will be named and described in a subsequent study that notably requires examining the type specimens of all valid names of cocculinids.

562

563 *Cocculina*

564 The species in the genus *Cocculina* as traditionally defined can be diagnosed from other
565 cocculinids by having a copulatory organ originated from the oral lappet (Marshall, [1985]
566 1986; Haszprunar, 1987; McLean & Harasewych, 1995; Strong *et al.*, 2003). This particular
567 condition was observed in the specimens of morphogroups MB (9 species), MCI (5 species)
568 and MCII (8 species). However, we consider that MCI alone represents *Cocculina* s.s. with
569 specimens identified as the type species *C. rathbuni* (MC12, collected in the waters off
570 Guadeloupe, French Caribbean, at depths of 600–900 m) in this clade (Fig. 8).

571 Morphogroup MB, recovered as the sister clade to MCI (*Cocculina* s.s.), mostly shows a
572 unique, spindle shape of the shell and a teleoconch sculpture with plainly raised radial ribs
573 (Table 1; Fig. 3C). These conditions are found in two described species, *Cocculina emsoni*
574 McLean & Harasewych, 1995 from around the Bahamas and *Cocculina angulata* Watson,
575 1886 from the Philippines. However, our MB specimens collected from Caribbean Sea (MB3)
576 have a narrow, oval shell shape compared to *C. emsoni* and MB specimens from IWP. Our
577 MB specimens and *C. emsoni* further share the loose reticulate sculpture of the protoconchs
578 (Fig. 10D) and narrow-shaped rachidian of the radula (Fig. 11E, F; McLean & Harasewych,
579 1995). *Cocculina angulata*, known only from the shell (Watson, 1886), also agrees with
580 conditions in many MB specimens, but we cannot robustly identify it with any one of our
581 species, including MB8 from the Philippines. We consider that the monophyletic nature and
582 morphological uniqueness may warrant a new genus (nGen3) for this group, but its
583 relationship with MCI (*Cocculina* s.s.) remains to be solved (Figs 6B, 10).

584 Although MCII is phylogenetically distant from MCI, their specimens are similar to each
585 other in external morphology. However, the two groups have different rachidian teeth of the
586 radula. The specimens of MCII have an “acuspate-flat” type (Fig. 11K, L) similar to those in
587 its sister clade MF (Fig. 11P–R). On the contrary, the rachidians in MCI and its sister lineage
588 MB are of different types: the former being “acuspate-flat,” “unicuspid broad” or “obsolete”
589 (Fig. 11G–J), while the latter are “unicuspid narrow” (Fig. 11E, F). However, we examined
590 only two radulae from MCII and more data are needed to confirm these as diagnostic traits of
591 different genera. Regarding the bathymetric distribution, the specimens of MCI were sampled
592 at shallower depths (50–365 m deep) than those of MCII (105–900 m) and MB (170–1250 m)

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3 593 (Fig. 6). This might further support the taxonomic uniqueness of the clade MCII as another
4 594 new genus (nGen1). The previous morphology based cocculinid phylogeny by Strong *et al.*
5 595 (2003) did not detect this putative genus within *Cocculina* probably as a result of the
6 596 geographic sampling centred in the Atlantic Ocean.

7 597 The phylogenetic positions of *Cocculina messingi* and *C. enigmadonta* Chen & Linse,
8 598 2020 are both unresolved. The former was grouped with the clade MA1 + ME without
9 599 support (Fig. 8); the latter was sister to *C. messingi* in the *cox1*-based ML tree, but again
10 600 without support (Fig. 6C). As we only get *Cox1* sequences for these two species, these
11 601 affinities are uncertain. The type specimen of *C. messingi* was collected around the Bahamas
12 602 at 412 m deep. Its copulatory organ originated from the right oral lappet conforms to the
13 603 diagnosis of *Cocculina s.l.*, but its teleoconch with raised concentric growth lines and fine
14 604 radial striae (McLean & Harasewych, 1995: fig. 12) is similar to the concentric sculpture we
15 605 classified (Fig. 3D), which can only be observed in nGen4 sp4 (Table S2). *Cocculina*
16 606 *enigmadonta* is the only cocculinid species found so far in the Southern Ocean, and also the
17 607 only one from the hydrothermal vent environment (Chen & Linse, 2020). It has a unique type
18 608 of the radula and a copulatory organ similar to that of *Paracocculina* (Chen & Linse, 2020:
19 609 fig. 5C). We thus leave *C. messingi* and *C. enigmadonta* in *Cocculina s.l.*, waiting for more
20 610 data for future reclassification.

21 611 Among other IWP species described under this genus, *Cocculina japonica* Dall, 1907
22 612 was easily recognized in our material and fell into *Cocculina s.s.* (MCI) in the present study.
23 613 The information available in the species descriptions of *C. capulus* Thiele, 1925, *C. dofleini*
24 614 Thiele, 1925, *C. fragilis* Thiele, 1925, *C. pellita* Thiele, 1925, *C. similis* Thiele, 1925 and *C.*
25 615 *vestita* Thiele, 1925, did not allow us to confidently attribute them to our SSHs or to the
26 616 clades identified here. Seven species names proposed by Schepman (1908) for Indonesian
27 617 material were also difficult to link to our specimens, because the original descriptions were
28 618 based mainly on the shell and little on radular morphology. *Cocculina ovata* Schepman, 1908
29 619 might be an exception. McLean (1987) attributed numerous specimens collected at 187–210
30 620 m deep in the Philippines to this species. Among our SSHs from similar depths of
31 621 neighbouring areas, the one made up of MC19 (Fig. 6C) showed the conchological features of
32 622 *C. ovata* redescribed by McLean (1987), perhaps suggesting its identity at the species level.
33 623 The shape of rachidian tooth of MC18 (Fig. 11I) did not exactly match the condition
34 624 described for *C. ovata* by Schepman (1908) and McLean (1987). However, this inconsistency

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3 625 might have resulted from worn teeth or different angles in SEM shots and examination of
4 626 more radulae is needed to confirm our species identification.

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7 627 Five more species have been described from the IWP under the genus *Cocculina*: *C.*
8 628 *pacifica* Kuroda & Habe, 1949, *C. tosaensis* Kuroda & Habe, 1949, *C. surugaensis*
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10 629 Hasegawa, 1997 and *C. tenuitesta* Hasegawa, 1997, all on sunken wood off Japanese coasts,
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12 630 and *C. delphinicola* Zhang & Zhang, 2018 from a dolphin skull at a depth of 300–400 m in
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14 631 the East China Sea (Zhang & Zhang, 2018). Although none of them were found in our
15
16 632 material, we believe that the first species should be moved from the genus to either
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18 633 *Fedikovella* (“*C.*” *pacifica*; see below) or Lepetellidae (“*C.*” *tosaensis*; see Hasegawa, 1997:
19 634 65). The sequences we gathered from GenBank similarly illustrated the difficulty of the
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21 635 morphological identification at both genus and species levels. Examples include published
22
23 636 sequences attributed to *Coccopigya punctoradiata* (Table S1) that were clustered with an
24
25 637 entirely different species (MC5) in a potential new genus (nGen1).

26 638 27 28 639 *Coccopigya*

29 640 *Coccopigya* is the most easily recognized genus in Cocculinidae. It is distinguished from
30
31 641 other cocculinids by having periostracal spines and by lacking epipodial tentacles (Marshall,
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33 642 [1985] 1986; Haszprunar, 1987). All specimens attributed to MG, corresponding to nine
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35 643 distinct species (Fig. 6A), displayed both conditions and were thus considered as the members
36
37 644 of *Coccopigya* (Tables 1, 2).

38 645 Specimens of MG2–MG4 and MG15 were identified respectively as *Coccopigya*
39
40 646 *punctoradiata* (Kuroda & Habe, 1949) and *Coccopigya okutanii* Hasegawa, 1997 based on
41
42 647 their morphology and collection sites (Japan). The specimens of MG11–MG13, gathered into
43
44 648 a single SSH, were attributed to *Coccopigya hispida* based on a published *cox1* sequence from
45
46 649 a paratype of the species (AY296823, voucher NMNZ M075188) (Fig. 6A).

47 650 48 651 *Cococrater*

49
50 652 The use of the right cephalic tentacle as a copulatory organ is considered plesiomorphic
51
52 653 within the Cocculinoidea and shared by the species of *Cococrater*, *Coccopigya*, *Fedikovella*,
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54 654 *Macleaniella* and *Teuthirostria* (Table 2; Strong *et al.*, 2003). This condition was observed in
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56 655 morphogroups MA, MD, MG and MH (Table 1; Fig. 6). Of these, MD and MG were
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58 656 attributed to *Fedikovella* and *Coccopigya*, respectively, and MH to an undescribed genus with
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60 657 ecological uniqueness (see below). The morphological and ecological features of

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4 658 *Macleaniella* and *Teuthirostria* do not match any of our specimens (see section *Macleaniella*
5 659 and *Teuthirostria*). We thus consider morphogroup MA as *Cococrater*.

6
7 660 The occurrence of *Cococrater* in the IWP region, including the type species *C. radiatus*
8 661 (with a type locality off western Sumatra), suggests a generic identity for one or both of the
9 662 two MA lineages. As we cannot exclude that MA is monophyletic (see above) and the
10 663 original description of *C. radiatus* by Thiele (1903) was too simple to be linked to any of our
11 664 SSHs, both lineages are assigned here to *Cococrater* with a possibility of establishing a new
12 665 genus in future pending more data on their relationships and the type specimen of *C. radiatus*.

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18 667 *Fedikovella*

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20 668 The genus *Fedikovella* contains two described species. The type species *Fedikovella*
21 669 *caymanensis* Moskalev, 1976 was described from a hadal depth (6800 m) in the Cayman
22 670 Trough. *Fedikovella beanii* (Dall, 1882) was described from the north-western part of the
23 671 Atlantic (lectotype USNM 333751 from 613 m deep, off Martha's Vineyard Island,
24 672 Massachusetts) and has been collected from the Lesser Antilles and Martinique at around 400
25 673 m to 1000 m deep (McLean & Harasewych, 1995). The concentric sculpture of the
26 674 protoconch and reticulate ribs of the teleoconch are diagnostic characters of the genus
27 675 (Moskalev, 1976; Marshall, [1985] 1986; McLean & Harasewych, 1995; Leal & Harasewych,
28 676 1999). The right cephalic tentacle of *F. caymanensis* is supposedly used as a copulatory organ
29 677 but not hypertrophied or modified (Leal & Harasewych, 1999). The rachidian, first and
30 678 second lateral teeth are all multicuspidid (Leal & Harasewych, 1999). These conditions were
31 679 found in our specimens of the clade MD (Tables 1, 2; Figs 10A–D, 11M, N).

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33 680 An additional MD specimen from the Lesser Antilles at 500–550 m deep (MNHN-IM-
34 681 2013-60187; Table S1) was grouped with the IWP specimens in the 28S analysis (Fig. S2),
35 682 supporting the identity of this clade as *Fedikovella* from a geographic perspective. This clade
36 683 also included species from off Faro, Portugal (M-unknown1, AORI_YK1662) and from off
37 684 Costa Rica, east Pacific (M-unknown2, SMNH-108733) (Fig. 8). "*Cocculina*" *pacifica*
38 685 described from off Japan should likewise be attributed to *Fedikovella* based on its shell,
39 686 radula and copulatory organ (Kuroda & Habe, 1949: pl. 3, fig. 5; Hasegawa, 1997: fig. 5).
40 687 *Fedikovella* thus occupies a wide range, both geographically (worldwide) and bathymetrically
41 688 (bathyal to hadal zones). We should stress that Strong *et al.* (2003) found *Fedikovella* +
42 689 *Teuthirostria* as sister to the rest of the cocculinids, but this sister group relationship

690 (*Fedikovella* versus all other examined cocculinids, as we did not include *Teuthirostria* in the
691 analysis) is only weakly supported in the molecular tree we obtained.

692

693 *Paracocculina*

694 *Paracocculina* contains two named species: the type species *P. laevis* collected from off
695 west of Sumatra Island at 614 m deep (Thiele, 1903) and *P. cervae* from New Zealand in a
696 depth range of 18–891 m (Marshall, [1985] 1986; Haszprunar, 1987). Marshall (1994)
697 recorded *P. cervae* from wood, whale bones and sunken algal holdfasts. This genus can be
698 differentiated from other cocculinids by their foot-originated copulatory organ (Haszprunar,
699 1987; but see Strong *et al.*, 2003: 122), a condition observed in all our specimens attributed to
700 the clade MF (Fig. 4B; Tables 1, S2). The MF specimens were collected from a wide
701 geographic area, ranging from the western Indian Ocean (Madagascar) to the western Pacific
702 (New Caledonia). Although we did not survey west off Sumatra with the type locality of *P.*
703 *laevis*, the identity of the clade MF as *Paracocculina* seems to be well justified with the
704 unique position of the copulatory organ.

705 Among GenBank data the sequence GQ160744 (Table S1; MNHN-IM-2009-5054 from
706 the Solomon Sea) was grouped with MF9 (Fig. S1). This GenBank sequence has been
707 attributed to “*Cocculina*” *subcompressa*, a species described from south-west of Timor Island
708 (216 m; Schepman, 1908). The widespread distribution of MF7–MF9, ranging from the
709 Philippines to New Caledonia, and from 350 m to 1000 m deep (Fig. 6C), may potentially
710 justify the species identification (as *Paracocculina subcompressa*). Unfortunately, Schepman
711 (1908) did not mention the position of the copulatory organ in the type material and our
712 identification thus remains tentative.

713

714 Clades ME and MH

715 Clade ME contained three species from the Bismarck Sea, the Solomon Sea and Vanuatu
716 (Fig. 6A). These species displayed a remarkably unique position of a presumed copulatory
717 organ on the right mantle margin close to the head (Table 1; Fig. 4D). No-one has yet reported
718 this type of copulatory organ for any cocculinids. Further examination of the anatomy and the
719 histology is thus mandatory. The phylogenetic position (Fig. 8) combined with the
720 morphology suggests to treat this clade as a new genus (nGen2) awaiting formal description.

721 Clade MH contained specimens from “non-wooden” habitats. The specimens of MH1
722 were found on nodes of deep-sea bamboo corals (Isididae), which are formed by a specific

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3 723 protein matrix (Ehrlich *et al.*, 2006), whereas MH2 were on the egg cases of chondrichthyes
4 724 (sharks and/or rays). We consider that such unique habitats and independent phylogenetic
5 725 position they occupy (Fig. 8) justify erection of another new genus (nGen4).
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727 *Macleaniella* and *Teuthirostria*

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12 728 These cocculinid genera were established for species from abyssal or hadal depths
13 729 (Moskalev, 1976; Leal & Harasewych, 1999). *Macleaniella moskalevi* is the monotypic
14 730 species of the genus, known exclusively from the lower abyssal and hadal zones of the Puerto
15 731 Rico Trench (5179–8595 m). It is easily recognized by its large internal septum of the shell as
16 732 a unique character within Cocculinidae (Leal & Harasewych, 1999). *Teuthirostria cancellata*
17 733 Moskalev, 1976, also the type and only species of the genus, was collected from a dead
18 734 cephalopod beak on 5200–5540 m bottom off northern Peru (Moskalev, 1976). This species
19 735 was recovered as a sister group to *Fedikovella* by morphology (Strong *et al.*, 2003). We did
20 736 not find any IWP specimen that was comparable in morphological or ecological
21 737 characteristics, suggesting that the two genera are endemic to the Atlantic (*Macleaniella*) or
22 738 the East Pacific (*Teuthirostria*), or that they are restricted to the lower abyssal and hadal
23 739 zones.
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CONCLUSIONS

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37 743 Our study based on broadly sampled material, constitute so far, the most comprehensive
38 744 biodiversity survey on cocculinid limpets. We identified 51 species from organic falls on the
39 745 deep-sea floor in the Indo-West Pacific. These included six named species (*Coccopigya*
40 746 *punctoradiata*, *Coccopigya hispida*, *Coccopigya okutanii*, *Cocculina japonica*, *Cocculina*
41 747 *ovata* and *Paracocculina subcompressa*) and 45 other, presumably new, species of the family.
42 748 We inferred the phylogenetic relationships among cocculinids for the first time based on a
43 749 multigene dataset. We recognized five named and four unnamed genera: *Cocculina* (8
44 750 species), *Cococrater* (7), *Coccopigya* (9), *Fedikovella* (5), *Paracocculina* (6), new genus 1
45 751 (nGen1: 8), nGen2 (3), nGen3 (8) and nGen4 (2); diagnostic morphological and ecological
46 752 traits are summarized for all genera in Table 2. These numbers of genera and species are
47 753 much higher than previously recognized in the Indo-West Pacific, although a larger part of the
48 754 oceans of the world remains to be sampled. We thus estimate that additional sampling efforts
49 755 will reveal more taxa, especially given the inherent patchiness of their habitats. Drawing a full
50 756 picture of the diversity of this perplexing animal group requires further research into other

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3 757 oceans and habitats.
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7 759 DATA AVAILABILITY
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10 761 The data underlying this article are available in the article and its online supplementary
11 762 material, and in the GenBank Nucleotide Database at <https://www.ncbi.nlm.nih.gov/genbank/>
12 763 and can be accessed with the GenBank accession numbers OL800712–OL801212 and
13 764 OL956554–OL956700.
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- 20 1070 **Table S1.** List of oceanographic expeditions and specimens analysed in this study.
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22 1071 **Table S2.** Morphological conditions of SSHs.
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24 1072 **Figure S1.** Maximum likelihood tree of cocculinids based on *cox1* dataset. Numbers at nodes
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26 1073 represent bootstrap values in percentage. Name of specimens collected during *Tropical*
27 1074 *Deep Sea Benthos* expeditions are shown as: voucher number, expedition, station, depth;
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29 1075 specimens collected around Japan: voucher number, locality or station, depth. See Table
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31 1076 S2 for details.
32 1077 **Figure S2.** Maximum likelihood tree based on “master” 28S dataset. Numbers at nodes
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34 1078 represent bootstrap values in percentage.
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36 1079 **Figure S3.** 28S subtrees.
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38 1080 **Figure S4.** Maximum likelihood tree based on combined three-gene dataset (*cox1*, 28S and
39 1081 *H3*). Numbers at nodes represent bootstrap values in percentage.
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41 1082 **Figure S5.** Bayesian tree based on combined three-gene dataset (*cox1*, 28S and *H3*). Numbers
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43 1083 at nodes represent posterior probability.
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1085 **Table 1.** Eight morphogroups of Cocculinidae and their habitats.

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	Shell shape	Teleoconch sculpture	Copulatory organ	Epipodial tentacles	Habitat
MA	round	radial or plainly raised radial	modified right cephalic tentacle	present	wood
MB	Spindle or narrow-round	plainly raised radial	on oral lappet	present	wood
MC	round	radial, smooth or concentric	on oral lappet	present	wood
MD	round	clathrate	right cephalic tentacle (unmodified)	present	wood
ME	round	smooth	on mantle margin	present	wood
MF	round	radial	on foot	present	wood
MG	round	radial with pits	modified right cephalic tentacle	absent	wood, leaf, bone
MH	round	concentric or smooth	modified right cephalic tentacle	present	chondrichthyan egg case, deep-sea coral

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Pre-review Only

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Table 2. Diagnoses of cocculinid genera (with unique conditions shown in **bold**).

Genus	Protoconch sculpture	Teleoconch sculpture	Copulatory organ	Epipodial tentacles	Shell shape	Habitat	References
<i>Cocculina</i>	reticulate	radial, smooth or concentric	on oral lappet or foot	present	round	wood, dolphin skull	Haszprunar, 1987; McLean & Harasewych, 1995; Zhang & Zhang, 2018
<i>Coccapigya</i>	reticulate	spinose	modified right cephalic tentacle	absent	round	wood, leaf, bone	Marshall, [1985] 1986; McLean & Harasewych, 1995; see main text for habitat
<i>Cococrater</i>	reticulate	radial or plainly raised radial	modified right cephalic tentacle	present	round	wood	Haszprunar, 1987; McLean & Harasewych, 1995
<i>Paracocculina</i>	reticulate	radial	on foot or oral lappet	present	round	wood	Strong <i>et al.</i> , 2003
<i>Fedikovella</i>	concentric	reticulate	right cephalic tentacle; unmodified	present	round	wood	Haszprunar, 1987; Strong <i>et al.</i> , 2003
<i>Teuthirostria</i>	smooth	reticulate	modified right cephalic tentacle	present	round	cephalopod beak	Moskalev, 1976; McLean & Harasewych, 1995; Leal & Harasewych, 1999
<i>Macleaniella</i>	smooth	radial (with an inner septum)	modified right cephalic tentacle	present	round	wood	Moskalev, 1976; Leal & Harasewych, 1999; Strong <i>et al.</i> , 2003
nGen1	reticulate	radial, smooth or concentric	on oral lappet	present	round	wood	This study
nGen2	reticulate	smooth	on mantle margin	present	round	wood	This study
nGen3	reticulate	plainly raised radial	on oral lappet	present	spindle or narrow round	wood	This study
nGen4	reticulate	concentric or smooth	modified right cephalic tentacle	present	round	chondrichthyan egg case, deep-sea coral	This study

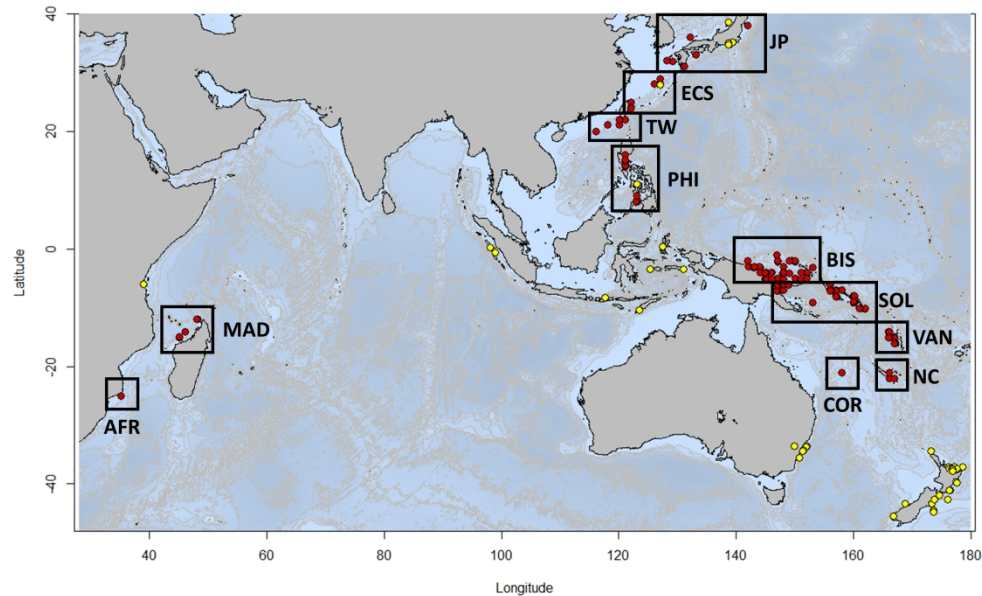


Figure 1. Localities of cocculinid specimens collected during our expeditions (red) and those in literature records (yellow; Watson, 1886; Schepman, 1908; Thiele, 1925; Marshall, 1985; Hasegawa, 1997; Lesicki, 1998; Zhang & Zhang, 2018) in Indo-West Pacific. The square indicates the 11 regions identified. AFR: Mozambique Channel; MAD: Madagascar; TW: Taiwan plus South China Sea; ECS: East China Sea; PHI: seas surrounding the Philippines; JP: seas surrounding Japan; BIS: Bismarck Sea; SOL: Solomon Sea; COR: Coral Sea; Van: seas surrounding Vanuatu; NC: seas surrounding New Caledonia.

254x190mm (300 x 300 DPI)

Sample collected from 28 expeditions in the Indo-West Pacific regions

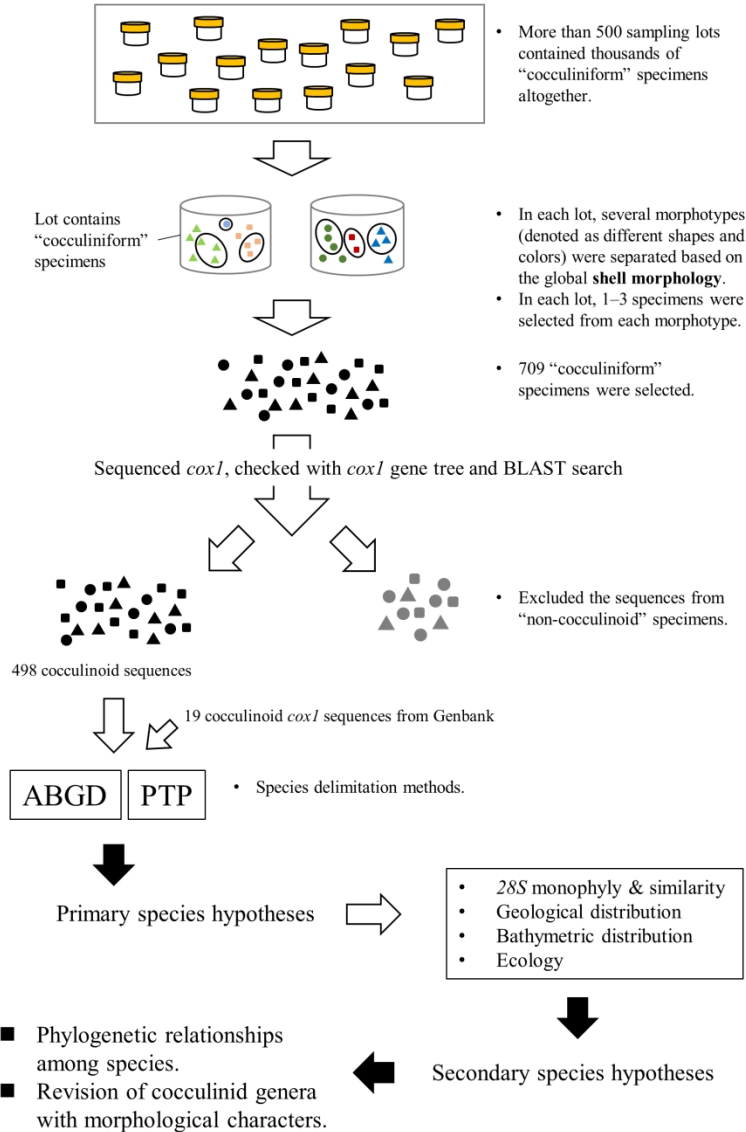


Figure 2. Diagram of integrative approach used for species delimitation.

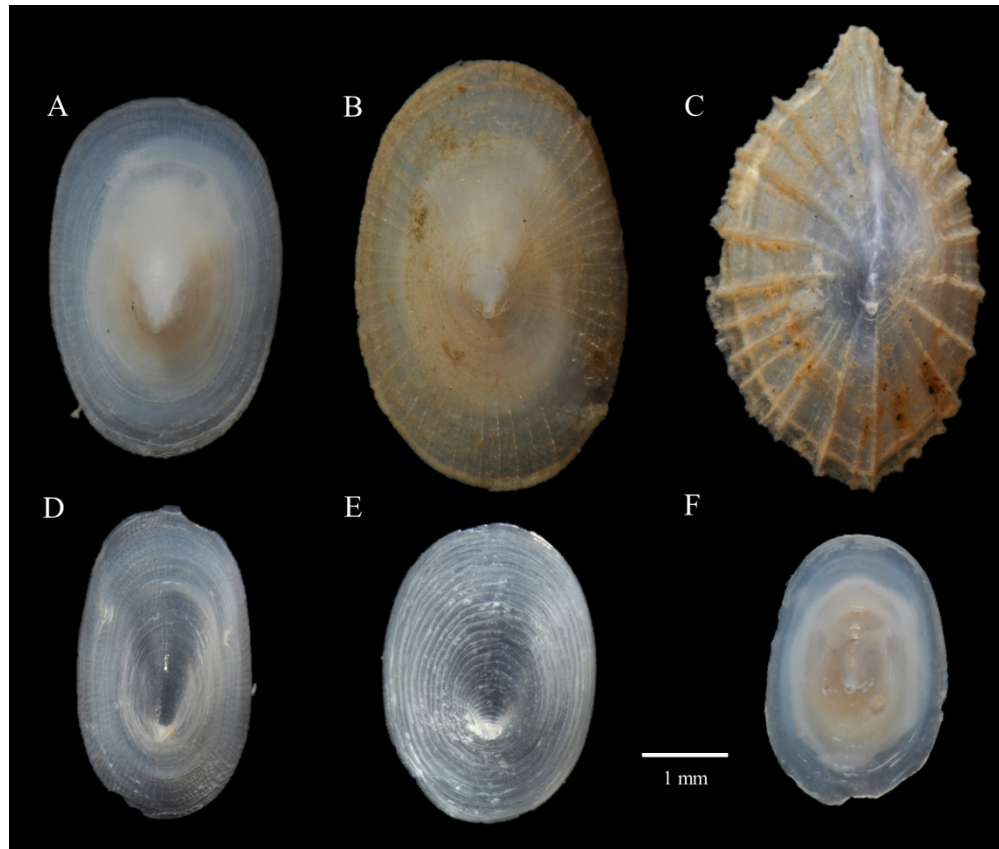


Figure 3. Character used in morphological examination. Teleoconch structure: (A) radially ribbed (MNHN-IM-2013-42733, MC10), (B) radially ribbed with pits and hairs (MNHN-IM-2013-42896, MG5), (C) plainly raised radially ribbed (MNHN-IM-2013-62346), (D) clathrate (MNHN-IM-2013-62341, MD5), (E) concentric (MNHN-IM-2009-11985, MH1), or (F) smooth (MNHN-IM-2013-42654, ME1).

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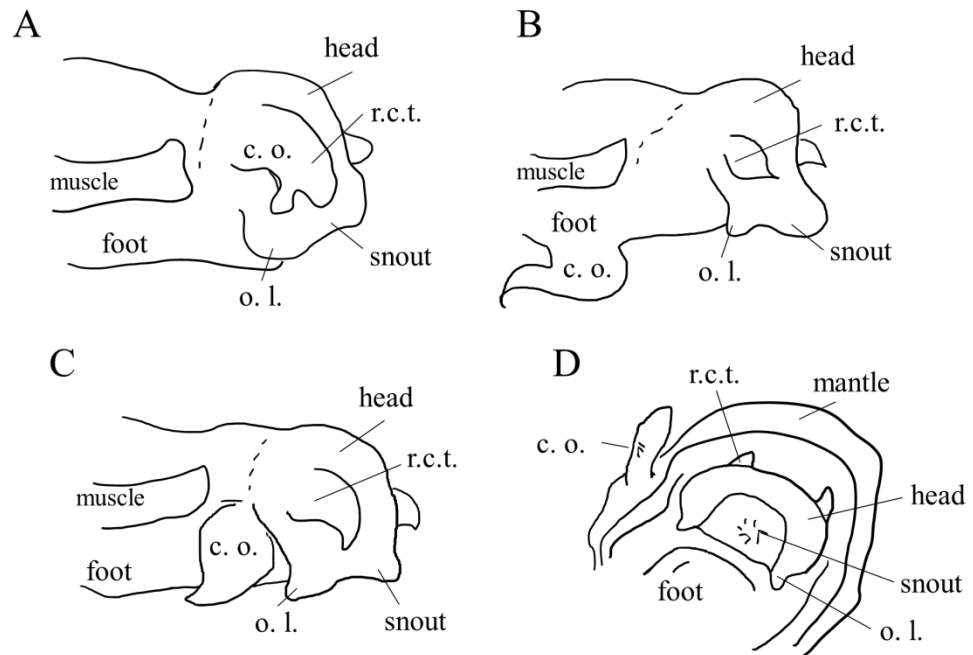


Figure 4. Character used in morphological examination. Copulatory organ: (A) associated with or branched from right cephalic tentacle, (B) originated from foot, (C) originated from oral lappet, or (D) originated from mantle margin. c.o.: copulatory organ, r.c.t.: right cephalic tentacle, o. l.: oral lappet.

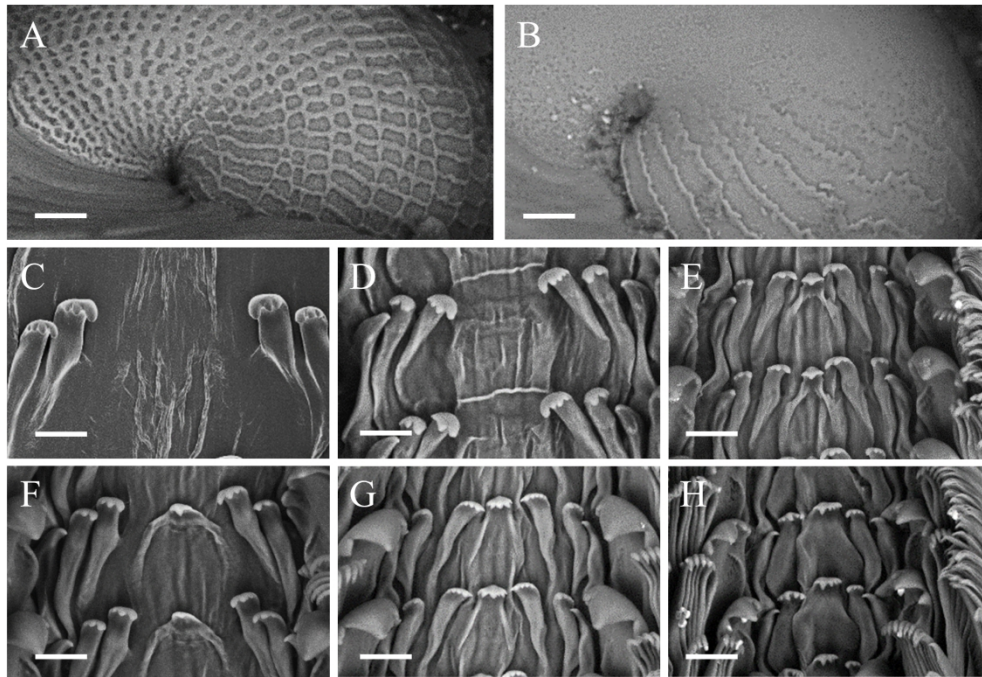


Figure 5. Characters used in morphological examination. Protoconch: (A) reticulate (MNHN-IM-2013-42752, MC2) or (B) concentric (MNHN-IM-2013-62343, MD8). Radula: (C) "obsolete," (*C. japonica*) (D) "acuspate-flat," (MNHN-IM-2013-42608, MC9) (E) "unicuspid narrow," (MNHN-IM-2013-42715, MB1) (F) "unicuspid broad," (MNHN-IM-2013-42803, MC14) (G) "multicuspid narrow," (MNHN-IM-2013-42570, MD3) or (H) "multicuspid broad" (MNHN-IM-2013-62344, MA5).

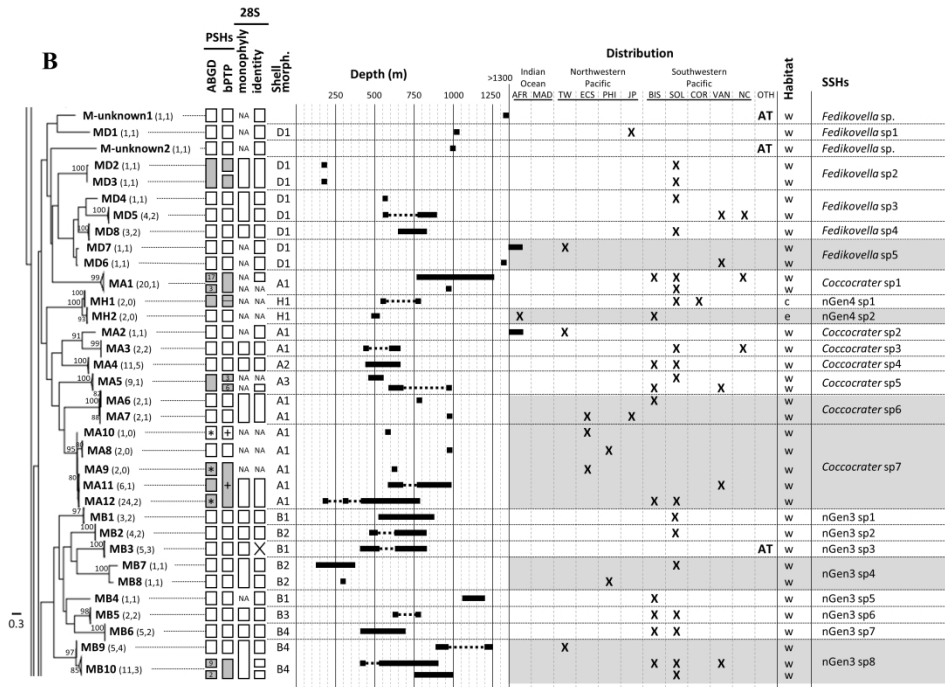


Figure 6. Maximum-likelihood tree based on *cox1* dataset of cocculinids with results of species delimitation analyses. Numbers at tree nodes represent bootstrap values in percentage (values < 70% not shown). Names of primary species hypotheses (PSHs) are followed by number of sequences determined (*cox1* and 28S). Boxes for PSHs are highlighted in gray when differently delimited in ABGD and PTP analyses (numerals in boxes represent numbers of *cox1* sequences; asterisk and plus for MA9–MA12 denote same PSH detected by ABGD and PTP, respectively). 28S sequence monophyly and identity are also shown as empty boxes (NA: no data); cross marks (X) indicate non-monophyly or genotype shared with other PSHs. The “shell morph.” denotes distinguishable forms of the shell with different numerals in each morphogroup (MA–MH); shell morph. C1 for MC1–MC3, for example, means that the three PSHs are identical in conchological characteristics. Known bathymetric and geographic distributions and habitat are also provided for each PSH (bathymetric distribution — sampled depth range shown in lines, dotted lines represent the estimated distributed range; geographic distribution — AFR: Mozambique channel, MAD: north Madagascar, TW: Taiwan and South China Sea, ECS: East China Sea, PHI: Philippines, JP: Japan, BIS: Bismarck Sea, SOL: Solomon Sea, COR: Coral Sea, VAN: Vanuatu, NC: New Caledonia, OTH: others, ME: Mediterranean, NZ: New Zealand, RS: Red Sea, AT: Atlantic, EP: East Pacific, WS: Weddell Sea; habitat — w: wood, l: leaf, b: bone, c: coral, e: egg case, vent*: hydrothermal vent area). Final, secondary species hypotheses (SSHs) are shown with generic and species names if available; those found from more than one area (northwestern Pacific, southwestern Pacific, and Indian Ocean) are highlighted in gray.

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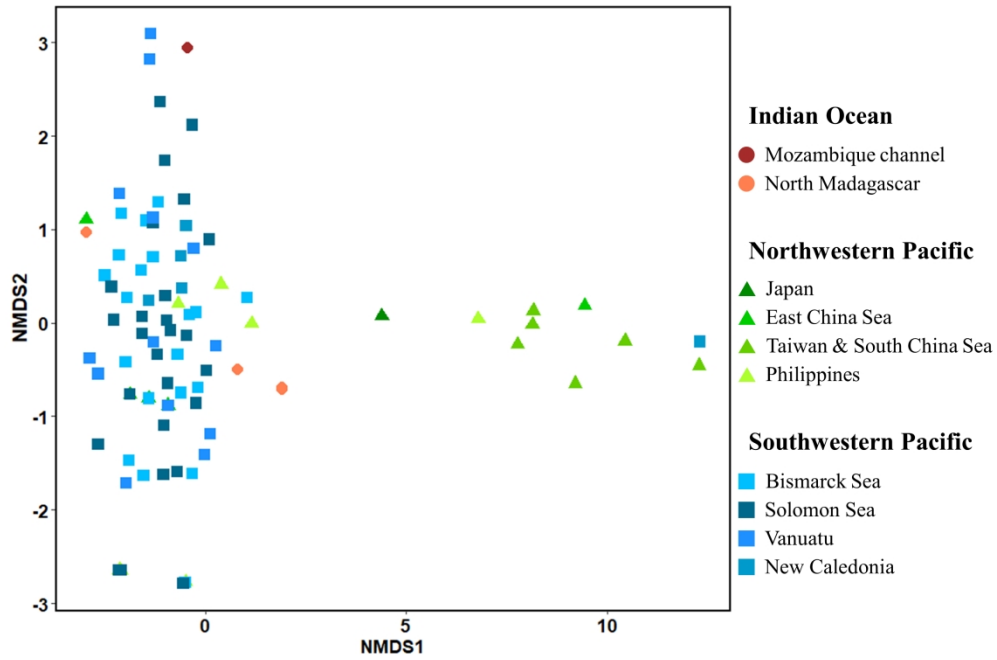


Figure 7. Nonmetric multidimensional scaling (NMDS) plot based on Jaccard index for cocculinid species composition among 157 stations in Indo-West Pacific.

254x190mm (300 x 300 DPI)

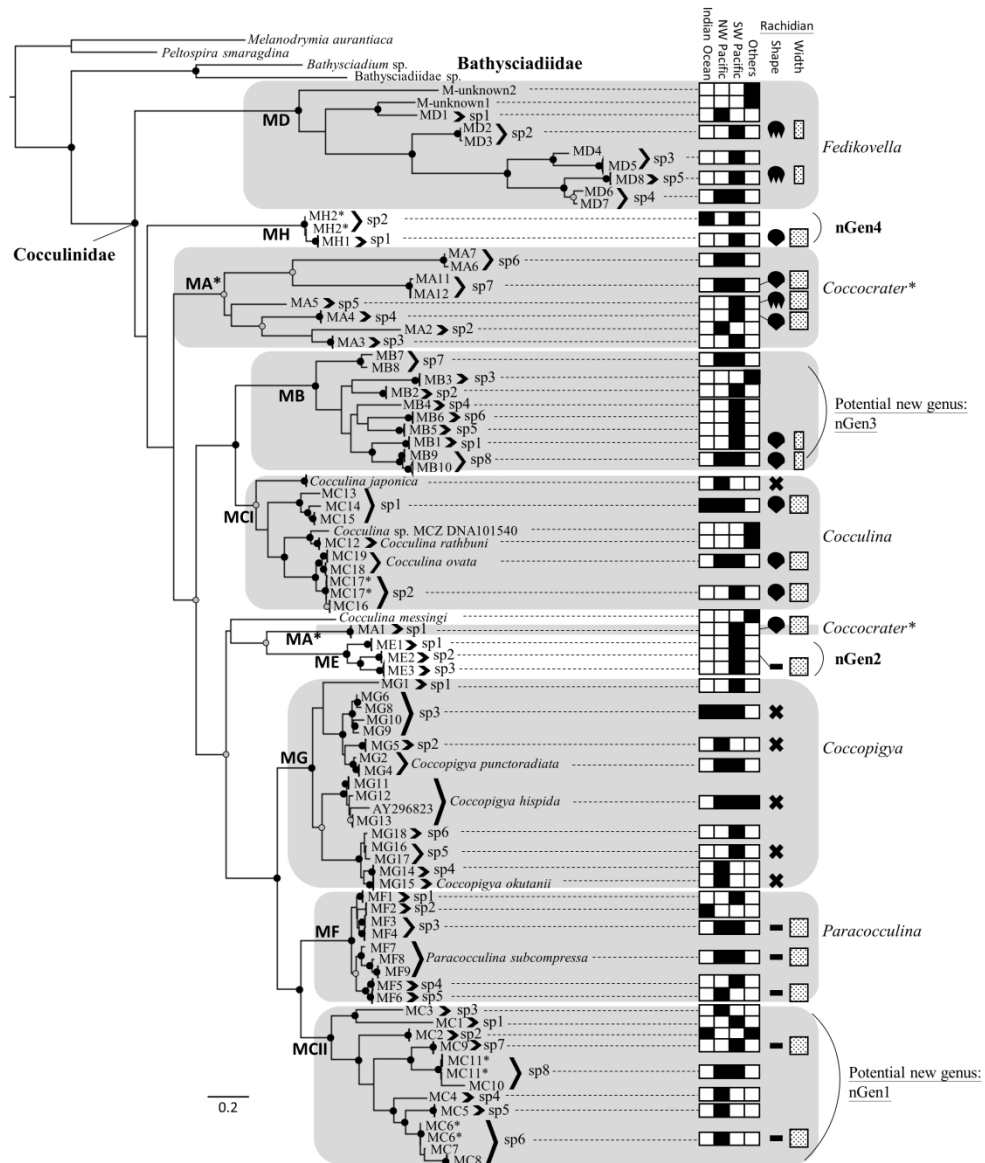


Figure 8. Combined three-gene ML tree for 138 cocculinid specimens (1,421 bp from *cox1*, 28S and H3). Nodal supports are shown as circles on nodes. Nodes supported by both bootstrap value (BS) $\geq 80\%$ and posterior probability (PP) of ≥ 0.95 are shown in black circles; nodes supported by only BS $\geq 80\%$ or PP ≥ 0.95 are shown in grey circles. Nodes with BS of $< 80\%$ and PP < 0.95 are not marked with a circle. Morphogroup types are plotted on the tree (Table S2). Geographic distribution of each species is shown with filled square(s). Different shapes of rachidian tooth of radula are denoted with following symbols: X, obsolete; —, acuspate; circle with three spike: multicuspid; circle with a single spike, unicuspid. Dotted squares, broad or narrow, indicate relative width of rachidian tooth. Asterisks represent non-monophyletic morphogroup.

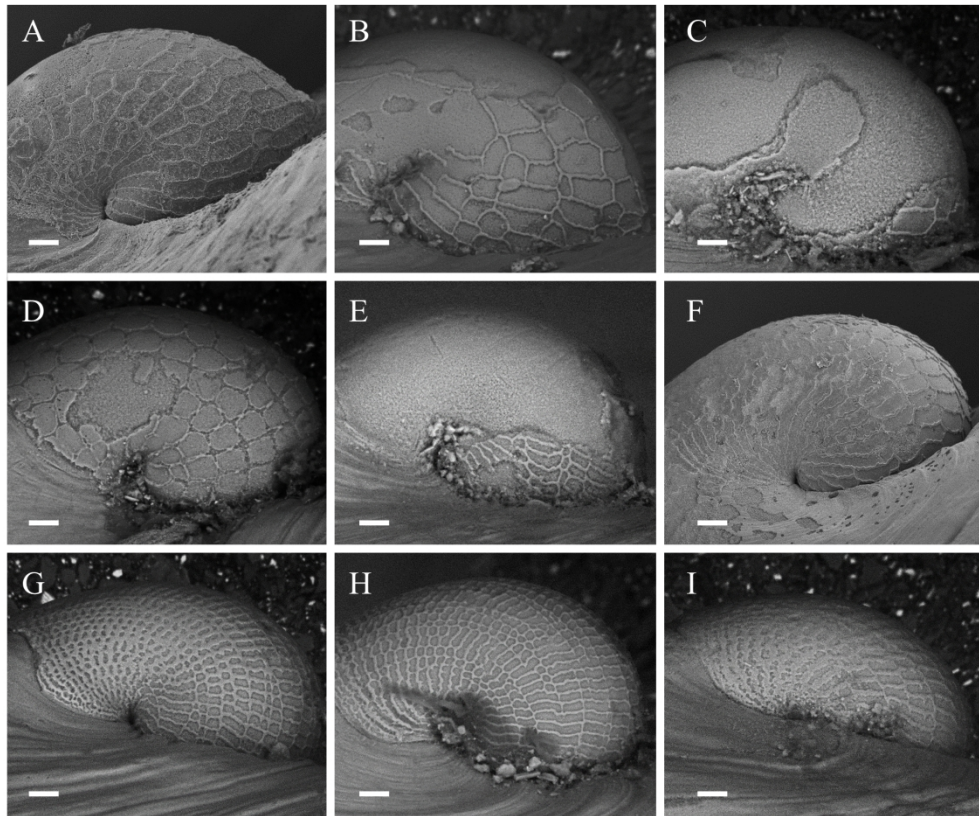


Figure 9. Protoconchs of sequenced specimens. (A) MNHN-IM-2013-59217 (MA4); (B) MNHN-IM-2013-42738 (MA8); (C) MNHN-IM-2013-42581 (MA12); (D) MNHN-IM-2013-62346 (MB7); (E) MNHN-IM-2013-42709 (MC15); (F) MNHN-IM-2013-42850 (MC18); (G) MNHN-IM-2013-42752 (MC2); (H) MNHN-IM-2013-42608 (MC9); (I) HS215 (MC5). Scale bars = 20 μ m.

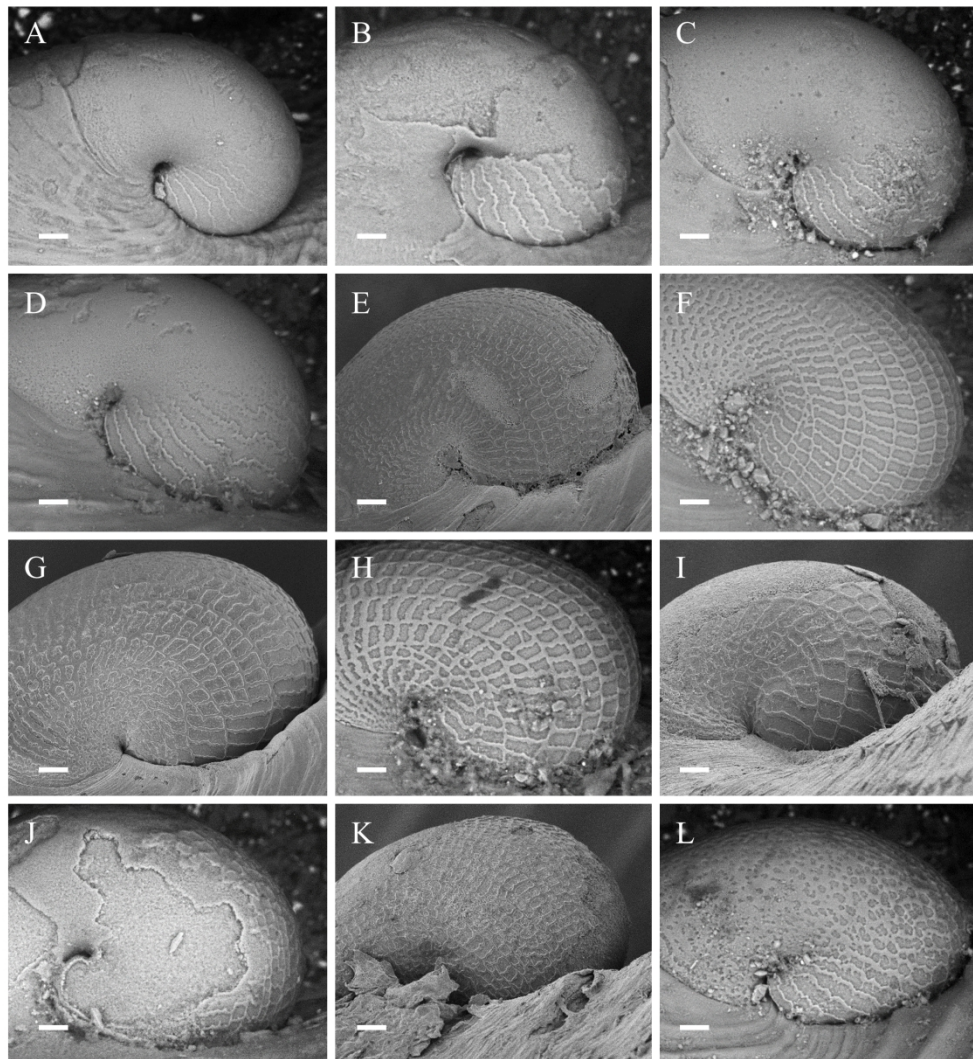


Figure 10. Protoconchs of sequenced specimens. (A) MNHN-IM-2013-42570 (MD3); (B) MNHN-IM-2013-42648 (MD4); (C) MNHN-IM-2013-62341 (MD5); (D) MNHN-IM-2013-62343 (MD8); (E) MNHN-IM-2013-42654 (ME1); (F) MNHN-IM-2013-42627 (MF9); (G) MNHN-IM-2013-42878 (MF6); (H) MNHN-IM-2013-42748 (MF2); (I) MNHN-IM-2013-42907 (MG13); (J) MNHN-IM-2013-42798 (MG8); (K) MNHN-IM-2013-42896 (MG5); (L) MNHN-IM-2009-11985 (MH1). Scale bars = 20 μ m.

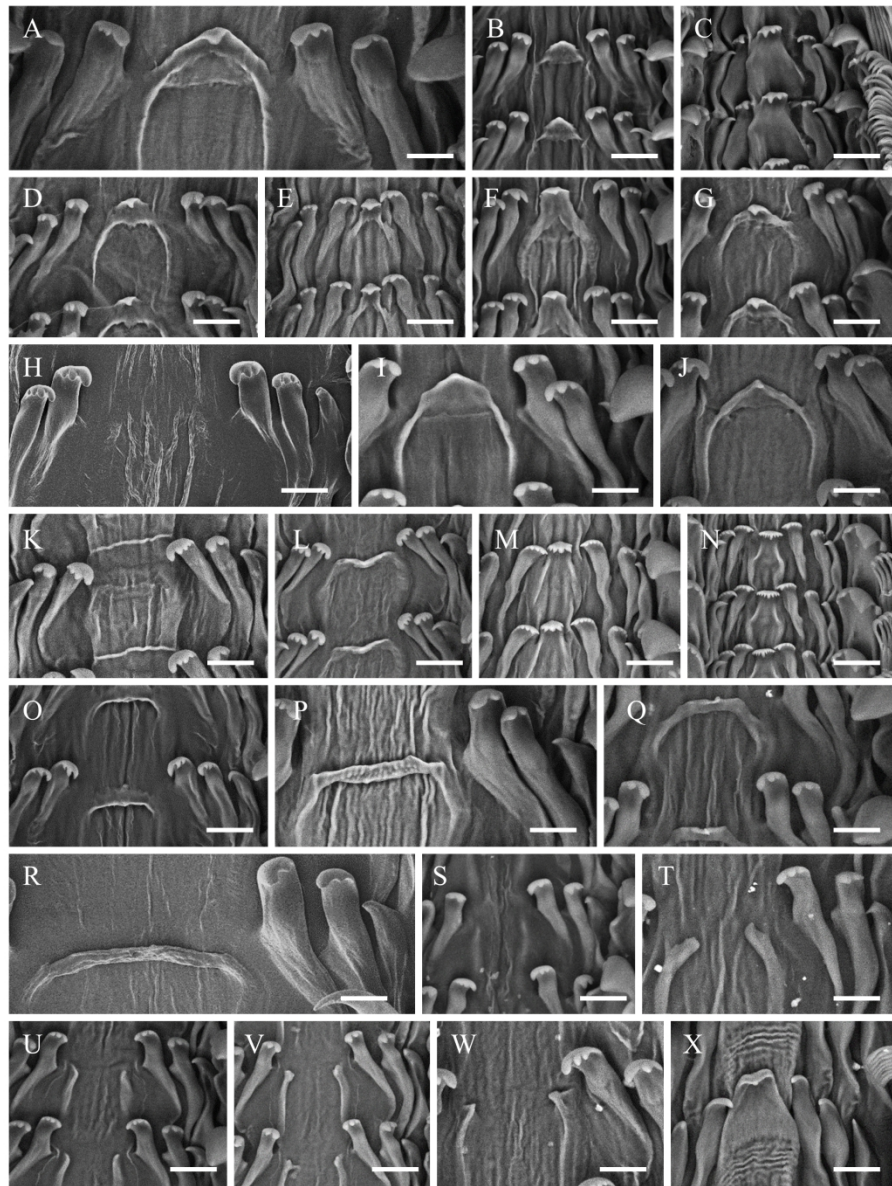


Figure 11. Radulae of sequenced specimens and an additional examined specimen of *C. japonica*. (A) MNHN-IM-2013-42671 (MA1), "unicuspid broad"; (B) MNHN-IM-2013-42687 (MA4), "unicuspid broad"; (C) MNHN-IM-2013-62344 (MA5), "multicuspid broad"; (D) MNHN-IM-2013-42828 (MA12), "unicuspid broad"; (E) MNHN-IM-2013-42715 (MB1), "unicuspid narrow"; (F) MNHN-IM-2013-42845 (MB10), "unicuspid narrow"; (G) MNHN-IM-2013-42803 (MC14), "unicuspid broad"; (H) *C. japonica*, "obsolete"; (I) MNHN-IM-2013-42799 (MC18), "acuspate-flat"; (J) MNHN-IM-2013-42968 (MC16), "unicuspid broad"; (K) MNHN-IM-2013-42608 (MC9), "acuspate-flat"; (L) HS217 (MC6), "acuspate-flat"; (M) MNHN-IM-2013-42570 (MD3), "multicuspid narrow"; (N) MNHN-IM-2013-42643 (MD8), "multicuspid narrow"; (O) MNHN-IM-2013-42620 (ME2), "acuspate-flat"; (P) MNHN-IM-2013-42992 (MF9), "acuspate-flat"; (Q) MNHN-IM-2013-40565 (MF6), "acuspate-flat"; (R) HS227 (MF3) "acuspate-flat"; (S) MNHN-IM-2013-42732 (MG10), "obsolete"; (T) MNHN-IM-2013-42863 (MG11), "obsolete"; (U) MNHN-IM-2013-42896 (MG5), "obsolete"; (V) MNHN-IM-2013-42874 (MG15), "obsolete"; (W) MNHN-IM-2013-42851 (MG16), "obsolete"; (X) MNHN-IM-2009-11985 (MH1), "unicuspid broad". Scale bars = 20 μ m.

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