Zoological Journal of the Linnean Society
August 2022, Volume 196 Issue 1 Pages 366-392
https://doi.org/10.1093/zoolinnean/zlac023
https://archimer.ifremer.fr/doc/00811/92280/

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

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Abstract :

The family Cocculinidae (Gastropoda: Cocculinida) consists of small, usually colourless benthic limpets living primarily at depths below 100 m, and on decaying plant or animal remains. These habitats are difficult to sample and the knowledge about Cocculinidae species diversity, biogeography, ecology and evolution is therefore 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' programme in the Indo-West Pacific (IWP). To propose a 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 gene 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 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 a higher taxonomic level, we identified ten main clades in the family. Although six of them matched existing genera, four others should be regarded as new genera awaiting formal description.

Keywords : classification, deep sea, organic fall, species delimitation, sunken wood, wooden, steps hypothesis

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

Among the numerous and small cryptic taxa inhabiting the deep sea, Cocculinidae Dall, 1882 (Gastropoda: Cocculinida) encompasses limpet-shaped gastropods that live attached to primarily organic substrates laying on the ocean floor such as sunken woods, cephalopod beaks and fish and whale bones. They are grazers with a rhipidoglossan radula to feed most probably on co-habiting micro-organisms that decompose these organic remains (Marshall, ["1985"]1986; Lesicki, 1998). Their unique habitats, small sizes and simple shell shapes make Cocculinidae one of the most taxonomically puzzling groups of gastropods. Presently, the *World Register of Marine Species*, lists 52 valid species classified under seven recognized genera (Moskalev, 1976; Marshall, [1985] 1986; Haszprunar, 1987; McLean, 1987; McLean & Harasewych, 1995; Warén, 1996; Hasegawa, 1997; Leal & Harasewych, 1999; Ardila & Harasewych, 2005; Zhang & Zhang, 2018; Chen & Linse, 2020).

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

Despite the establishment of the classification, the monophyly of the defined genera and the phylogenetic hypotheses were not steadily tested, especially not with molecular tools. Strong *et al.* (2003) conducted the first and only phylogenetic study for the Cocculinidae. They examined 31 morphological characters for 15 cocculinoid species, resulting in a single most parsimonious tree. The monophyly of superfamily Cocculinoidea (Cocculinidae + Bathysciadiidae), family Cocculinida, and the genera *Cocculina* Dall, 1882 and *Coccopigya* B. A. Marshall, 1986 were all supported. In contrast, *Paracocculina* Haszprunar, 1987 and *Coccocrater* Haszprunar, 1987 were recovered as paraphyletic. *Fedikovella* and *Teuthirostria* Moskalev, 1976 collectively formed a monophyletic group sister to all other cocculinids.

Members of Cocculinidae have been recorded from all oceans of the world. Their lecithotrophic larvae (Young *et al.*, 2013) suggests limited dispersal ability and many geographically restricted species have been defined. In the Indo-West Pacific (IWP), the focused area of the present study, 30 species in four genera are currently recognized. *Cocculina*, the most studied genus of the family, contains 20 IWP species (e.g. Watson, 1886; Schepman, 1908; Thiele, 1925; Kuroda & Habe, 1949; Hasegawa, 1997; Zhang & Zhang, 2018). *Coccopigya* includes seven IWP species with five possible endemics in the waters around New Zealand (Marshall, [1985] 1986) and two others distributed in the north-western Pacific including the seas around Japan, Korea and Taiwan (Kuroda & Habe, 1949; Hasegawa, 1997). *Coccocrater* contains the type species *C. radiatus* (Thiele, 1903) from off Sumatra as the only IWP member of the genus. *Paracocculina* is composed only of two IWP species, namely *P. cervae* (C. A. Fleming, 1948) from off New Zealand and *P. laevis* (Thiele, 1903) from off Sumatra, Indonesia.

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

MATERIAL AND METHODS

SAMPLE COLLECTION AND SELECTION

The specimens examined were mostly collected during 21 IWP expeditions of the *Tropical Deep-Sea Benthos* (TDSB) program led by the Muséum national d'Histoire naturelle (MNHN) and the Institut de Recherche pour le Développement (IRD), in collaboration with the National Taiwan University (NTU) and the National Taiwan Ocean University (NTOU), between 2004 and 2018: AURORA 2007, BIOPAPUA, BOA1, CONCALIS, DONGSHA 2014, EBISCO, EXBODI, KAVALAN 2018, KAVIENG 2014, MADEEP, MAINBAZA,

MIRIKY, NANHAI 2014, NORFOLK 2, PANGLAO 2004, PANGLAO 2005, PAPUA NIUGINI, SALOMON 2, SALOMONBOA 3, SANTO 2006 and TAIWAN 2013. During the listed expeditions (see for more details https://expeditions.mnhn.fr) specific efforts were made by the on-board scientific teams to collect the fauna associated with plant remains (reviewed notably in Samadi *et al.*, 2010 and Pante *et al.*, 2012). The sampling covered the areas surrounding Taiwan (including the South China Sea and East China Sea), the Philippines (Bohol and Sulu Seas and the Pacific coast), Papua New Guinea (Bismarck Sea and Solomon Sea), Solomon Islands (Solomon Sea), New Caledonia (Coral Sea), Vanuatu (Coral Sea) and Madagascar (in the Mozambique channel) (Fig. 1). Most specimens were found attached on organic substrates collected by dredging or trawling at depths ranging from 100 to 1500 meters. Some specimens were collected from organic substrates that were deployed on the deep-sea floor as traps to attract the recruitment of larvae (Samadi *et al.*, 2010 for more details). Additional specimens were collected from areas surrounding Japan and from East China Sea (Fig. 1; Table S1). We also included several Caribbean specimens from the expedition KARUBENTHOS 2 from TDSB program available in the collection of Muséum national d'Histoire naturelle (MNHN) to increase sample diversity and to assign generic names to clades; the type species of *Cocculina* and *Macleaniella* were described from this area.

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

DNA SEQUENCING

We extracted total genomic DNA from the muscle tissue of 710 specimens using either the automated extractor LabTurbo 48 Compact System and LGD 480-220 kits (Taigene BioSciences Corp., Taiwan) or the NucleoSpin 96 Tissue kit (Macherey-Nagel, France) with the automated pipetting system epMotion 5075 according to the manufacturers' instruction.

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

We performed the PCR reactions in 20 µl, using 1–3 µl of DNA, 1× reaction buffer, 1.25 mM $MgCl_2$, 0.26 mM dNTP, 0.3 µM of each primer and 1.5 unit of Q-Bio Taq (MPBiomedicals, LLC., USA). We started the PCR with a cycle of 94°C for 5 min, followed by 35 cycles of denaturation step (94°C, 30 s), annealing step (51°C for *cox1*, 56°C for *28S* and 53°C for *H3*, 30 s) and elongation step (72°C, 45 s for *cox1*, 1 min for *28S* and 30 s for *H3*) and a final elongation step for 5 min (72°C). The purification and sequencing for PCR products were mainly carried out by Eurofins Scientific (France). For others the purification was conducted with the AMPure magnetic bead clean-up protocol (Agencourt Bioscience Corp.) and the sequencing was performed at the Center of Biotechnology, National Taiwan University.

The obtained DNA sequence chromatograms were visualized, edited and assembled using CodonCode Aligner v.6.0.2 (Codoncode Corporation, Dedham, MA, USA). The edited sequences were manually aligned and compiled using the software Se-Al v. 2.0 (Rambaut, 1996). However, some *H3* sequences (n = 39) presented more than one peak at multiple nucleotide positions of the sequences. We thus suspected a presence of paralogs or contaminant sequences. To resolve this, we pooled the PCR products of those 39 problematic *H3* samples to construct a library using the NEB next Library preparation kit (New England

Biolabs, MA, USA) with a single *H3* PCR per tag (Hinsinger *et al.*, 2015) for next generation sequencing (NGS) under the Ion Torrent system (Life technologies, France). Template amplifications of the library were performed by emulsion PCR on an Ion OneTouch robotic system, and the subsequent sequencing was performed by an Ion Torrent PGM sequencer using Hi-Q chemistry (Life technologies, France). The sequences were demultiplexed *a posteriori* (Hinsinger *et al.*, 2015) and assembled using Geneious R9 (Biomatters Ltd., Auckland, New Zealand). To rule out the possibility of contamination, we compared the obtained sequences using the BLAST search as implemented in NCBI GenBank and topologies of phylogenetic trees inferred from individual gene fragments.

PRIMARY SPECIES HYPOTHESES (PSHs)

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

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

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

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

SECONDARY SPECIES HYPOTHESES (SSHs)

To decide if we turn the detected PSH into secondary species hypotheses (SSHs) we used additional criteria as described in Figure 2. The main additional sources of evidences were firstly the nuclear genetic data that are unlinked to the mitochondrial data used to establish the PSH, and then the comparisons of the geographic range and the ecological data (depth and habitat) with the sister PSH.

The recovery of a given PSH as a clade in both mitochondrial- and nuclear-gene trees strongly supports the distinctiveness of the species (Pante *et al.,* 2015). Consequently, we reconstructed phylogenetic trees using *28S* sequences (for more details notably on alignment methods see “Phylogenetic inference”). We tried to sequence a fragment of the *28S* gene for up to five representatives of each PSH defined with the *cox1* gene and for another specimen from the Caribbean Sea for which we failed to obtain a *cox1* sequence (MNHN-IM-2013-60187; Table S1). The first 415 base pairs of the *28S* fragment were too conserved at the species level, but useful at a deeper phylogenetic scale. Consequently, we reconstructed a tree based only on this conserved part (hereafter referred to as the *28S*-gene “master” tree) to define main lineages. We then reconstructed a subtree for each main lineage by aligning the entire length of the *28S* fragment, which were partly too variable to be aligned with other lineages (hereafter referred to as the *28S*-gene "sub" trees). We regarded a PSH as a potential SSH when its component individuals constituted a supported phylogenetic clade in the *28S* subtree or if they shared an identical *28S* sequence.

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

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

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

BETA DIVERSITY ANALYSIS

To estimate the dissimilarity of cocculinid species diversity among stations from the 11 geographic regions defined above, we used non-metric multidimensional scaling (NMDS) plots based on the Jaccard similarity coefficient with the presence/absence data matrix, implemented in the R packages "vegan" (Oksanen *et al*., 2019).

PHYLOGENETIC INFERENCE WITHIN THE COCCULINIDAE

To examine the relationships among the delimited species (SSHs) and to revise the genus-level classification of the Cocculinidae, we inferred phylogenetic trees based on three genes. To complement the *cox1* and *28S* datasets we employed a *H3* dataset obtained using both Sanger and NGS technics. This *H3* dataset contained representatives from each SSH, including sequences extracted from GenBank (Table S1). We aligned the *cox1* and *H3* sequences by eye and the *28S* using the automatic multiple alignment tool implemented in MAFFT ver. 7 with default parameters (Katoh & Standley, 2013).

We compiled a combined dataset with the *cox1*, *28S* and *H3* sequences for a common set of 140 taxa, including two distant outgroups of Neomphalida (*Melanodrymia aurantiaca* Hickman, 1984 and *Peltospira smaragdina* Warén & Bouchet, 2001) and the two bathysciadiids used for species delimitation. The dataset included at least one representative of each SSH (Table S1).

We reconstructed phylogenetic trees with the partitioned ML method using the *cox1* dataset, reduced *cox1* dataset for bPTP, *28S* master dataset, *28S* sub datasets, *H3* dataset and the combined dataset implemented in RAxML v.8.0 (Stamatakis, 2014). The GTR+G substitution model was employed for the analyses because RAxML only provides GTR-related models of rate heterogeneity. Partitions were set by genes and for *cox1* and *H3* by codon positions. Nodal support was assessed by bootstrapping (Felsenstein, 1985) with 1000 pseudoreplicates.

For the combined dataset, we also performed a Bayesian inference (BI) using MrBayes v.3.2.6 (Ronquist *et al*., 2012) on the CIPRES Science Gateway (Miller *et al*., 2010), with eight Markov chains in two parallel runs for 30000000 generations, a sampling frequency of one tree per thousand generations and a heating temperature of 0.02. The convergence of the likelihood scores for parameters was further evaluated using Tracer v.1.6 (Rambaut *et al*., 2014) to make sure that all ESS values were over 200. To be consistent with the ML analysis, we used the same partitions and the same GTR+G substitution model for the BI method.

MORPHOLOGICAL CHARACTERIZATION

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

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

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

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

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

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

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

(AORI), the University of Tokyo.

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

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

RESULTS

DATASETS FOR SPECIES DELIMITATION

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

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

(809 bp, 21 samples left) and put them aside in a new dataset, *28S*-h (816 bp, 18 taxa). Finally, the inferred tree shows eight groups (Fig. S2). Phylogenetic trees inferred from the *28S* subdatasets are shown in Figure S3A–H.

SPECIES DELIMITATION ANALYSES

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

REGIONAL SPECIES DIVERSITY

We included the 187 IWP stations in our NMDS analysis. The stress value reached close to 0 (around 0.0007) after 200 NMDS runs, suggesting the presence of outlier stations. This result was not surprising since some stations contained a single, unique species. For a better recognition of underlying patterns, we removed 25 dissimilar stations that showed NMDS1/NMDS2 over 20 or below -20. The re-analysis with 162 stations resulted in the final stress value of 0.022. NMDS plots showed two main groups. One contained most stations in the south-western Pacific and Indian Ocean, and part of the stations in the north-western Pacific; the other contained many of the stations in the north-western Pacific and one station in the south-western Pacific (Fig. 7).

PHYLOGENETIC RELATIONSHIPS AMONG SPECIES AND MORPHOGROUPS

The combined dataset of three genes (*cox1*, *H3* and *28S*) consisted of 1,421 aligned sites and 140 terminals, including 136 cocculinids, two bathysciadiids and two neomphaloids. The topologies of the inferred ML and BI trees were similar to each other but with a noticeable difference in the position of the clade MH (Figs S4, S5). The ML tree is shown in Figure 8 with reference to PSH names. The Cocculinidae consisted of ten major clades and a single species (*Cocculina messingi*) that did not fall into any one of the major clades. The latter may be due to the quantity of missing data. Seven out of the ten clades were strongly supported with a bootstrap value (BS) of ≥ 80% and posterior probability (PP) of ≥ 0.95. Six of the seven well-supported clades corresponded to the morphogroups MD, MH, MB, ME, MG and MF. Conversely, the morphogroups MA and MC appeared to be non-monophyletic (Fig. 8). Morphogroup MC consisted of two distantly related clades that were denoted as MCI and MCII; the former was recovered as a sister clade to MB and the latter to MF. One species of MA was distinct from others in the same morphogroup and instead sister to the clade ME. However, this relationship was not supported with a significant BS value (73%) and we cannot exclude that MA is monophyletic. Overall, relationships among the ten major clades were not resolved expect the sister group relationships between MB and MCI (BS = 92%, PP = 1.00), MF and MCII (86%, 1.00) and MG and MF + MCII (90%, 1.00) (Figs 8, S4, S5).

PROTOCONCH AND RADULA

To further diagnose the cocculinid clades we used two morphological characters: the protoconch and radula. However, the protoconch of adult cocculinids are often eroded, potentially due to high dissolution rates of calcium carbonate in the deep sea. Cocculinid radulae are also known to be similar within a genus (e.g. Haszprunar, 1987; Marshall, [1985] 1986; McLean & Harasewych, 1995). Thus, we selected only 21 and 24 representative specimens for the examination of the protoconch (Figs 9, 10) and radula (Fig. 11), respectively.

Most protoconchs had a reticulated sculpture, whereas the individuals of clade MD displayed a concentric sculpture in the initial part of shell formation (Fig. 10A–D). The radula showed more variation between and within the clades (Fig. 8). Clade MA had two different types of the rachidian tooth: "unicuspid broad" and "multicuspid broad" (Fig. 11A–D). Among the species assigned to the polyphyletic morphogroup MC, those of clade MCI had two types of "unicuspid broad" and "obsolete" (Fig. 11G–J), whereas two species from MCII shared the same "acuspate-flat" rachidian (Fig. 11K, L). One species examined for clade ME had the rachidian tooth of the "acuspate flat" (Fig. 11O). On the other hand, the clades MB, MD, MF and MG were diagnosed by their "unicuspid narrow" (Fig. 11E, F), "multicuspid narrow" (Fig. 11M, N), "acuspate-flat" (Fig. 11P–R) and "obsolete" (Fig. 11S–W) types of the rachidian, respectively. Finally, the only specimen examined for the clade MH had a rachidian tooth that can be classified into the "unicuspid broad" type (Fig. 11X). This specimen was also unique in having unicuspid first lateral teeth; all other radulae examined here had multiple cusps in the first lateral tooth.

DISCUSSION

SPECIES DIVERSITY IN THE INDO-WEST PACIFIC

The integrative taxonomy as implemented in this study revealed a remarkable species richness of cocculinids with 51 delimited species occurring in the IWP. However, only 29 extant species have been described from the IWP and specimens examined in previous studies originated from a few restricted areas. These include five *Coccopigya* and one *Paracocculina* species around New Zealand and off the east coast of Australia (Fleming, 1948; Marshall, [1985] 1986), six *Cocculina* and two *Coccopigya* species around Japan (Kuroda & Habe, 1949; Hasegawa, 1997, 2009; Zhang & Zhang, 2018), one *Cocculina* species around the Philippines (Watson, 1886), one *Coccocrater*, eight *Cocculina*, one *Paracocculina* species around Indonesia (Thiele, 1903; Schepman, 1908) and five *Cocculina* species off West Africa (Thiele, 1925). Our specimens were collected mainly in the vicinity of Papua New Guinea, the Solomon Islands, New Caledonia and Vanuatu, but also from around Madagascar, in the South China Sea, near Taiwan, the East China Sea and Japan. There is thus a relatively small overlap between our sampling areas and the known distribution ranges of the described cocculinid species. This suggests that many, if not most, of the species we delimited in this study are new to science as discussed below.

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

Regional endemicity was observed for most of the IWP cocculinid species we identified. This general trend contrasts with the case of nGen4 sp2 that gathered specimens collected in two distant regions: the Mozambique Channel and the Bismarck Sea (Fig. 6B). The genetic divergence between the samples from these regions was low (i.e. *cox1* distance = 0.64%) despite a geographic distance of more than 5000 km between them. Strikingly, we collected this species always on chondrichthyan egg cases and sister to nGen4 sp1 that was collected exclusively on the skeleton of deep-sea corals. We should stress that plant remains are highly abundant around tropical islands, and these were thus well represented in the catches available in the MNHN's collection. Animal remains seem to be much more unevenly distributed on the deep-sea floor and were much rarer in our samples. The two species were never present in the abundant wood material and we can therefore regard them as truly specialized to either egg cases or coral skeletons. Their robust sister relationship (Figs 6B, 8) and vast geographic range of nGen4 sp1 suggest that habitat shift from the plesiomorphic wood to other biogenic substrates has been uncommon in the evolutionary history of the Cocculinidae. We found few specimens of *Coccopigya hispida* from the specimens collected during the experimentally deployed leaves and bones in New Caledonia (MG13, Fig. 6A; Samadi *et al*., 2010), but those cases might represent rare, opportunistic use of substrates by an essentially wood-dwelling species. Additional efforts for sampling species associated to animal remains are still required to better understand the ecological radiation of cocculinid limpets.

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

CLASSIFICATION OF COCCULINID GENERA

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

Cocculina

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

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

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

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

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

Among other IWP species described under this genus, *Cocculina japonica* Dall, 1907 was easily recognized in our material and fell into *Cocculina s.s.* (MCI) in the present study. The information available in the species descriptions of *C. capulus* Thiele, 1925, *C. dofleini* Thiele, 1925, *C. fragilis* Thiele, 1925, *C. pellita* Thiele, 1925, *C. similis* Thiele, 1925 and *C. vestita* Thiele, 1925, did not allow us to confidently attribute them to our SSHs or to the clades identified here. Seven species names proposed by Schepman (1908) for Indonesian material were also difficult to link to our specimens, because the original descriptions were based mainly on the shell and little on radular morphology. *Cocculina ovata* Schepman, 1908 might be an exception. McLean (1987) attributed numerous specimens collected at 187–210 m deep in the Philippines to this species. Among our SSHs from similar depths of neighbouring areas, the one made up of MC19 (Fig. 6C) showed the conchological features of *C. ovata* redescribed by McLean (1987), perhaps suggesting its identity at the species level. The shape of rachidian tooth of MC18 (Fig. 11I) did not exactly match the condition described for *C. ovata* by Schepman (1908) and McLean (1987). However, this inconsistency might have resulted from worn teeth or different angles in SEM shots and examination of more radulae is needed to confirm our species identification.

Five more species have been described from the IWP under the genus *Cocculina*: *C. pacifica* Kuroda & Habe, 1949, *C. tosaensis* Kuroda & Habe, 1949, *C. surugaensis* Hasegawa, 1997 and *C. tenuitesta* Hasegawa, 1997, all on sunken wood off Japanese coasts, and *C. delphinicula* Zhang & Zhang, 2018 from a dolphin skull at a depth of 300–400 m in the East China Sea (Zhang & Zhang, 2018). Although none of them were found in our material, we believe that the first species should be moved from the genus to either *Fedikovella* ("*C.*" *pacifica*; see below) or Lepetellidae ("*C.*" *tosaensis*; see Hasegawa, 1997: 65). The sequences we gathered from GenBank similarly illustrated the difficulty of the morphological identification at both genus and species levels. Examples include published sequences attributed to *Coccopigya punctoradiata* (Table S1) that were clustered with an entirely different species (MC5) in a potential new genus (nGen1).

Coccopigya

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

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

Coccocrater

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

Macleaniella and *Teuthirostria* do not match any of our specimens (see section *Macleaniella* and *Teuthirostria*). We thus consider morphogroup MA as *Coccocrater*.

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

Fedikovella

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

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

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

Paracocculina

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

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

Clades ME and MH

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

Clade MH contained specimens from "non-wooden" habitats. The specimens of MH1 were found on nodes of deep-sea bamboo corals (Isididae), which are formed by a specific protein matrix (Ehrlich *et al*., 2006), whereas MH2 were on the egg cases of chondrichthyes (sharks and/or rays). We consider that such unique habitats and independent phylogenetic position they occupy (Fig. 8) justify erection of another new genus (nGen4).

Macleaniella and *Teuthirostria*

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

CONCLUSIONS

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

DATA AVAILABILITY

The data underlying this article are available in the article and its online supplementary material, and in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/genbank/ and can be accessed with the GenBank accession numbers OL800712–OL801212 and OL956554–OL956700.

ACKNOWLEDGEMENTS

We thank all PIs and participants of the "*Tropical Deep-Sea Benthos*" expeditions AURORA 2007, BIOPAPUA, BOA1, CONCALIS, DONGSHA 2014, EBISCO, EXBODI, KAVALAN 2018, KAVIENG 2014, MADEEP, MAINBAZA, KARUBENTHOS 2, MIRIKY, NANHAI 2014, NORFOLK 2, PANGLAO 2004, PANGLAO 2005, PAPUA NIUGINI, SALOMON 2, SALOMONBOA 3 and TAIWAN 2013 conducted by MNHN and IRD, but also NTU and NTOU for Taiwanese expeditions. Additional to MNHN, IRD, NTU and NTOU supports, some of these expeditions were conducted with financial supports notably from Flotte Océanographique Française (DOI numbers: 10.17600/15005400, 10.17600/10100040, 10.17600/5100060, 10.17600/8100010, 0.17600/5100080, 10.17600/11100080, 10.17600/14004400, 10.17600/14004000, 10.17600/3100030, 10.17600/18000841, 10.17600/4100090, 10.17600/7100070), INEE-CNRS and Labex BCDiv (ANR-10-LABX-0003-BCDiv). We thank B. Buge for the curation of specimens; J. Abdelkrim, A. Dettaï and all staff from the Service de Systématique Moléculaire (SSM-UMS2700) for their help in managing and analysing molecular data; L. Genio and M. Cunha, University of Aveiro, for a specimen from the expedition James Cook 10 during the EU Hermes Project; A. Warén, P. Bouchet, V. Héros, P. Lozouet, P. Maestrati, L. Galindo, M. Caballer for discussion and collection management; S. Pont, P. Maestrati and L. Corbari for SEM shots. This research was mainly supported by research funding from the Ministry of Science and Technology, Taiwan (MOST 102- 2923-B-002-001-MY3, MOST 107-2611-M-002-007-, MOST 108-2611-M-002-012-MY2 and MOST 110-2611-M-002-013- to W-J Chen), the French National Research Agency (ANR 12-ISV7-0005-01 to S Samadi) and Japan Society for the Promotion of Science (KAKENHI 18H02494 and 19KK0385 to Y Kano).

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SUPPORTING INFORMATION

Table S1. List of oceanographic expeditions and specimens analysed in this study.

Table S2. Morphological conditions of SSHs.

Figure S1. Maximum likelihood tree of cocculinids based on *cox1* dataset. Numbers at nodes represent bootstrap values in percentage. Name of specimens collected during *Tropical Deep Sea Benthos* expeditions are shown as: voucher number, expedition, station, depth; specimens collected around Japan: voucher number, locality or station, depth. See Table S2 for details.

Figure S2. Maximum likelihood tree based on "master" *28S* dataset. Numbers at nodes represent bootstrap values in percentage.

Figure S3. *28S* subtrees.

Figure S4. Maximum likelihood tree based on combined three-gene dataset (*cox1*, *28S* and *H3*). Numbers at nodes represent bootstrap values in percentage.

Figure S5. Bayesian tree based on combined three-gene dataset (*cox1*, *28S* and *H3*). Numbers at nodes represent posterior probability.

Table 1. Eight morphogroups of Cocculinidae and their habitats.

	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

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
Cocculina	reticulate	radial, smooth or concentric	on oral lappet or foot	present	round	wood, dolphin skull	Haszprunar, 1987; McLean & Harasewych, 1995; Zhang & Zhang, 2018
Coccopigya	reticulate	**spinose**	modified right cephalic tentacle	**absent**	round	wood, leaf, bone	Marshall, [1985] 1986; McLean & Harasewych, 1995; see main text for habitat
Coccocrater	reticulate	radial or plainly raised radial	modified right cephalic tentacle	present	round	wood	Haszprunar, 1987; McLean & Harasewych, 1995 Strong *et al*., 2003
Paracocculina	reticulate	radial	on foot or oral lappet	present	round	wood	Haszprunar, 1987; Strong *et al*., 2003
Fedikovella	**concentric**	reticulate	right cephalic tentacle; unmodified	present	round	wood	Moskalev, 1976; McLean & Harasewych, 1995; Leal & Harasewych, 1999
Teuthirostria	smooth	reticulate	modified right cephalic tentacle	present	round	**cephalopod beak**	Moskalev, 1976; Leal & Harasewych, 1999; Strong *et al*., 2003
Macleaniella	smooth	**radial (with an inner septum)**	modified right cephalic tentacle	present	round	wood	Leal & Harasewych, 1999
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

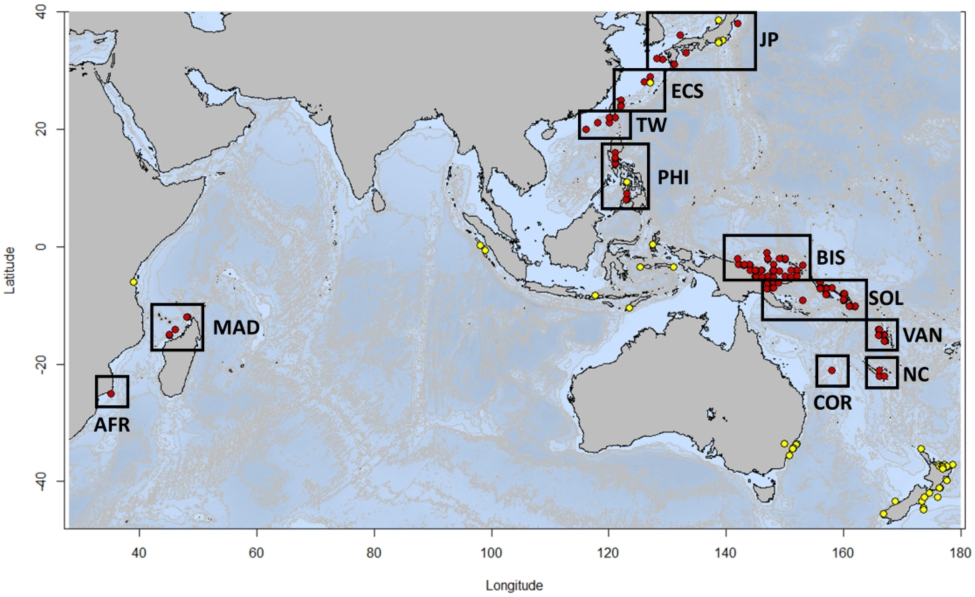

Figure 1. Localities of cocculinid specimens collected during our expeditions (red) and those in literature records (yellow; Watson, 1886; Schepman, 1908; Thiele, 1925; Marshall, 1985; Hasegawa, 1997; Lesicki, 1998; Zhang & Zhang, 2018) in Indo-West Pacific. The square indicates the 11 regions identified. 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)

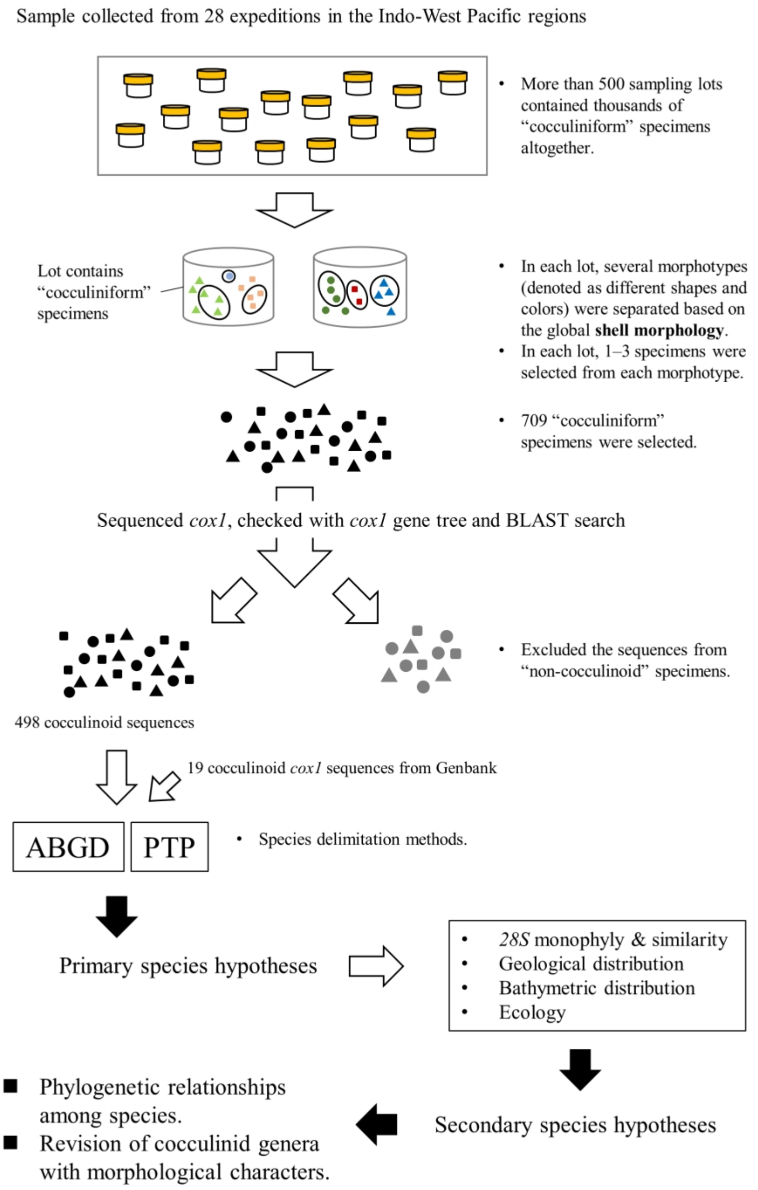

Figure 2. Diagram of integrative approach used for species delimitation.

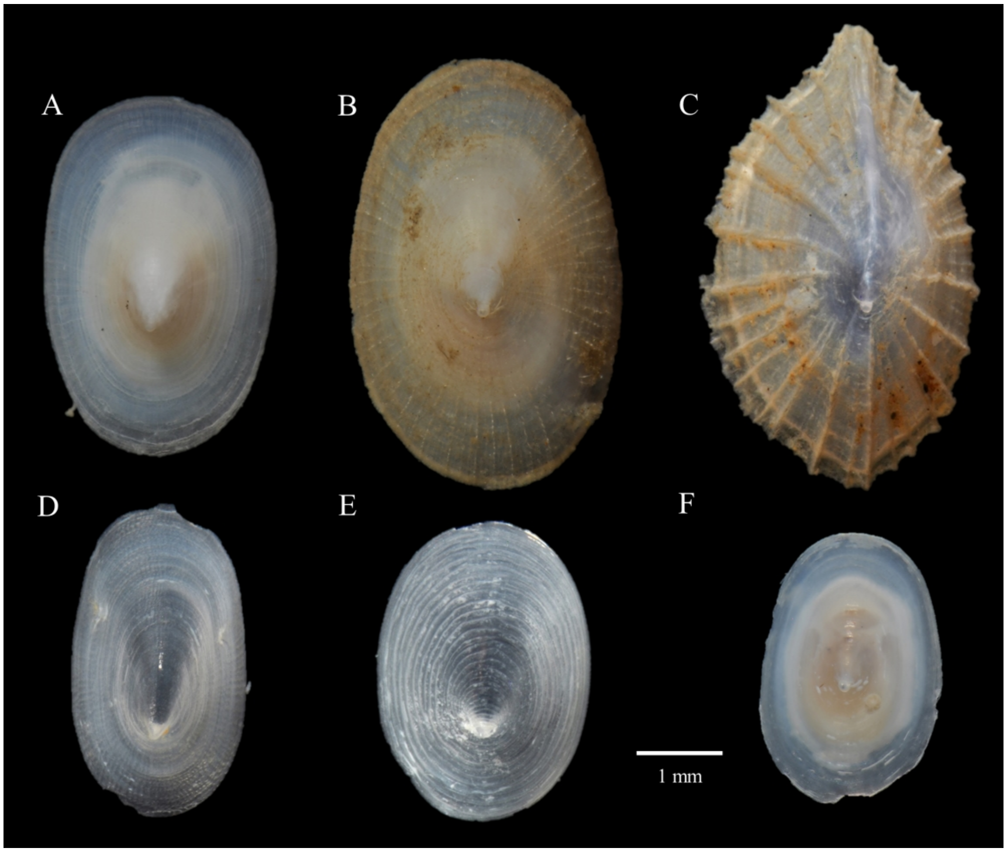

Figure 3. Character used in morphological examination. Teleoconch structure: (A) radially ribbed (MNHN-IM-2013-42733, MC10), (B) radially ribbed with pits and hairs (MNHN-IM-2013-42896, MG5), (C) plainly raised radially ribbed (MNHN-IM-2013-62346), (D) clathrate (MNHN-IM-2013-62341, MD5), (E) concentric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

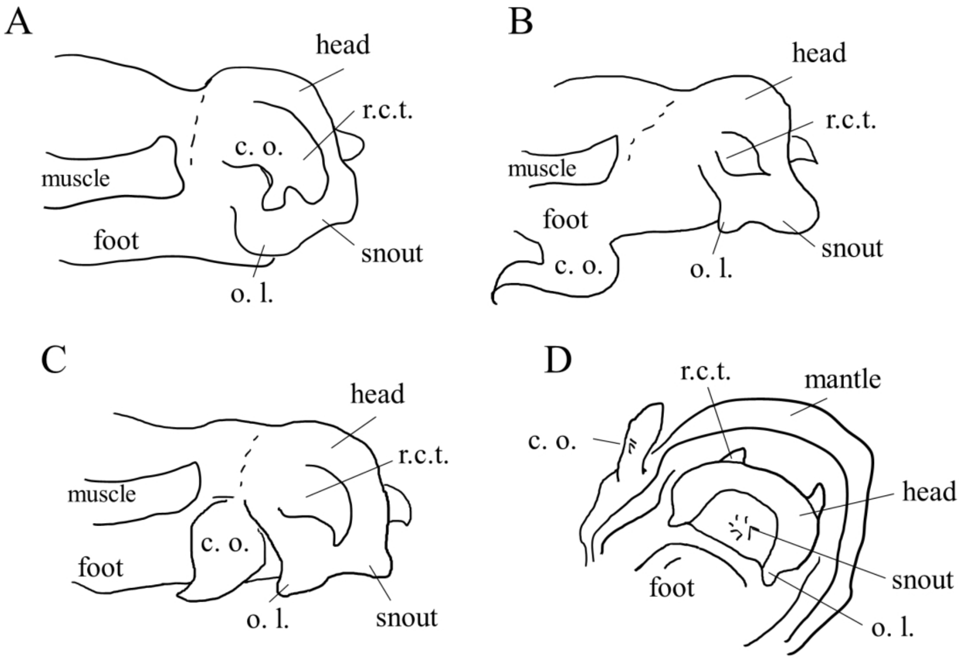

Figure 4. Character used in morphological examination. Copulatory organ: (A) associated with or branched from right cephalic tentacle, (B) originated from foot, (C) originated from oral lappet, or (D) originated from mantle margin. c.o.: copulatory organ, r.c.t.: right cephalic tentacle, o. l.: oral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

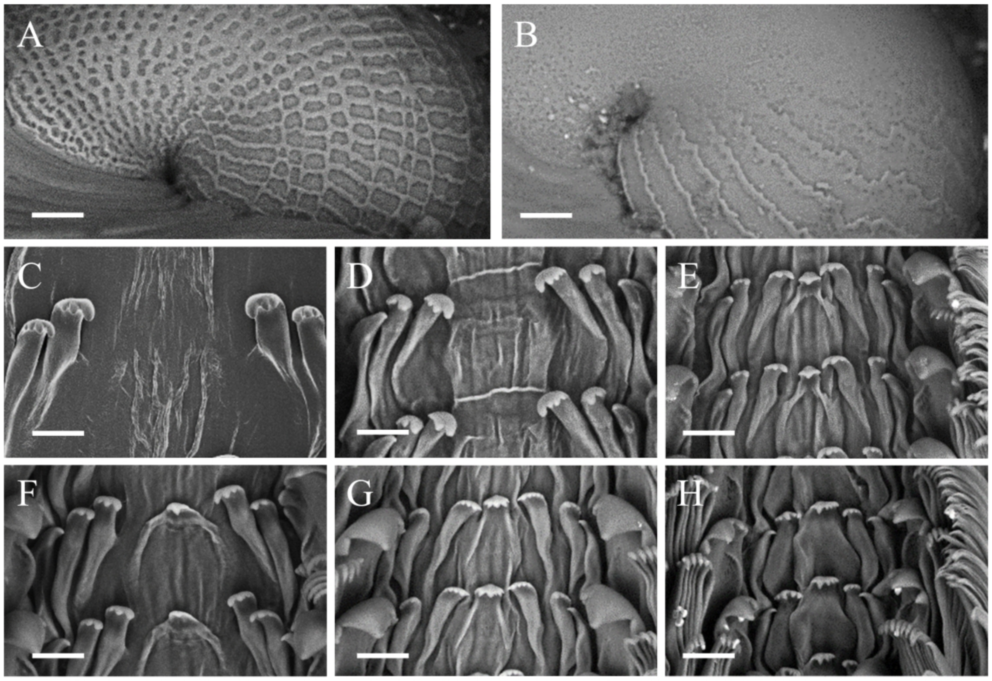

Figure 5. Characters used in morphological examination. Protoconch: (A) reticulate (MNHN-IM-2013-42752, MC2) or (B) concentric (MNHN-IM-2013-62343, MD8). Radula: (C) "obsolete," (C. japonica) (D) "acuspate-flat," (MNHN-IM-2013-42608, MC9) (E) "unicuspid narrow," (MNHN-IM-2013-42715, MB1) (F) "unicuspid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

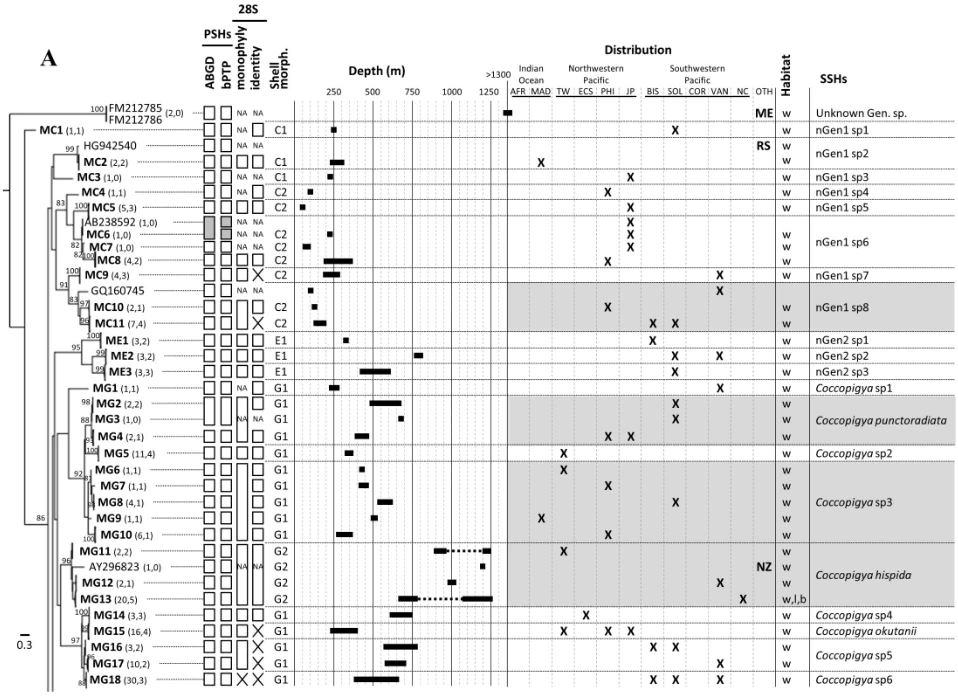

Figure 6. Maximum-likelihood tree based on cox1 dataset of cocculinids with results of species delimitation analyses. Numbers at tree nodes represent bootstrap values in percentage (values < 70% not shown). Names of primary species hypotheses (PSHs) are followed by number of sequences determined (cox1 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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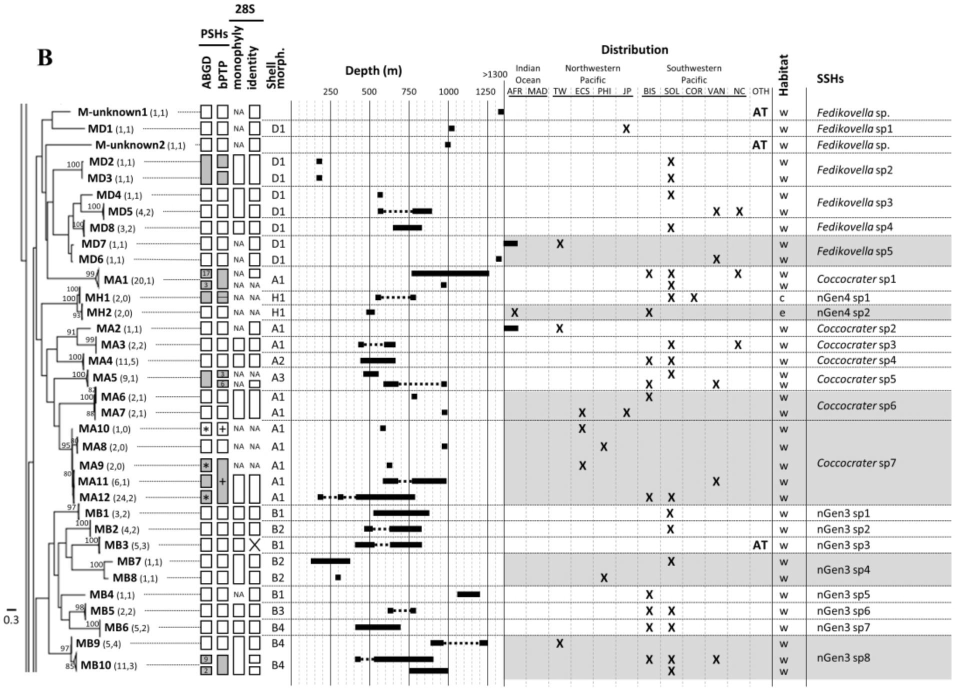

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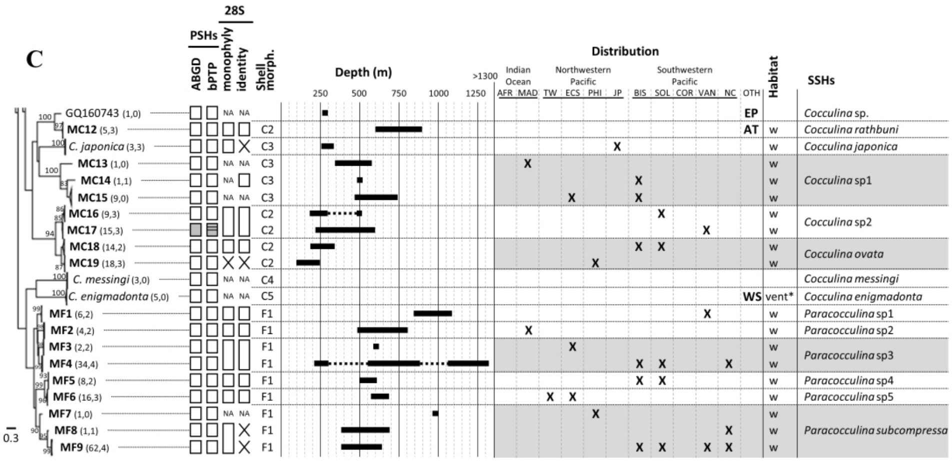
285x199mm (300 x 300 DPI)



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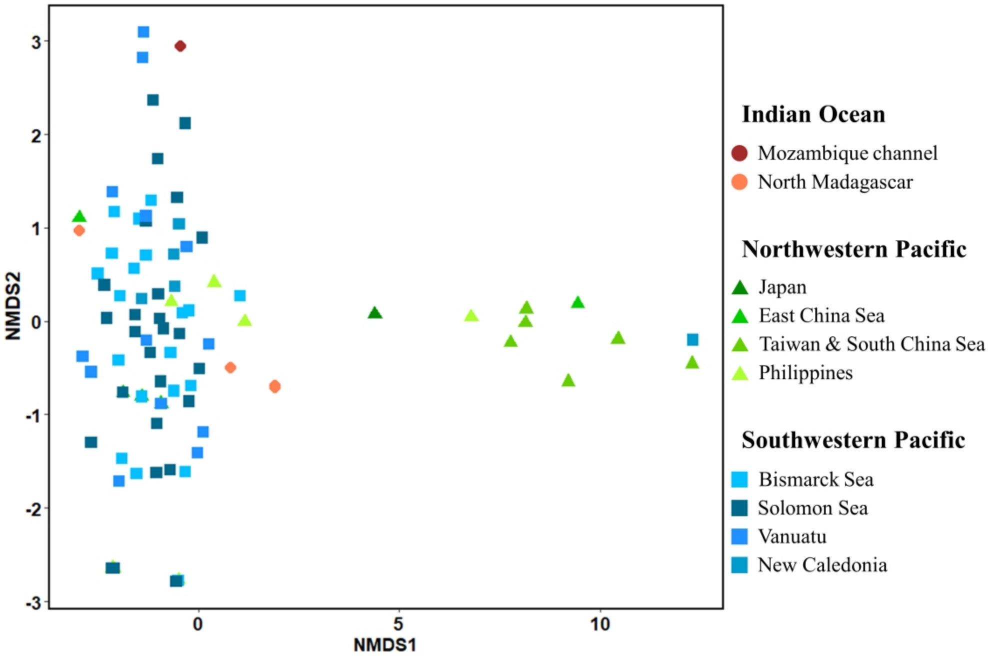

Figure 7. Nonmetric multidimensional scaling (NMDS) plot based on Jaccard index for cocculinid species composition among 157 stations in Indo-West Pacific.

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) ≥ 80% and posterior probability (PP) of ≥ 0.95 are shown in black circles; nodes supported by only BS ≥ 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.

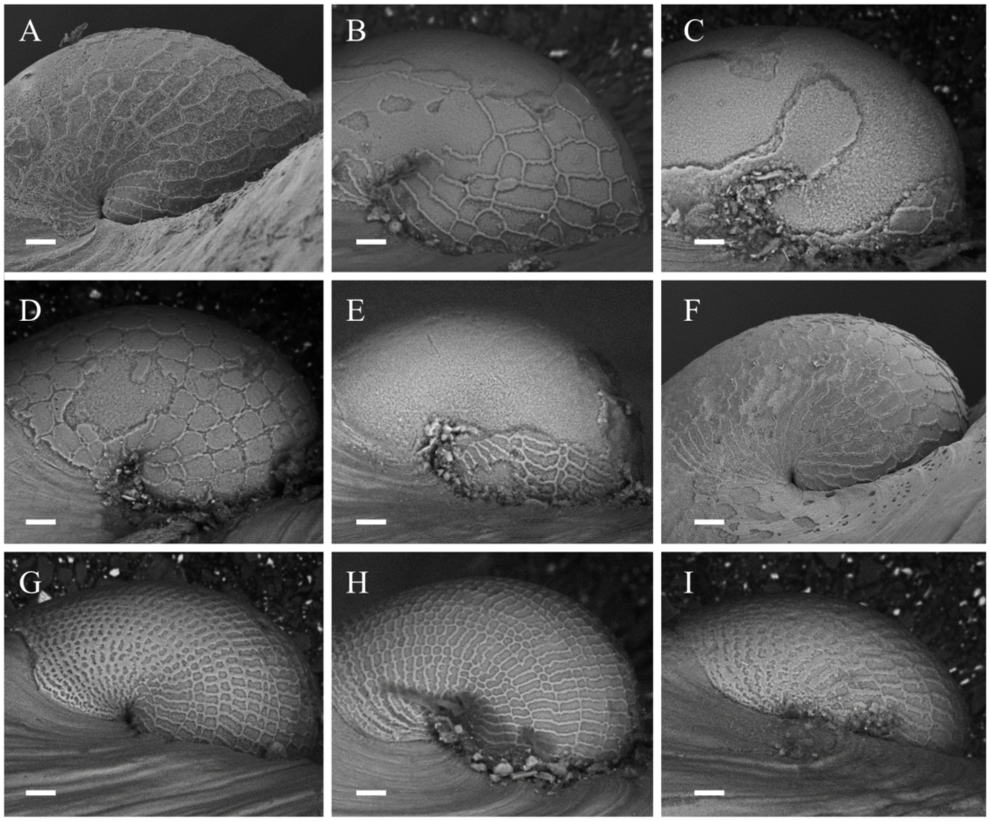

Figure 9. Protoconchs of sequenced specimens. (A) MNHN-IM-2013-59217 (MA4); (B) MNHN-IM-2013-42738 (MA8); (C) MNHN-IM-2013-42581 (MA12); (D) MNHN-IM-2013-62346 (MB7); (E) MNHN-IM-2013-42709 (MC15); (F) MNHN-IM-2013-42850 (MC18); (G) MNHN-IM-2013-42752 (MC2); (H) MNHN-IM-2013-42608 (MC9); (I) HS215 (MC5).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

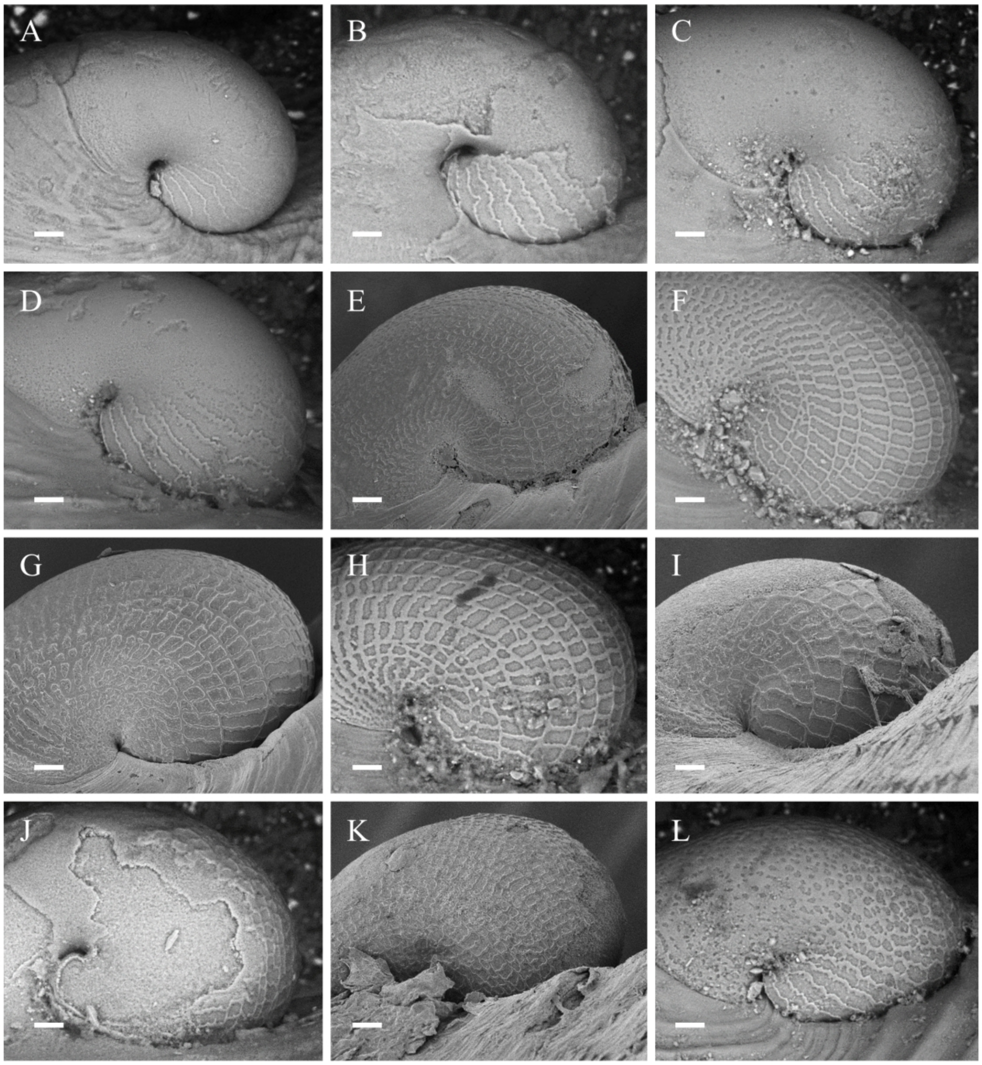

Figure 10. Protoconchs of sequenced specimens. (A) MNHN-IM-2013-42570 (MD3); (B) MNHN-IM-2013-42648 (MD4); (C) MNHN-IM-2013-62341 (MD5); (D) MNHN-IM-2013-62343 (MD8); (E) MNHN-IM-2013-42654 (ME1); (F) MNHN-IM-2013-42627 (MF9); (G) MNHN-IM-2013-42878 (MF6); (H) MNHN- IM-2013-42748 (MF2); (I) MNHN-IM-2013-42907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

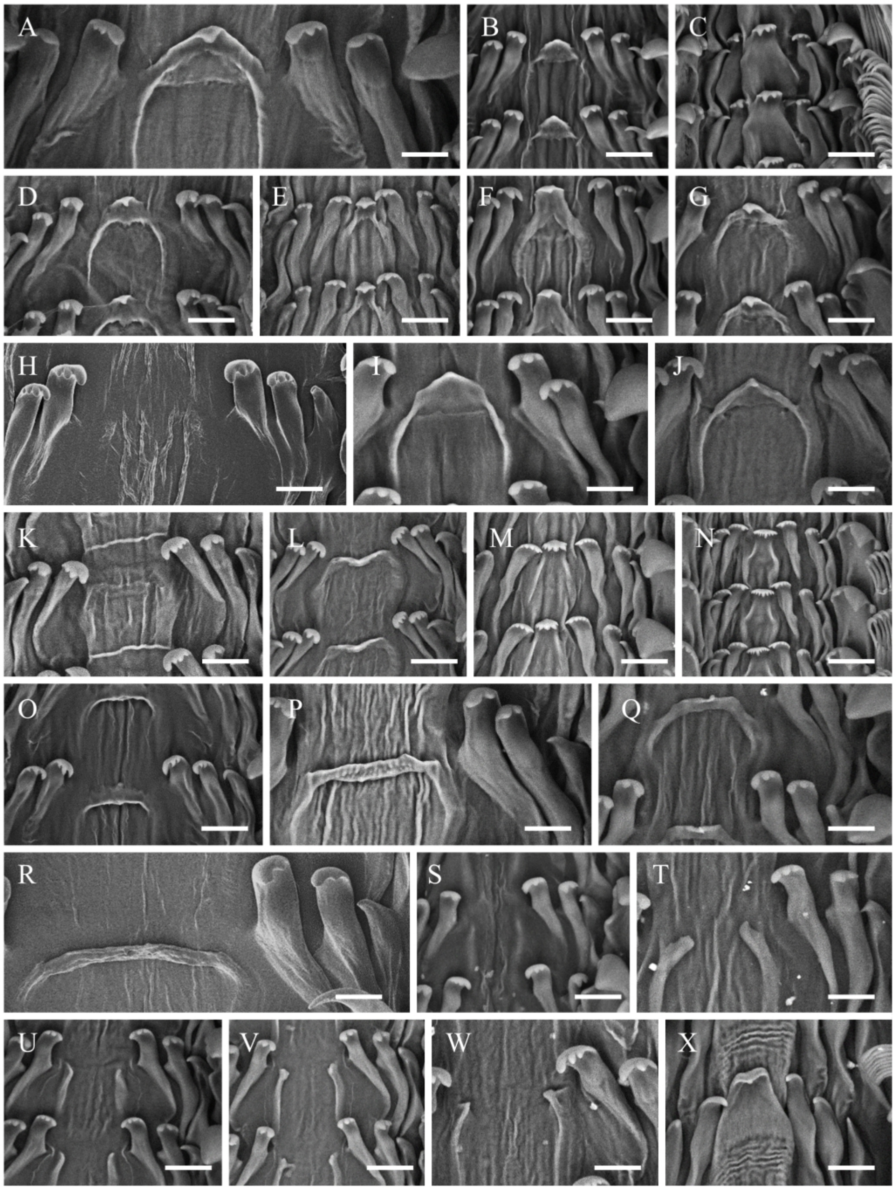

Figure 11. Radulae of sequenced specimens and an additional examined specimen of C. japonica. (A) MNHN-IM-2013-42671 (MA1), "unicuspid broad"; (B) MNHN-IM-2013-42687 (MA4), "unicuspid broad"; (C) MNHN-IM-2013-62344 (MA5), "multicuspid broad"; (D) MNHN-IM-2013-42828 (MA12), "unicuspid broad"; (E) MNHN-IM-2013-42715 (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 μm.