



Systematics of deep-sea starfish order Brisingida (Echinodermata: Asterozoa), with a revised classification and assessments of morphological characters[☆]

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ABSTRACT

Brisingida Fisher 1928 is one of the seven currently recognised starfish orders, and one of the least known taxa as being exclusive deep-sea inhabitants. Modern deep-sea expeditions revealed their common occurrences in various deep-sea settings including seamounts, basins and hydrothermal vent peripheral, underlining the necessity of clarifying their global diversity and phylogeny. In this study, we present a comprehensive molecular phylogeny of Brisingida which encompasses the highest taxonomic diversity to date. DNA sequences (COI, 16S, 12S and 28S) were obtained from 225 specimens collected in the global ocean, identified as 58 species spanning 15 of the 17 extant genera. Phylogenetic relationship was inferred using both maximum likelihood and Bayesian inference methods, revealing polyphyletic families and genera and indicating nonnegligible bias in prior morphology-based systematics. Based on the new phylogeny, a novel classification of the order, consisting of 5 families and 17 genera, is proposed. Families Odinellidae, Brisingasteridae and Novodiniidae (sensu Clark and Mah, 2001) were resurrected to encompass the genera *Odinella*, *Brisingaster* and *Novodinia*. Brisingidae and Freyellidae were revised to include 11 and 3 genera, respectively. A new genus and species, two new subgenera and seven new combinations are described and a key to each genus and family is provided. Transformations of morphological traits were evaluated under the present phylogenetic hypothesis. A series of pedomorphic characters were found in many genera and species, which led to a high degree of homoplasy across phylogenetically distant groups. Our results provide new insights in the phylogeny and ontogeny of the order, and highlight the necessity to evaluate character convergence under sound phylogenetic hypothesis.

1. Introduction

The deep ocean is amongst the most understudied territories on earth. Modern ocean expeditions and research have shown that the deep-sea is a heterogeneous environment that harbours a variety of habitats and a high level of biodiversity (Gjerde, 2006). The inaccessibility and fragility of deep-sea specimens have been a major obstacle in taxonomic, phylogenetic and biogeographic studies in the deep-sea

context. Order Brisingida Fisher, 1928, belonging to superorder Forcipulatacea, is an important group of starfish in the deep-sea benthic communities. They are commonly spotted in various marine habitats from the tropical oceans to the Antarctic, such as in trenches (e.g., Mu et al., 2018; Zhang et al., 2019), seamounts (e.g., Zhang et al., 2020) and hydrothermal vent fields (e.g., Desbruyeres et al., 2006; Kim and Hammerstrom, 2012; Gerdes et al., 2021), living solitarily or forming dense assemblages at favourable conditions (Mah, 2016; Amon et al.,

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2017; Woods et al., 2022) (Fig. 1). Their multiple flexible arms and long spines are used for suspension feeding, primarily on food particles brought by bottom current (Emson and Young, 1994; Gale et al., 2013; Mah, 2016). They were used as an indicator taxon to reflect habitat suitability near hydrothermal vents in a recent study, which suggested that relict sulfide chimneys, in contrast to active chimneys and basalt substrate, provided best living condition for these suspension feeders, where their density could be up to 300 ind./m² (Woods et al., 2022).

Brisingida are listed as a Vulnerable Marine Ecosystem habitat indicator by the South Pacific Regional Fisheries Management Organisation (SPRMFO) (Tracey et al., 2008; Parker et al., 2009) and as such are relied upon to suggest regulatory frameworks and conservation measures in areas where human activities can impact on deep-sea ecosystems. However, our understanding on the systematics of this deep-sea taxon is largely restricted to morphological descriptions published in the late 19th and early 20th century (e.g., Sladen, 1889; Fisher, 1917, 1919), and limited molecular data and phylogenetic research were available up to now. There is an urgent need to resolve the phylogeny and taxonomy of this important deep-sea community component, as they will provide baseline data on biodiversity, including phylogenetic

diversity (see Brondizio et al., 2019), which is an essential index for conservation and restoration of marine ecosystems.

1.1. Historical classification of Brisingida

Differing from the common five-armed starfish, Brisingida possess 6–20 long, slender arms and a small disk, superficially resembling ophiuroids (e.g., *Ophiocamax*) and crinoids (e.g., Pentametrocrinidae) (Fisher, 1928; Downey, 1986). This remarkable form of life was first reported in the Norwegian sea and was named after *brisingamen*, a necklace belonging to the Norwegian goddess Freya (Asbjørnsen, 1856). Later on, multiple species and genera were reported from the world ocean (Sladen, 1889; Perrier, 1882, 1885; Koehler, 1909a, b; Fisher, 1906, 1917, 1919), and the suborder Brisingina was established (Fisher, 1928). Tortonese (1958) raised the suborder to order Euclasteroidea, but soon it was renamed as Brisingida by Downey (1986). In the latter study, Downey proposed a two-family classification of the order, encompassing Brisingidae and Freyellidae. This classification is largely adopted in the present day (Mah, 2023), but controversies remained in regard to familial level classification.

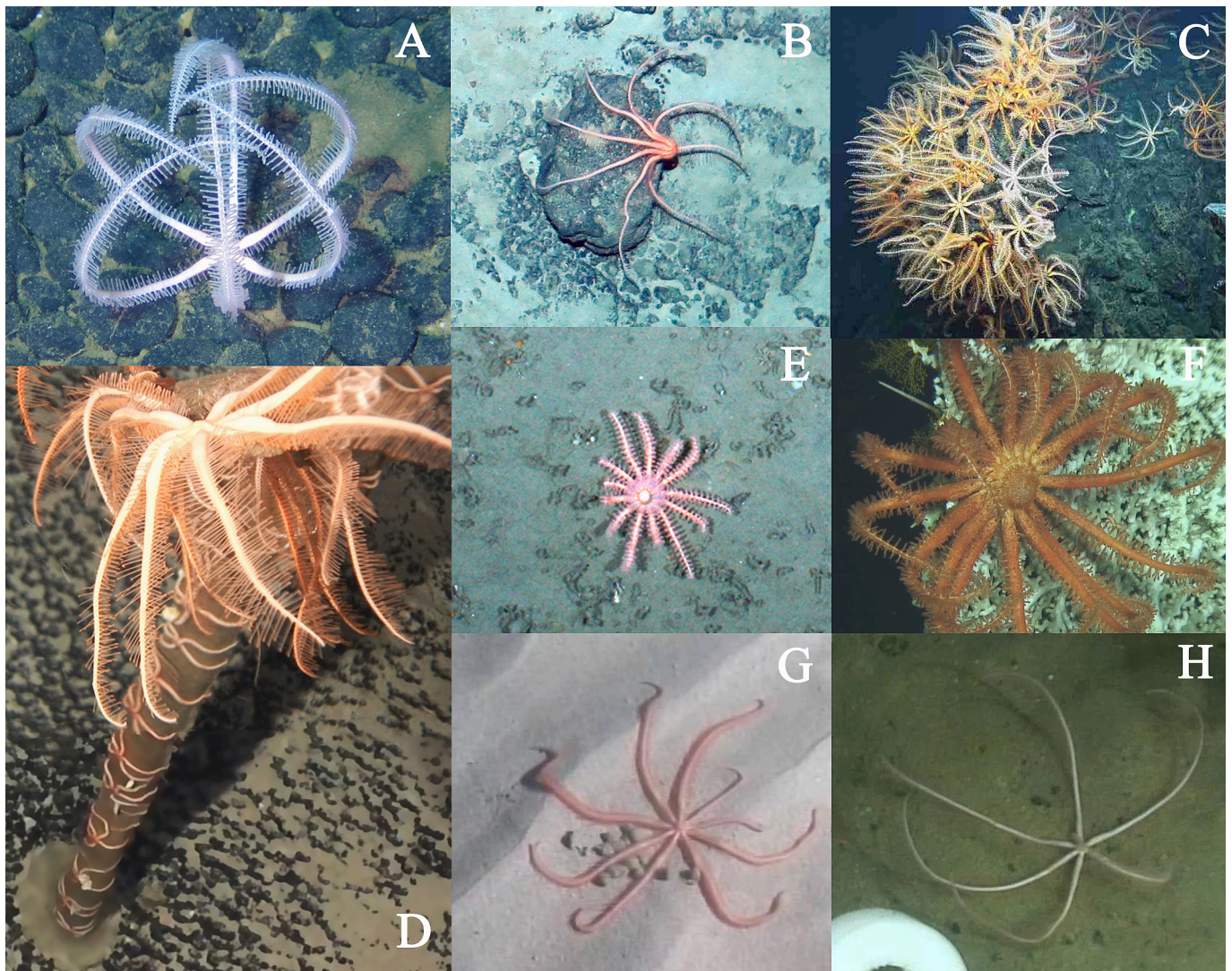


Fig. 1. Brisingida species from various deep-sea habitats. A. *Freyastera basketa*, on polymetallic nodules, Lamont seamount, Northwest Pacific, 4832 m; B. *Freyella* sp. 1 in a spawning gesture, Kyushu-Palau Ridge, Philippine Sea, 3283 m; C. *Freyella* sp. 1, at hydrothermal vent field, Lau basin, Southwest Pacific, 2528 m. Photo © Hourdez and Jollivet, 2019; D. *Colpaster patricki*, attached to a sponge, Weijia seamount, Northwest Pacific, 1935 m (Zhang, et al., 2020); E. *Odinella nutrix*, Antarctic Peninsula, 567 m. Photo © AWI/IPÖ (Piepenburg et al., 2013, 2017); F. *Novodinia antillensis*, attached to stony coral, Kyushu-Palau Ridge, Philippine Sea, 535 m; G. *Hymenodiscus* cf. *fragilis*, Weijia seamount, Northwest Pacific, 1957 m; H. *Freyastera* sp., Kyushu-Palau Ridge, Philippine Sea, 3641 m.

Mah (1998a, b) conducted a cladistic analysis on Brisingida using 24 morphological characters and proposed a new classification diagram for the order, which included six families, Odiniellidae, Brisingasteridae, Novodiniidae, Brisingidae, Freyellidae and Hymenodiscidae (Fig. 2) (also see Clark and Mah, 2001). The former three families are all monogeneric, composed of *Odinella*, *Brisingaster* and *Novodinia*, respectively, which belonged to Brisingida in Downey's classification (1986). Genera *Hymenodiscus*, *Astrolirus* and *Parabrisinga* were removed from Brisingidae to form a new family Hymenodiscidae (Mah, 1998b). This work provided novel insights on the phylogenetic relationship among Brisingida genera, but taxonomic description and diagnosis of the new families were not properly published later on, leading to a stage when both classifications were adopted in later works by different authors (Mah, 1999; Clark and Mah, 2001; McKnight, 2006; Campos et al., 2010; Mah and Foltz, 2011). Mah and Blake (2012) in their review of the global diversity and phylogeny of Asterozoa resurrected the 2-families classification, which henceforth became prevailing (Mah, 2016; Fau and Villier, 2020; Zhang et al., 2019; Zhang et al., 2020; Mah, 2022). The validity of the two classifications and whether they reflected the phylogeny of Brisingida is thoroughly investigated in the present study.

1.2. Molecular phylogeny of Brisingida

Few studies have attempted to resolve the phylogeny of Brisingida using molecular data, owing to the paucity of DNA sequences and difficulty in precise identification. Recent molecular phylogenetic studies, which were devoted to the superorder Forcipulatacea (including orders Forcipulata and Brisingida), suggested the monophyly of Brisingida and its affinity to family Asterozoa, but the sizes of the datasets were

quite limited, including nine brisingid species at most (Foltz et al., 2007; Mah and Foltz, 2011). In 2019 and 2020, the authors published three new species of Brisingida and constructed phylogenetic trees targeting at Brisingida using *COI*, *16S*, *H3*, *12S* and *18S* genes (Zhang et al., 2019; Zhang et al., 2020). In these studies, both Brisingidae and Freyellidae were found to be polyphyletic, with *Odinella*, *Brisingaster* and *Novodinia* and a *Freyella* sp. formed an outlying clade from the rest of Brisingida genera, whereas *Freyellaster fecundus* (Fisher, 1905) from Freyellidae clustered with Brisingidae species (Zhang et al., 2020). Genus *Hymenodiscus* was found to be polyphyletic, and *Astrolirus* was closer to *Brisinga* than to *Hymenodiscus*, which was not consistent with the morphology-based phylogenetic hypothesis (Mah, 1998a) (Fig. 2). However, many of the sequences used in these studies were not linked with morphological data and came from unidentified or even misidentified specimens, which significantly limits evolutionary inference. These molecular phylogenies also suffered from an underrepresentation of the less frequent or diverse genera.

In the present study, Brisingida specimens from 20 museum/institute collections were studied by both morphological and molecular approaches. Here we present the most comprehensive molecular phylogeny of Brisingida, which encompasses the highest taxonomic diversity (15 of 17 genera) to date. The new phylogenetic hypothesis revealed noneligible issues in the present classification scheme and suggested misinterpretation on phylogeny caused by a series of homoplastic characters used in previous taxonomic studies. We revised the classification of Brisingida accordingly, and evaluated key morphological characters used in prior taxonomic works. The new phylogeny and classification, as well as DNA reference data and a diagnostic key will serve as powerful tools for future identification of Brisingida.

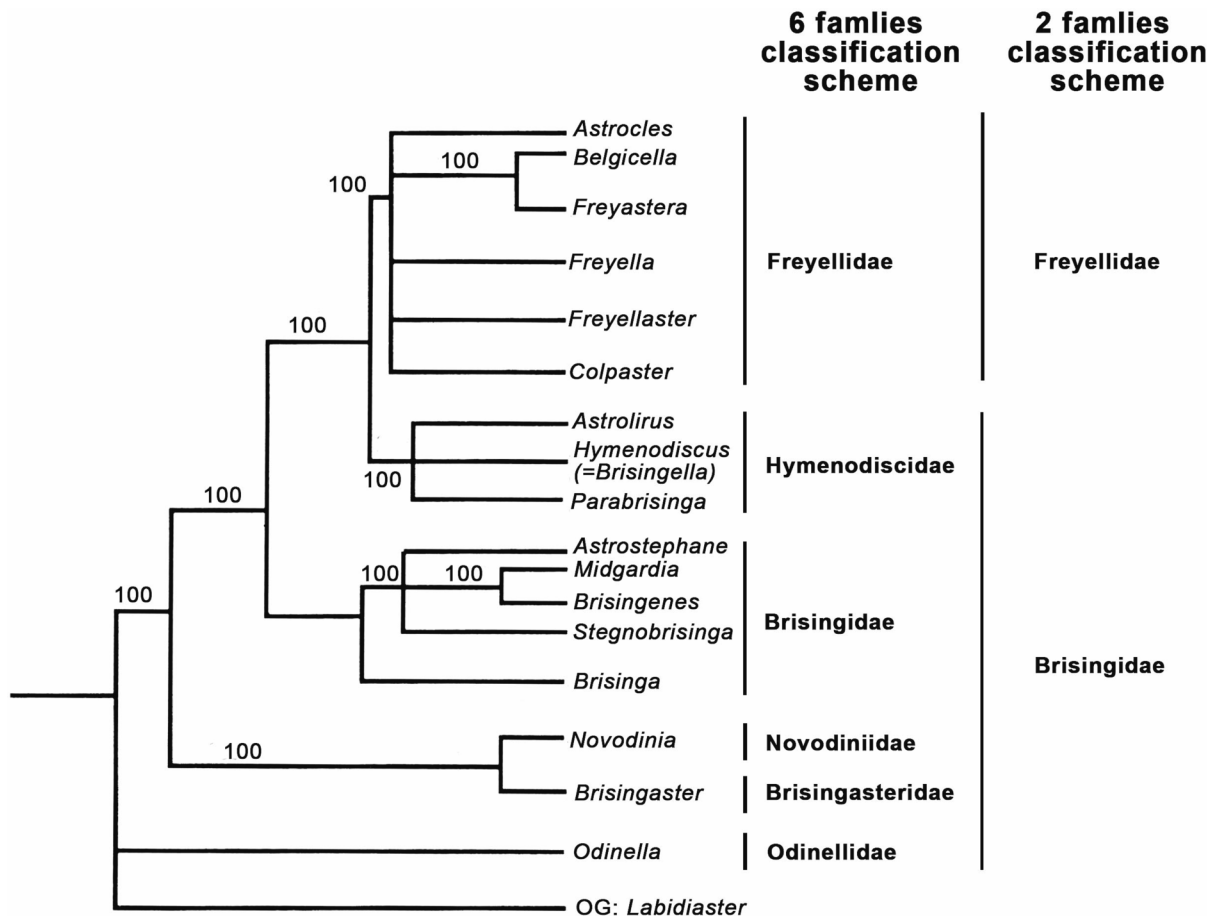


Fig. 2. Diagram of the 6-families classification of Brisingida (Mah, 1998a, 1998b) versus the 2-families classification (Downey, 1986; Clark & Downey, 1992). Tree topology follows Mah (1998a) (50 % majority rule consensus tree based on 24 morphological characters).

2. Material and methods

2.1. Taxon sampling

A total of 423 specimens of Brisingida were examined in the present study, including 333 specimens fixed and preserved in 75 %–96 % ethanol, 23 fixed in formalin and preserved in 70 %–96 % ethanol, and 67 dry specimens. Specimens examined were deposited in CAS, DZMB, ICML-UNAM, IDSSE, IFREMER, IOCAS, IORAS, MNHN, MOM, NBC, NHMUK, NIWA, NMV, RBCM, SCRIPPS, SIOMNR and ULB. Additional photos of type specimens held at USNM, NMNS and IORAS were also studied. A summary of specimens and species examined is shown in Table 1 and their global distribution illustrated in Fig. 3. Voucher numbers and sampling information of specimens as well as GenBank accession numbers are listed in Supplementary Table 1.

Institutional abbreviations

CAS: California Academy of Science, US

DZMB: German Center for Marine Biodiversity Research,

Table 1

Summary of specimens and species examined in the present study.

Family Genus	No. of specimens examined	No. of specimens sequenced	No. of species examined ^a / no. of species currently recognised (Mah, 2023)	No. of species sequenced ^a / no. of species currently recognised (Mah, 2023)
Family Brisingidae Sars, 1875				
<i>Astrolirus</i> Fisher, 1917	18	16	3/2	3/2
<i>Astrostephane</i> Fisher, 1917	9	9	1/2	1/2
<i>Brisinga</i> Asbjørnsen, 1856	63	51	14/19	8/19
<i>Brisingaster</i> de Loriol, 1883	27	14	1/1	1/1
<i>Brisingenes</i> Fisher, 1917	17	14	3/5	1/5
<i>Hymenodiscus</i> Perrier, 1884	31	10	10/16	2/16
<i>Miðgardia</i> Downey, 1973	5	5	2/1	2/1
<i>Novodinia</i> Dartnall et al., 1969	77	28	13/13	7/13
<i>Odinella</i> Fisher, 1940	12	7	1/1	1/1
<i>Parabrisinga</i> Hayashi, 1943	3	0	1/1	0/1
<i>Stegnobrisinga</i> Fisher, 1916	1	0	1/3	0/3
Family Freyellidae Downey, 1986				
<i>Astrocles</i> Fisher, 1917	4	2	1/3	1/3
<i>Belgicella</i> Ludwig, 1903	2	1	1/1	1/1
<i>Colpaster</i> Sladen, 1889	2	1	2/2	1/2
<i>Freyastera</i> Downey, 1986	62	30	14/8	12/8
<i>Freyella</i> Perrier, 1885	76	31	24/29	12/29
<i>Freyellaster</i> Fisher, 1918	11	7	6/5	3/5

^a The number of species examined and sequenced includes unidentified or undescribed (potentially new) species.

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ICML-UNAM: Institute of Marine Sciences and Limnology, National Autonomous University of Mexico, Mexico

IDSSE: Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences, China

IFREMER: Institut Français de Recherche pour l'Exploitation de la Mer, France

IOCAS: Institute of Oceanology, Chinese Academy of Sciences, China

IORAS: P. P. Shirshov Institute of Oceanology, Russian Academy of Sciences, Russia

MNHN: Muséum national d'Histoire naturelle, France

MOM: Musée Océanographique de Monaco, Monaco

NBC: Naturalis Biodiversity Center, Netherlands

NHMUK: Natural History Museum, UK

NIWA: National Institute of Water and Atmospheric Research, New Zealand

NMNS: National Museum of Nature and Science, Japan

NMV: Museums Victoria, Australia

NOC: National Oceanography Center, UK

RBCM: Royal British Columbia Museum, Canada

SCRIPPS: Scripps Institution of Oceanography, US

SIOMNR: Second Institute of Oceanography, Ministry of Natural Resources, China

ULB: Université Libre de Bruxelles, Belgium

USNM: National Museum of Natural History, Smithsonian Institution, US

2.2. DNA extraction and sequencing

Of all specimens examined, 225 specimens were used for DNA sequencing and phylogenetic reconstruction. Genomic DNA was extracted from tube feet tissues of each ethanol-preserved specimen using Qiagen DNeasy Blood & Tissue Kit or Macherey–Nagel NucleoSpinR 96 Tissues kits following the manufacturers' protocols. DNA markers from mitochondrial genome, cytochrome oxidase subregion I (*COI*), 16S ribosomal DNA (*16S*), 12S ribosomal DNA (*12S*), and from nuclear, 28S ribosomal DNA (*28S*), were amplified with standard polymerase chain reaction (PCR). For PCR reaction, a mixture of 25 µL containing 12.5 µL Vazyme 2 × Phanta Max Master Mix, 9.5 µL ddH₂O, 1 µL forward primer, 1 µL reverse primer and 1 µL template DNA was used. Alternatively, Qiagen Taq DNA polymerase was used in a mixture of 20 µL containing 0.12 µL polymerase, 15.44 µL ddH₂O, 2 µL 10X Standard Taq Reaction Buffer, 0.8 µL dNTPs, 1 µL DMSO, 0.32 µL forward primer, 0.32 µL reverse primer and 1 µL template DNA. A dilution (1:5) of template DNA was made for better PCR performance when using Taq polymerase. PCR program was run under the following conditions: pre-denaturation at 95 °C for 4 min; 35–45 cycles of denaturation at 95 °C for 45 s, annealing at 50 °C (for *COI*, *12S*), 52 °C (for *16S*) or 55 °C (for *28S*) for 40 s, and extension at 72 °C for 1 min. Primer pairs used in this study were shown in Table 2.

PCR products were purified and sequenced in both directions using the forward and reverse primers by Sanger Sequencing or Next Generation Sequencing (NGS) on Illumina MySeq platform using multiplexing method detailed by Hinsinger et al (2015). The reads from NGS were assembled using Geneious Prime 2021.2 (Kearse et al., 2012) by comparing with closely related sequences acquired from Sanger Sequencing or from public database.

2.3. Sequence alignment and phylogenetic analyses

Reference data of Brisingida were obtained from GenBank and BOLD database (Table 4, Suppl. Table 2). Two Asteroidea species, *Asterias amurensis* and *Marthasterias glacialis*, were chosen as outgroups and were sequenced for the four genes. All sequences were aligned using MUSCLE algorithm (Edgar, 2004) in Geneious Prime (Kearse et al., 2012). The conserved regions of 16S alignment were selected by Gblocks 0.91b

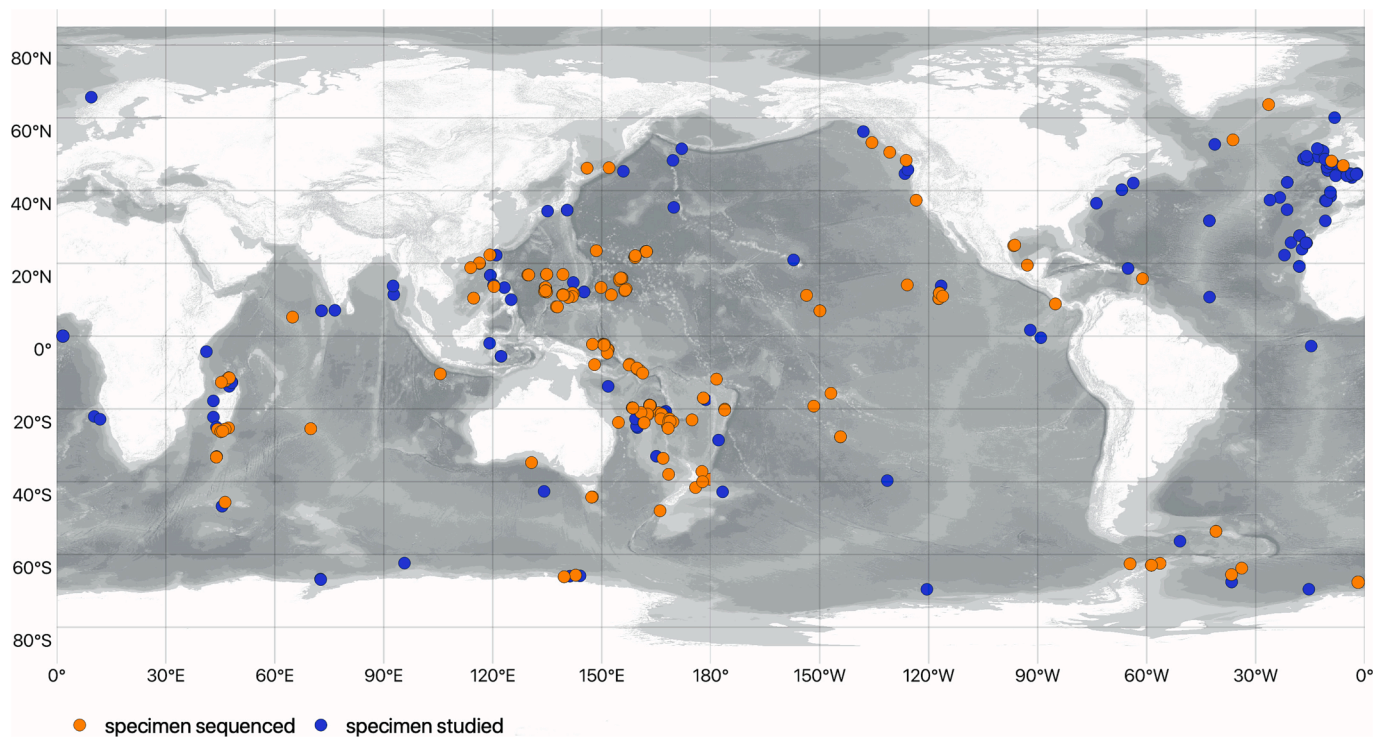


Fig. 3. Brisingida specimens studied in the present research. Orange dots show specimens used for both morphological and molecular analyses. Blue dots show specimens examined for morphology only. (For interpretation of the references to colour in this Fig. legend, the reader is referred to the web version of this article.)

Table 2

Primers and PCR annealing temperature (Ta) used in the present study.

Gene	Primer	Direction	Sequence (5'–3')	Reference
COI (~690 bp)	ECOLa	Forward	ACCATGCAACTAAGACGATGA	Knott & Wray, 2000
	EchinoF1deg	Forward	TTTCAACAAAACAYAAGGNCATNGG	Agnès Dettai (per. comm.)
	EchinoR1deg	Reverse	TAAACITCAGGGTGTCCRAARAATCA	Agnès Dettai (per. comm.)
	HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al., 1994
16S (~570 bp)	16Sar	Forward	CGCTGTATTATCAAAAACAT	Palumbi et al., 1991
	16Sbr	Reverse	CCGGTCTGAACTCAGATCACGT	Palumbi et al., 1991
12S (~530 bp)	12Saf	Forward	CTTAGCAACCGATTGGTCCTAGTCC	Zulliger and Lessios, 2010
	12Sar	Reverse	GCTGGTAAGGTTTTTCGTGGGTTATCG	Zulliger and Lessios, 2010
28S (~790 bp)	LH-28SF3	Forward	GGATCAGCCCAGCGCCGAAT	Hemery et al., 2012
	LH-28SR3	Reverse	TAGACTCCTTGGTCCGTGTTTC	Hemery et al., 2012
28S (~270 bp)	28SF	Forward	AGAAACTAACMAGGATTCYYTAGTA	Foltz et al., 2007
	28SR	Reverse	ACTTCCCTCAYGGTACTTGT	Foltz et al., 2007

(Castresana, 2000) under less stringent selection options. DAMBE 7 (Xia, 2018) was used to test for substitution saturation in protein coding gene *COI* and saturation were not detected. Pairwise genetic distances for *COI* sequences were calculated using the Kimura's two-parameter model (K2P) (Kimura, 1980).

Alignments of *COI*, *16S*, *12S* and *28S* genes were concatenated for phylogenetic analyses. The complete concatenated dataset includes all specimens and sequences acquired from the present study and reference databases. To assess the transformation of key morphological characters, a reduced dataset including 37 terminal taxa with both molecular and morphological data available was generated. Both datasets were used for phylogenetic reconstruction with Maximum Likelihood and Bayesian inference methods.

Prior to phylogenetic reconstruction, partitions were set for each gene and for each codon position of *COI*. ModelFinder (Kalyaanamoorthy et al., 2017) implemented in IQ-TREE v2.0 (Minh et al., 2020) was used to predict the best fit evolution models for each partition. The following models were selected and used in Maximum Likelihood analysis: *COI* (1st codon site: TIM2 + F + I + G4; 2nd codon site: SYM + I + G4 for the complete dataset and TIM3e + I + G4 for the reduced

dataset; 3rd codon site: TIM2 + F + I + G4), *16S* (TIM3 + F + I + G4), *12S* (TIM2 + F + I + G4), *28S* (GTR + F + G4). Maximum likelihood analysis with 100,000 ultrafast bootstrap (Hoang et al., 2018) replicates were performed in IQ-TREE.

Bayesian reconstruction was conducted using MrBayes v3.2.7 (Huelsenbeck and Ronquist, 2001). As TIM2 and TIM3 models were not implemented in MrBayes, the closest over-parameterized model (GTR) was used for Bayesian Inference analysis (Lecocq et al., 2013). Four parallel runs of 10,000,000 generations were executed with 4 chains, sampling every 1,000 generation to estimate the posterior probabilities. The first 25 % trees were discarded as burn-in. TRACER v. 1.7 (Rambaut et al., 2018) was used to check the estimated sample sizes (ESS) as a measure of run convergence. The tree topologies were observed and edited in Figtree v1.4.3.

2.4. Morphological examination and character transformation

Specimens were examined under a stereoscope or microscope. Morphological description and terminology followed taxonomic literatures (e.g., Sars, 1875; Sladen, 1889; Fisher, 1917, 1919, 1928; Downey,

1986). Photographs of the detailed diagnostic characters were captured by a stereoscope (Zeiss Axio Zoom. V16) or digital camera (Canon EOS 60D or Olympus TG-6). Ossicles were prepared and examined following protocols of Fau and Villier (2020) and Esteban-Vázquez et al. (2021).

Key morphological characters were mapped on a phylogenetic tree derived from a reduced molecular dataset including 37 (+2 outgroup) terminal taxa. These characters include the general form (character 1–3), plate characters (character 4–7, 14–15) and spine characters (character 11–13), which were adopted as diagnostic characters in prior taxonomic studies (Perrier, 1885; Sladen, 1889; Fisher, 1917, 1919, 1928; Downey, 1986; Mah, 1998b). Characters and their states were defined and each terminal taxa were scored accordingly (Suppl. Table 3). The ancestral character states were reconstructed in Mesquite 3.70 (Maddison and Maddison, 2021) using the most parsimonious reconstruction (MPRs) (Swofford and Maddison, 1987) and likelihood reconstruction with MK1 model (Lewis, 2001). The characters examined and their states are listed in Table 3.

Table 3
Morphological characters and states of Brisingida examined in the present study.

Character	State 0	State 1	State 2
1. Number of arms	>10 arms	7–10 arms	6 arms
2. Number of gonads on each arm	One pair of gonads	Numerous gonads	
3. Papulae	Numerous	Reduced or absent	
4. Shape of oral plate (from actinal view)	Fan shape	Trapezoid shape	
5. Position of the first inferomarginal plates	In contact with the first adambulacrals and odontophore	Not in contact with the first adambulacrals and odontophore (appearing later on arm)	
6. Arrangement of inferomarginal plates on arm (beyond genital area)	Correspond to every 3rd or more adambulacrals plates	Correspond to every 2nd adambulacrals plates	Correspond to every adambulacrals plates
7. First pair of adambulacrals plates on adjacent arms	In contact laterally	Not in contact laterally	
8. First pair of inferomarginal plates on adjacent arms	In full contact laterally	In contact only at adoral end (forming a reversed Y with the odontophore)	
9. First inferomarginal plates largely inserting in the first pair of adambulacrals plates	Absent	Present	
10. A partial fusion between the first and second adambulacrals plates	Absent	Present	
11. A lateral comb of dorsal-lateral spines	Present	Absent	
12. Shape of proximal subambulacrals spine ends	Acicular	Modified comparing to distal subambulacrals spines	
13. Shape of oral spines	Straight	Bent at base	
14. Costae	Present	Absent	
15. Intercostal area armature	Bare	Pavement of plates	Isolated discoid plates

3. Results

3.1. Specimens examination and revision

A total of 423 Brisingida specimens, collected from 1873 to 2022, were examined in the present study, including 72 type specimens of 44 species from 9 genera (Suppl. Table 1). The examination of type specimens and historical specimens suggested necessary taxonomic revisions of several currently accepted species and genera. These include synonymizing *Belgicella racowitzana* Ludwig, 1903 and *Freyastera benthophila* (Sladen, 1889) to *Freyella benthophila* comb. nov., the revision of *Astrolirus patricki* Zhang et al., 2020 to *Colpaster patricki* comb. nov., and the restoration of *Freyella edwardsi* Perrier 1882. In addition, several specimens with DNA information previously published on GenBank (Ward et al., 2008; Mah and Foltz, 2011; Mu et al., 2018; Glover et al., 2016) were also re-examined and their identification were corrected based on morphological examination and/or molecular data comparison. The revised identification of GenBank sequences and their corresponding accession numbers were shown in Table 4.

3.2. Phylogenetic inference

We successfully obtained 697 new gene sequences from 225 specimens collected from 1985 to 2022, which substantially enriched the DNA reference library of Brisingida. Together with the reference data, a total of 769 sequences from 245 specimens representing 60 taxa were used for phylogenetic analyses (Suppl. Table 4). Both BI and ML trees derived from the complete dataset are largely resolved with high supports at most nodes (Fig. 4, Suppl. Fig. 1). The posterior probability is greater than 0.95 at 89 % of all the nodes, while the ultrafast bootstrap value is greater than 95 at 84 % of all the nodes. The topologies of the two trees are generally congruent, with only six nodes of the BI tree not supported by the ML tree. Five of these nodes are at species level within *Freyella*, *Freyastera* and *Freyellaster*, whereas one node is at genus level suggesting an unresolved position of a potential new genus, Brisingidae gen. nov. (Fig. 4). As the scope of this study is to address the systematic issues at higher taxonomic level of Brisingida, the unresolved phylogeny

Table 4
Brisingida sequences from GenBank with revised identifications.

Published identification & voucher number	Revised identification	GenBank accession no.	Note
<i>Hymenodiscus</i> sp. MNHNP EcAh 6036	<i>Astrostephane moluccana</i>	EU707747 (H3), EU722977 (16S)	Original specimen re-examined (MNHNP EcAh 6036 = MNHN-IE-2022-2082)
<i>Astrostephane moluccana</i> NIWA 33311	<i>Brisingenes</i> sp. 1	GQ288556 (16S)	Original specimen re-examined (NIWA 33311)
<i>Freyastera benthophila</i>	<i>Freyella macropedicellariae</i>	NC039982 (complete mitogenome)	Original specimen re-examined (IDSSE-EEB-HX01)
<i>Freyastera benthophila</i> CLM-212	<i>Freyella macropedicellariae</i>	EU707770 (H3), EU722993 (16S)	16S matched with specimen IDSSE-EEB-HX01 (100 %)
<i>Hymenodiscus</i> sp. USNM E47614	<i>Brisinga</i> sp.	EU707659 (H3), EU722922 (16S)	Photos of original specimen examined (USNM E47614)
<i>Freyella</i> sp. 1 RDW-2008	<i>Novodinia novaezelandiae</i>	EU869921 (COI)	COI matched with specimen NIWA 31992 (99.85 %)
<i>Freyastera</i> cf. <i>benthophila</i> NHM-413	<i>Freyastera</i> cf. <i>tuberculata</i>	KU519550 (COI), KU519518 (16S)	Revised by Bribiesca-Contreras et al., 2022
<i>Freyastera</i> cf. <i>benthophila</i> NHM-421	<i>Freyastera</i> cf. <i>tuberculata</i>	KU519551 (COI)	Revised by Bribiesca-Contreras et al., 2022

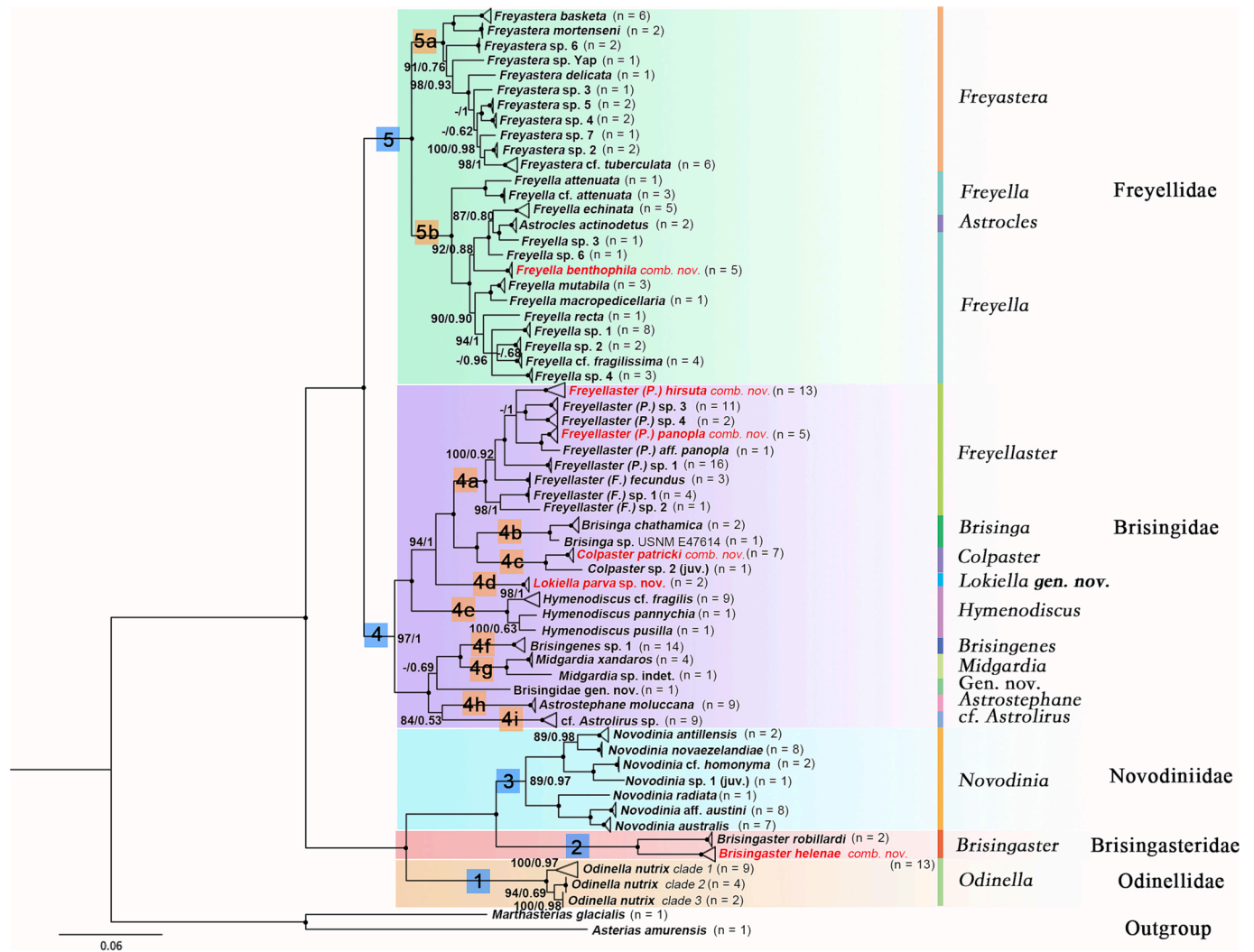


Fig. 4. Phylogenetic tree of Brisingida using four genes (*COI*, *16S*, *12S*, *28S*). The dataset includes 245 specimens of 58 species from 15 genera and 1 potential new genus, as well as two outgroup species. Leaves were collapsed by species for better readability. Number of specimens (n) sequenced for each taxon were provided at tip label. Tree topology follows that of the Bayesian tree. Ultrafast bootstrap support (%) / posterior probability (PP) were shown at each node. Solid circles indicate UFBS $\geq 99\%$ and PP ≥ 0.99 . “-” means that the node is not found in ML tree. Proposed classification in the present study is shown in the tree. Species names in red indicate new species or combinations. Clades and subclades were numbered from 1–5 and 4a–5b to represent each family and genus as discussed in the main text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

at species level which might require more data and finer sampling is not focused in the discussion herein. The trees derived from the reduced datasets are also congruent at family and genus levels (Fig. 6).

The phylogenetic trees reflect a comprehensive picture of the phylogeny of Brisingida. The order is well-supported as monophyletic, which is in accordance with previous molecular studies (Foltz et al., 2007; Mah and Foltz, 2011; Sun et al., 2022). However, a high degree of incongruence between the molecular phylogeny and morphology-based phylogeny and classification (Fig. 2) within the order was discovered. On the familial level, both family Brisingidae (Fig. 4, clades 1, 2, 3, 4a, 4b, 4d–i) and Freyellidae (Fig. 4, clades 5, 4a, 4c) appeared to be polyphyletic. On the generic level, the most speciose genera, *Brisinga* and *Freyella*, are not monophyletic either. *Brisinga chathamica* McKnight, 1973 and *Brisinga* sp. USNM E47614 formed a sister clade (clades 4b) to *Colpaster* species, whereas several other *Brisinga* species (i.e., *B. panopla* Fisher, 1906, *B. hirsuta* Perrier, 1894) clustered with *Freyellaster* species (clades 4a). *Freyella* (clade 5b) was paraphyletic by the interposition of a *Freyastera* species (*Freyastera benthophila*) and an *Astrocles* species (*Astrocles actinodetus* Fisher, 1917). All the other genera, including *Odinella*, *Brisingaster*, *Novodinia*, *Colpaster*, *Hymenodiscus*, *Brisingenes*,

Astrostephane, *Midgardia*, *Astrolirus* (cf.) and a new genus *Lokiella* gen. nov., were supported as monophyletic. In addition, a potential new genus, *Brisingida* gen. nov., was also supported as a clade derived from the other genera, but since only one specimen was found for this taxon, it is not described in the present study. Taxonomic revisions are made for polyphyletic families and genera based on the new phylogeny. The composition of each clade and the corresponding taxonomic revision are listed as follows.

Clade 1: genus *Odinella* (Fig. 5A), herein proposed to be raised as family *Odinellidae* (a name first proposed by Mah, 1998b).

Clade 2: genus *Brisingaster* (Fig. 5B), herein proposed to be raised as family *Brisingasteridae* (a name first proposed by Mah, 1998b).

Clade 3: genus *Novodinia* (Fig. 5C), herein proposed to be raised as family *Novodiniidae* (a name first proposed by Mah, 1998b).

Clade 4: family *Brisingidae* (revised). The revised family encompasses genera *Astrolirus*, *Astrostephane* (Fig. 5I), *Brisinga* (Fig. 5E), *Brisingenes* (Fig. 5H), *Colpaster* (Fig. 5F), *Freyellaster* (Fig. 5D), *Hymenodiscus* (Fig. 5G), *Lokiella* gen. nov. (Fig. 9), *Midgardia*. Two

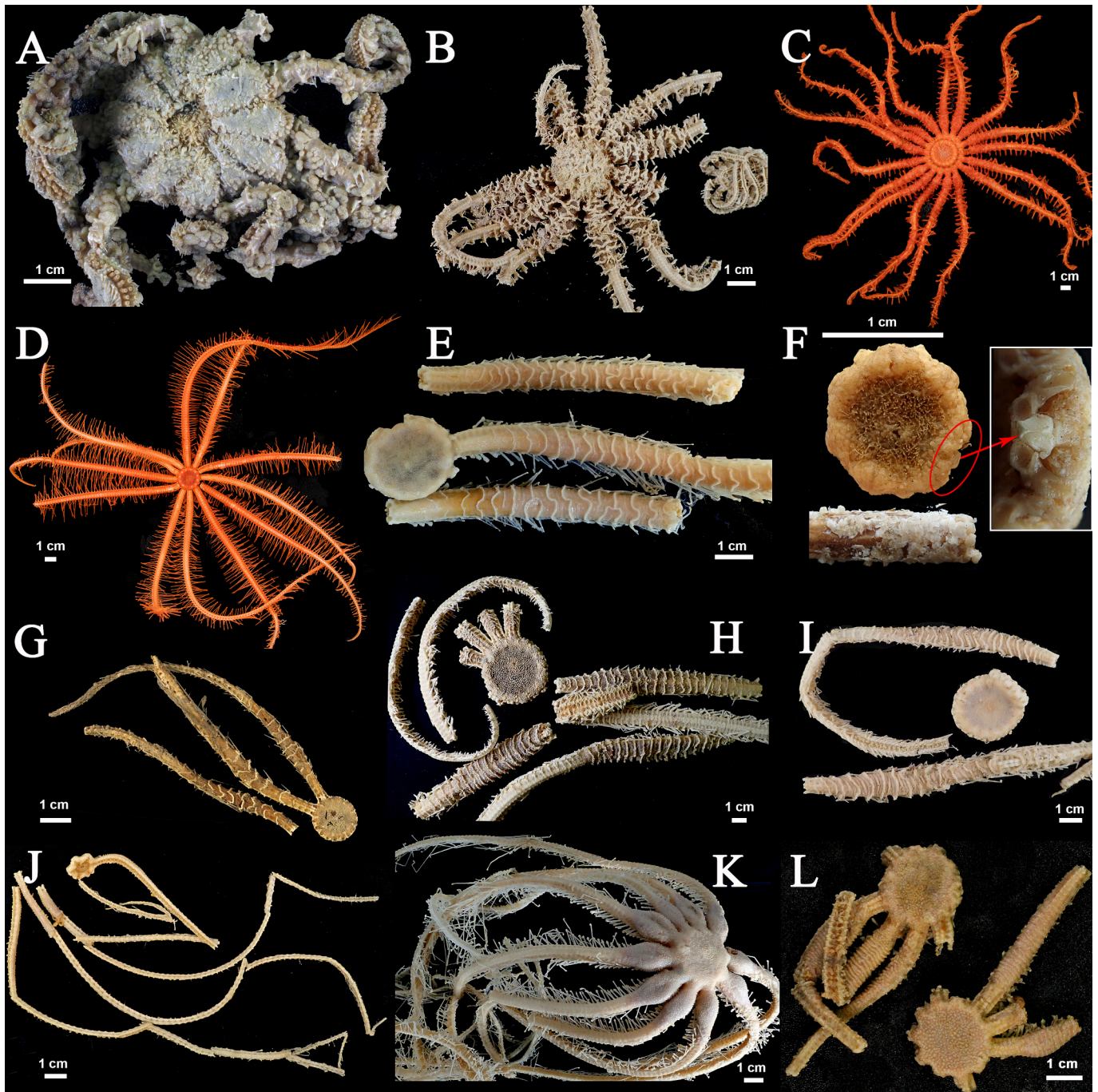


Fig. 5. Photographs of Brisingida specimens of different genera. A. *Odinella nutrix*, MNHN-IE-2019-342; B. *Brisingaster helenae* comb. nov., MNHN-IE-2019-4248; C. *Novodinia antillensis*, RSIOAST0093; D. *Freyellaster (Pseudobrisinga)* sp. 1, RSIOAST0088; E. *Brisinga cricophora* holotype NHMUK 1890.5.7.1043; F. *Colpaster scutigerula*, holotype NHMUK 1890.5.7.1081, red circle and inset show the first pair of inferomarginal plates inserting in between the first pair of adambulacral plates; G. *Hymenodiscus coronata*, MNHN-IE-2019-5468; H. *Brisingenes* sp. 1, NIWA 33311; I. *Astrostephane moluccana*, MNHN-IE-2022-2081; G. *Freyastera tuberculata*, type NHMUK 1890.5.7.1076; H. *Freyella remex*, holotype NHMUK 1890.5.6.1075; L. *Astrocles actinodetus* CASIZ 144002. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

genera not represented in the tree, *Parabrisinga* and *Stegnobrisinga* Fisher 1916, also belong to this family.

Clade 5: family Freyellidae (revised). The revised family encompasses three genera, *Astrocles* (Fig. 5L), *Freyastera* (Fig. 5J) and *Freyella* (Fig. 5K).

3.3. Character transformations

Transformations of 15 characters were traced on the phylogenetic

reconstruction of the reduced dataset, containing 37 species from 15 genera. *Astrolirus panamensis* (Ludwig, 1905), whose DNA were not obtained, was tentatively put in the tree in the place of cf. *Astrolirus* to represent the genus. Many of the morphological characters mapped onto the phylogenetic tree were retrieved as homoplastic, including several key characters that were used to delineate families and genera (Sladen, 1889; Fisher, 1917, 1928; Downey, 1986; Clark and Downey, 1992). Namely, the abactinal armature of arm (Char. 14 and 15), the adjoining degree of the first pair of adambulacral plates (Char.7) and first pair of

inferomarginal plates (Char. 8), etc. On the other hand, characters that have been overlooked in recent taxonomic works were found to be synapomorphies of certain lineages, such as the position of the first pair of inferomarginal plates (Char. 5), the partial fusion between the first and second adambulacral plates (Char. 10). The assessment of character transformations allows to link molecular phylogeny with morphological taxonomy and produces reliable indices for morphological delineation of families and genera.

3.3.1. Papulae (Char. 3)

Papulae function as respiration organs in starfish, the reduction of which in deep-sea starfish were regarded as an adaptation to deep-sea environment (Mironov et al., 2016; Hayashi, 1943). Brisingida genera *Odinella*, *Brisingaster* and *Novodinia* have notably numerous and well-developed papulae on the abactinal disk and in arm genital areas, homologous to those in Forcipulatida starfish. Genera *Brisingenes* and *Midgardia* were described to have a circle of papulae on the disk margin (“reduced papulae” in Mah, 1998a), two at each radial area, which was believed to be an important diagnostic character to distinguish *Brisingenes* from *Brisinga*, and *Midgardia* from *Stegnobrisinga* (Fisher, 1917; Downey, 1973). Papulae were absent in the rest of Brisingida genera.

All specimens of *Odinella*, *Brisingaster* and *Novodinia* examined in the present study are equipped with numerous and well-developed papulae, consistent of prior studies. However, in 5 of 12 *Brisingenes* specimens examined, the “reduced papulae” are absent. In contrast, in specimens of other genera, sporadic “reduced papulae” were also found, such as in the type specimen of *Freyellaster scalaris* (USNM 39899, photos available on <https://collections.nmnh.si.edu/search/iz/>), *Freyellaster* (*Pseudobrisinga*) sp. 1 (RSIOAS083) and *Astrostephane moluccana* (MNHN-IE-2007-3287) (Fig. 6C). Therefore, the presence or absence of “reduced papulae” on disk margin could not be regarded as a constant character to distinguish genera or species. The numerous well-developed papulae, on the other hand, is a sound character to suggest phylogenetic and taxonomic relationships.

The reduction of the papulae is a synapomorphy of clades 4 and 5.

3.3.2. Oral plate (Char. 4)

The oral plate is situated on the margin of the actinostome (mouth), forming a part of the rigid oral ring in Brisingida, together with the first ambulacral plates. The general shape of the oral plate in Brisingida is irregular (Fau and Villier, 2020). Without dissecting the oral ring, only the spine-bearing part (termed as *body*, Fau and Villier, 2018, 2020) of the plate is visible from the actinal view. In *Odinella*, *Brisingaster* and *Novodinia*, the *body* of the oral plate is expanded fan-wise at its proximal end towards the furrow, almost touching its companion across the furrow (Fisher, 1919; Mah, 1998a, b) (Suppl. Fig. 4B). In other Brisingida genera, the *body* of the oral plate is trapezoid-shaped from the actinal view, with the distal end wider than the proximal end (Suppl. Fig. 4C).

The fan-shaped oral plate (from actinal view) is a synapomorphy of clades 1, 2 and 3.

3.3.3. First inferomarginal plate (Char. 5)

The external skeleton system is largely reduced in Brisingida, notably in the presence of only one series of marginal plates. This single series of marginal plate located from arm base to the terminal plate was usually interpreted as the “infero-” marginal plates (Sladen, 1889; Fisher, 1919, 1928), though its homology to inferomarginal plates in Forcipulatida is unclear. The position of the first inferomarginal plates to differentiate Brisingida genera was first brought to notice by Fisher (1917). Our analysis of character transformation reinforced the significance of this character in phylogeny. In genera *Freyella*, *Freyastera* and *Astrocles*, the first inferomarginal plates are not in contact with the first adambulacral plates and the odontophore, instead they occur on arms at some distance from the disk, thus the first and several proximal adambulacral plates are not accompanied by inferomarginal plates (Fig. 7D).

In all the other Brisingida genera, the first inferomarginal plates occur above the first adambulacral plates and in contact with the odontophore (Fig. 7C). The first two or more inferomarginal plates are continuously distributed above the first several adambulacral plates, then become isolated alongside the arm (Fig. 7B).

The first inferomarginal plates not in contact with the first adambulacral plates is a synapomorphy of clade 5 (Fig. 7A).

3.3.4. A partial fusion between first and second adambulacral plates (Char. 10)

Fisher (1917) proposed the term “syzygy or non-muscular symphysis” to describe a specific articulation between the proximal adambulacral and ambulacral plates in certain Brisingida genera. However, the term he used was not well-illustrated, thus the interpretation of the structure varied in later works and its importance was overlooked (Mah, 2016; Zhang et al., 2020). The examination of specimens, especially under SEM, allows for redefinition of this character. The general articulation between consecutive adambulacral plates of Brisingida was illustrated by Fau and Villier (2020), which consists of a large, central area of muscle or collagen fiber (*interadam*) and one articulation surface (*interada*). The articulation surface of the first and second adambulacral plates in certain genera, however, was specified with reduced muscular area and expanded imperforate stereom especially prominent at the actinal part. This specific kind of articulation is visible on the actinal side as a suture between the two plates, so that the two plates seem to be fused together (Fig. 7G, H). We herein term this specific articulation as a “partial fusion” between two plates, in replacement of Fisher’s “non-muscular symphysis” (muscle or collagen fiber still present in such an articulation) or a “syzygy” (a term used to describe a specific articulation in crinoids (Roux et al., 2002) different from the articulation observed in Brisingida). The function of a partial fusion between the first and second adambulacral plates is unclear yet, but it is noticed that species with a partial fusion between the 1st/2nd adambulacral tend to have their arm broken at this articulation very often (e.g., Brisingidae species), whereas species lacking the partial fusion do not show any preferred breakage point (e.g., *Freyella*, *Odinella*). Similar trend was also found in crinoids, as autotomy and regeneration frequently take place at syzygial articulation (Amézière et al., 2021).

A partial fusion between the first two adambulacrals is observed in all the specimens examined on clades 2 and 4, even at early stage of growth (e.g., *B. helenae* MNHN-IE-2022-2122, $r = 4$ mm), appearing to be a character shared by *Brisingaster* and Brisingidae (restricted) (Fig. 7F). Partial fusion is absent from specimens in clades 1, 3 and 5. However, *Freyastera mexicana* was described to have a partial fusion between the between the 1st/2nd adambulacral (Clark, 1939). In specimens examined in the present study, many *Freyastera* and *Novodinia* have their arms broken at 1st/2nd adambulacrals thus a partial fusion is unclear (Fig. 7E). Based on specimens with this character examinable, both genera have no specified articulation between the 1st/2nd adambulacrals.

3.3.5. Lateral comb of spines (Char. 11)

In Brisingida, the abactinal skeleton is generally reduced and almost completely absent beyond the genital region. However, in *Odinella*, *Brisingaster* and *Novodinia*, beyond the genital region, usually 2–4 dorsal plates remain alongside the arm, appearing at regular interval and in contact with the inferomarginal plate. Each of these plates bears one dorsal-lateral spine, similar in form with the lateral spine, thus appear as a transverse line of spines on each side of the arm from the genital area to the arm tip (Fig. 6E). This structure is referred to as “lateral series or comb of spines” (Perrier, 1885; Sladen, 1889; Fisher, 1928). The dorsal-lateral plates were considered as the extension of the costal plates, but in nature they are probably homologous to the superomarginal plates and abactinal plates in Forcipulatida. These dorsal-lateral plates and lateral comb of spines are absent in all the other Brisingida genera (Fig. 6F).

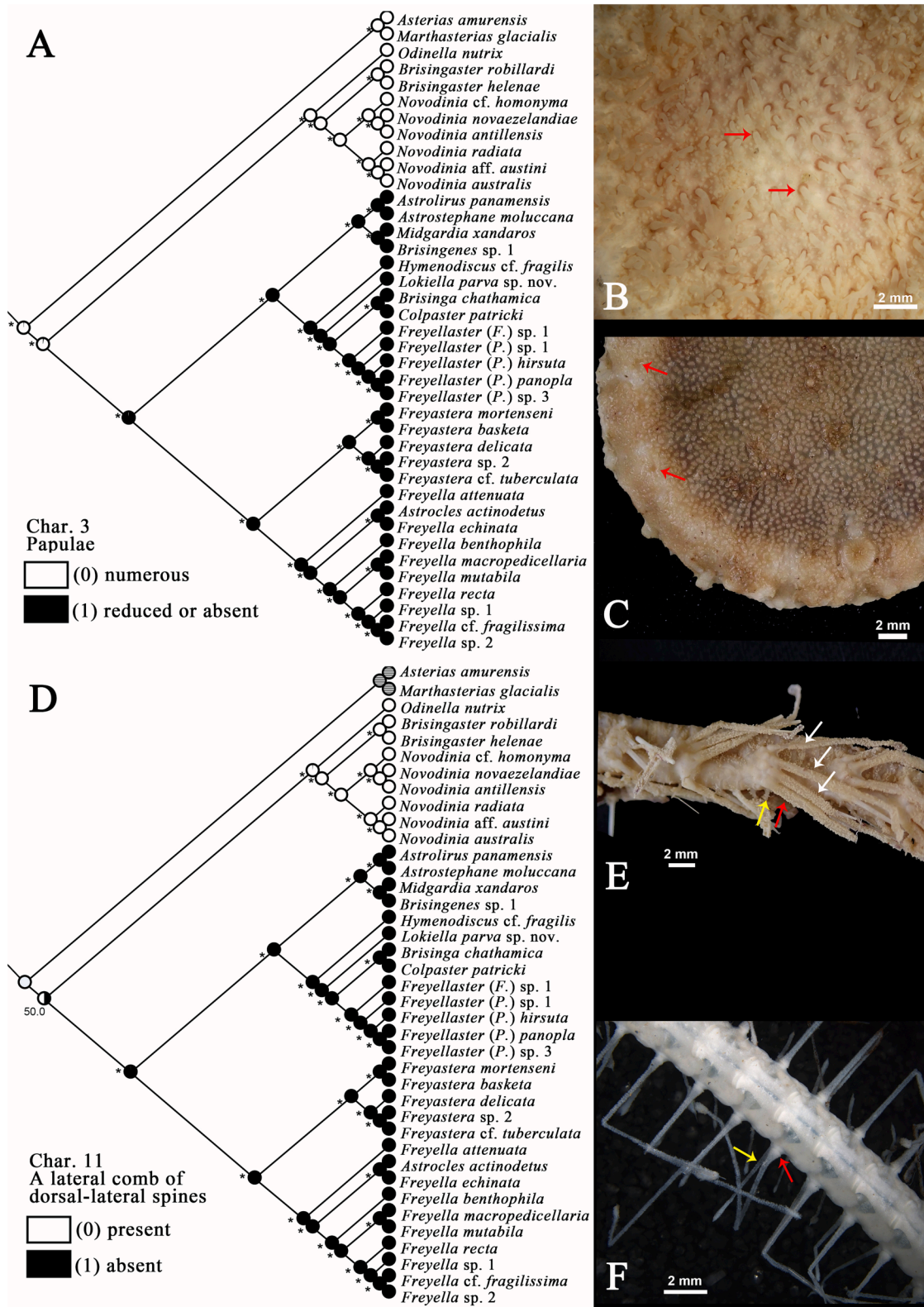
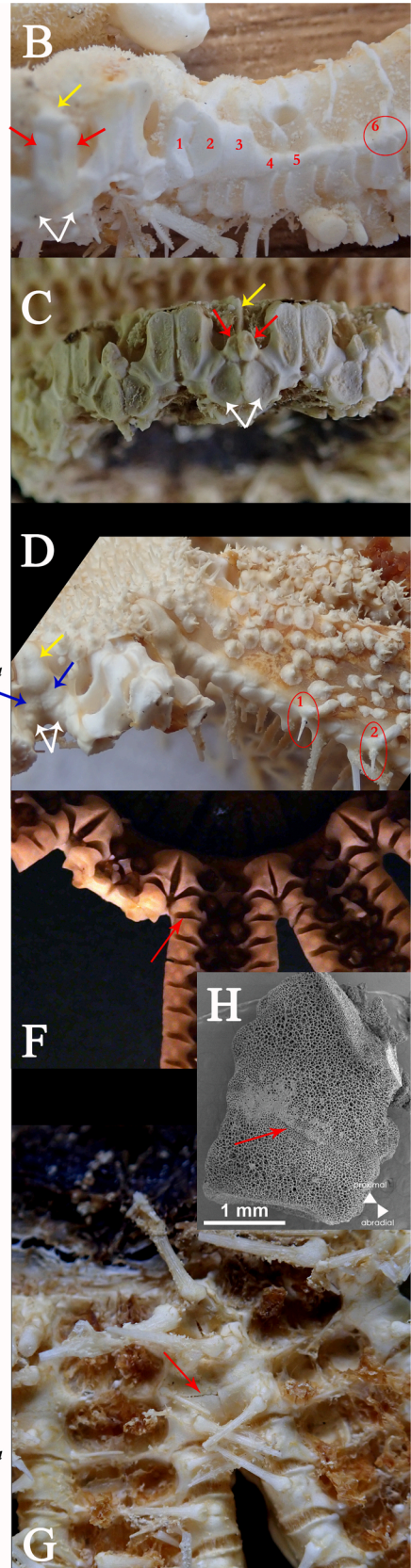
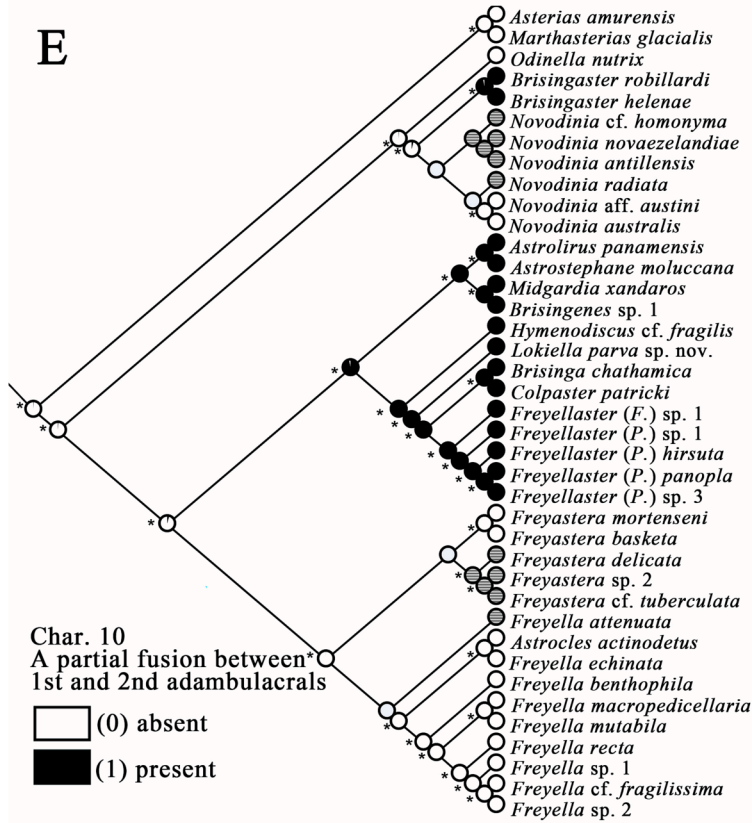
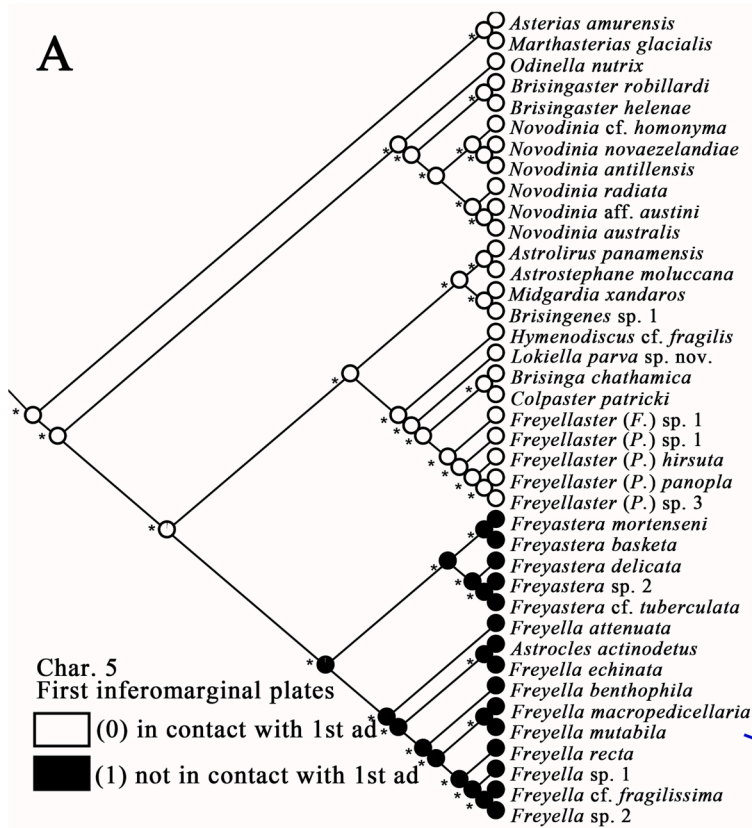


Fig. 6. Character transformations. A. Maximum likelihood transformations of character 3: papulae. Proportional likelihood scores of the most likely state at each node are provided. “***” indicates the most likely state has an estimated proportional likelihood of >95 %. B. *Novodinia antillensis* RSIOAST0093, numerous papulae on abactinal disk; C. *Astrostephane moluccana* MNHN-IE-2007-3287, a few indistinct papulae or pore on the margin of disk. D. Maximum likelihood transformations of character 11: a lateral comb of dorsal-lateral spines. E. *Novodinia radiata*, MNHN-IE-2022-2132, a lateral comb of spines present.; F. *Freyastera delicata*, RSIOAST022, no dorsal-lateral spine, only a single lateral spine present. Red arrow shows the lateral spine on inferomarginal plate. Yellow arrow shows the subambulacral spine on adambulacral plate. White arrows show a line of dorsal-lateral spines on dorsal-lateral plates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



(caption on next page)

Fig. 7. Character transformations. A. Maximum likelihood transformations of character 5: position of the first inferomarginal plates. Proportional likelihood scores of the most likely state at each node are provided. “*” indicates the most likely state has an estimated proportional likelihood of >95 %. B. *Brisinga* aff. *eucoryne* MNHN-IE-2022-2162, the first inferomarginal plates in contact with the first adambulacral plates and the odontophore; C. *Brisingenes* sp. 1, NIWA 33311, the first inferomarginal plates in contact with the first adambulacral plates and the odontophore; D. *Freyella* sp. 5, MNHN-IE-2019-5464, the first inferomarginal plates not in contact with the first adambulacral plates, occurring at about 7th adambulacral plate. The Arabic numerals indicate the 1st–6th inferomarginal plates. Yellow arrows show the odontophore; red arrows show the first inferomarginal plates; white arrows show the first adambulacral plates; blue arrows show the distal part of the oral plates. E. Maximum likelihood transformations of character 10: a partial fusion between the first and second adambulacral plates. Terminal circles with grey lines indicate the character is not applicable or not available. F. *Astrocles actinodetus*, CASIZ 144002, a partial fusion absent, instead an articulation not differentiated from the followings present between the first two adambulacral plates; G. *Brisingenes* sp. 1, NIWA 33311, a partial fusion (a suture from actinal view) present between the first two adambulacral plates; H. *Astrolirus panamensis* CASIZ 122095, a SEM photo of the first two adambulacral plates with a partial fusion (a suture shown by the red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The presence of lateral comb of spines is a synapomorphy of clades 1, 2 and 3 (Fig. 6D).

3.3.6. Abactinal arm armature (Char. 14 & 15)

Several brisingid genera developed a kind of specified, elongate and robust plate called “costae plates”, which overlap with each other and form several transverse lines called “costae” or “transverse ridges or ribs” roofing the genital area (Sladen, 1889; Fisher, 1906) (Fig. 8B, E–F). In contrast, the genital area in the other genera is armed by pavement of scale-like, thin plates, with no specified costal plates developed (Fig. 8C). The presence/absence of costae was believed to be an important diagnostic character to differentiate Brisingidae and Freyellidae (Downey, 1986). However, when mapped on the phylogenetic tree, this character is shown to be highly homoplastic. Loss or acquisition of costae occurs several times within the clade 4 (Brisingidae; Fig. 8A), thus making this character of very little phylogenetic significance.

In taxa with costae, the skeleton arrangement of the intercostal area varies greatly among or even within genera. *Novodinia*, *Midgardia*, *Astrolirus* and *Stegnobrisinga* are characterized by having a pavement of thin, flat, irregular plates between costae, forming a relatively crowded abactinal armature. In *Novodinia*, these intercostal plates are generally equipped with spinelets (Fig. 8F), whereas in *Midgardia*, *Astrolirus* and *Stegnobrisinga*, the plates are spinless and very delicate (Fig. 8E). The homology of these intercostal plates is pending further investigation. In genus *Brisinga* and the new subgenus *Freyellaster* (*Pseudobrisinga*), the intercostal area is equipped with a few of many isolated discoid plates, each bear 1 short spinelet (Fig. 8G, H). These plates are quite indiscernible in wet specimens thus could have been neglected in previous description of *Brisinga* species. In *Brisingenes*, *Hymenodiscus* and *Astrotephane*, the intercostal integument is devoid of plates, except for occasional occurrence of incomplete secondary costae (Fig. 8B). In all, the different states of abactinal armature were found to not be of any phylogenetic significance (Fig. 8D).

3.3.7. Other characters

Several other characters examined showed rather complicated transformations and appeared to be convergent across phylogenetically distant group. Some of them (Characters 2, 6, 9, 12, 13) do not appear to bear phylogenetic signals, but could serve as good taxonomic indicators, as they were found to be consistent within genus and could be used to differentiate close genera (Suppl. Figs. 3, 5 and 8–10). The arrangement of the first pair of adambulacral plates (Char. 7) and first pair of inferomarginal plates (Char. 8) which used to be regard as key characters to delimitate certain genera are found to be homoplastic and largely related to heterochrony (Suppl. Figs. 6 and 7) (see Section 4.4). The number of arms (Char. 1) is highly variable within family and genera (Suppl. Fig. 2) and is not a reliable character to be used for identification, nor of phylogenetic purposes. However, a reduction of arm number might be related to depth or other ecological restraints (see Section 4.4).

3.4. Systematic accounts

Based on the molecular phylogeny and evaluation of morphological

characters, we propose a novel classification of the order, which consists of 5 families and 17 genera. Major taxonomic revisions include 1) the resurrection of families Odiniellidae, Brisingasteridae and Novodiniidae (*sensu* Clark and Mah, 2001) to encompass the genera *Odinella*, *Brisingaster* and *Novodinia*, 2) revision and redefinition of families Brisingidae and Freyellidae, which consequently include 11 and 3 genera, respectively, 3) a new genus and species, *Lokiella parva* gen. & sp. nov., two new subgenera *Freyellaster* (*Freyellaster*) subgen. nov., *Freyellaster* (*Pseudobrisinga*) subgen. nov. and seven new species combinations. The readers may refer to Fig. 4 and the taxonomic key for the complete classification scheme proposed in this study.

Order Brisingida Fisher, 1928

Family Odiniellidae Mah, 1998b

Included genus: *Odinella* Fisher, 1940

Diagnosis. Arms 12–16. The first pair of inferomarginal plates above the first pair of adambulacral plates, in contact with the odontophore. Articulation between the first and second adambulacral plates not differentiated from that of the later adambulacrals. Papulae present on both disk and proximal part of arms. One pair of gonads on each arm. Genital area with specialized brooding chambers composed of a dense reticulation with numerous irregularly placed abactinal spines, which dorsolaterally and laterally interlock with those of adjacent rays and sometimes form permanent bridges. Inferomarginal plates occur on every 3–4 adambulacral plates. One lateral spine and several dorsal-lateral spines form a comb on each side of the arm. Abactinal spines and lateral spines sheathed in ovoid sacculi. Proximal subambulacral spines with sharp end. Oral plate fan-shaped from oral view, with expanded proximal wing. Ambulacral ossicles wing shaped (not vertebrae-shaped).

Family Brisingasteridae Mah, 1998b

Included genus: *Brisingaster* de Loriol, 1883

Diagnosis. Arms 10–12. The first pair of inferomarginal plates above the first pair of adambulacral plates, in contact with the odontophore. A partial fusion between the first and second adambulacral plates, appearing externally as a suture between the two plates. Papulae present on both disk and proximal part of arms. Abactinal disk skeleton an open reticulum. Costae well-developed on arms, sometimes interconnected. Intercostal area skin bare. Gonads numerous, arranged in series on each arm. Inferomarginal plates occur on every 3–4 adambulacral plates. One lateral spine and several dorsal-lateral spines form a comb on each side of the arm. Proximal subambulacral spines with modified end, flattened or capitate. Oral plate fan-shaped from oral view, with expanded proximal wing almost closing the adambulacral furrow.

Family Novodiniidae Mah, 1998b

Included genus: *Novodinia* Dartnall et al., 1969

Diagnosis. Arms 11–19. The first pair of inferomarginal plates above the first pair of adambulacral plates, in contact with the odontophore. Articulation between the first and second adambulacral plates not

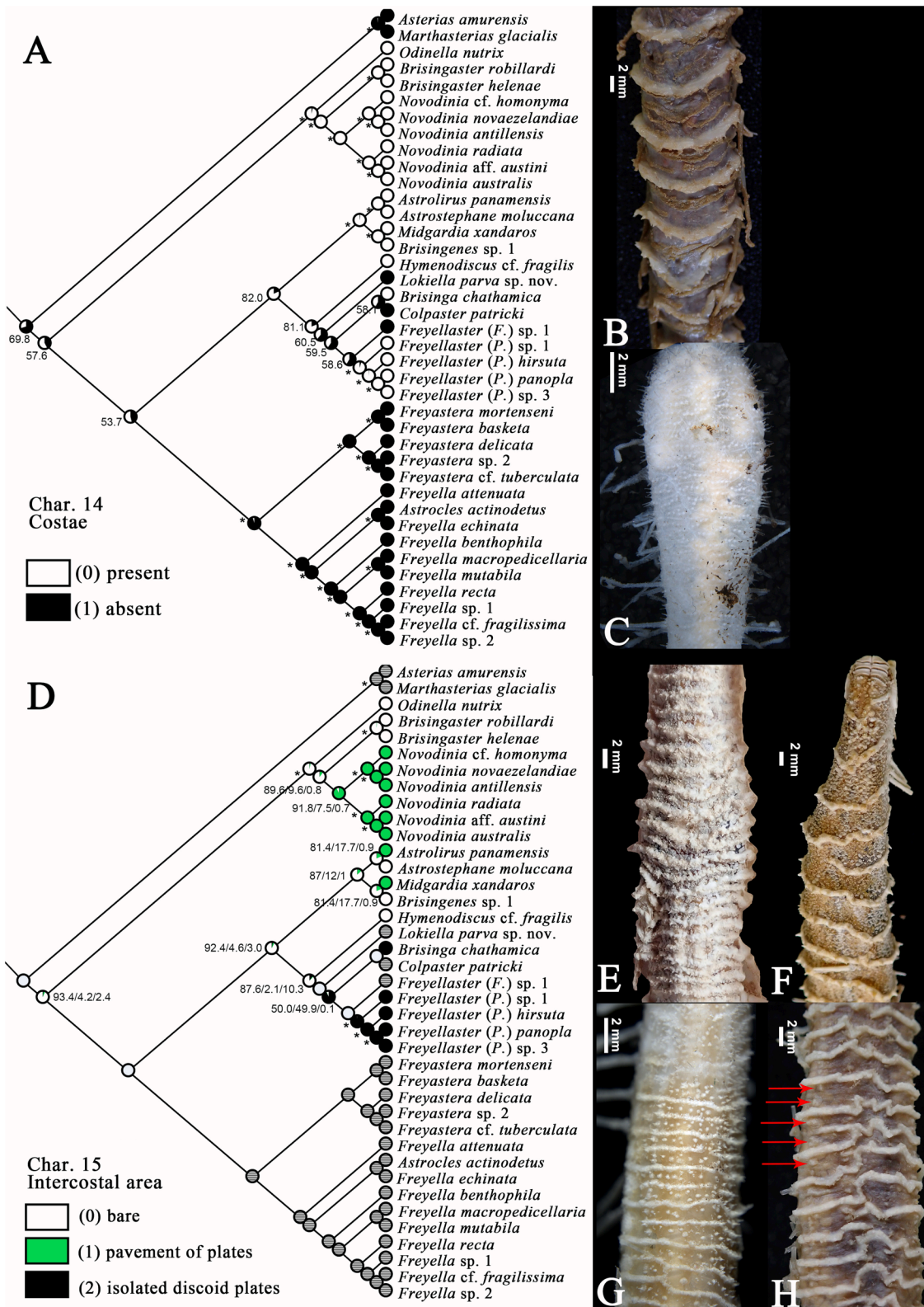


Fig. 8. Character transformations. A. Maximum likelihood transformations of character 14: costae in abactinal arm genital area. Proportional likelihood scores of the most likely state at each node are provided. “*” indicates the most likely state has an estimated proportional likelihood of >95 %; B. *Astrostephane moluccana*, MNHN-IE-2022-2085, costae present, with bare intercostal area; C. *Freyastera delicata*, RSIOAST022, costae absent. A dense pavement of scale like plates in the abactinal genital area; D. Maximum likelihood transformations of character 15: intercostal area armature. Proportional likelihood scores at each node are provided in order of most likely state to the least likely state, separated by “/”. Terminal circles with grey lines indicate the character is not applicable or not available. E. *Midgardia xandaros*, MNHN-IE-2022-2093, intercostal area with pavement of spineless plates; F. *Novodinia radiata*, MNHN-IE-2031-4344, intercostal area with pavement of spineless plates; G. *Freyellaster (P.)* sp. 1, RSIOAST0084, intercostal area with isolated discoid plates; H. *Freyellaster (P.) panopla*, MNHN-IE-2017-17244, intercostal area with isolated discoid plates. Red arrows show a line of discoid plates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

differentiated from that of the later adambulacrals. Papulae present on both disk and proximal part of arms, usually solitary. Abactinal disk and arm plates fenestrate. A pair of gonads on each arm. Abactinal arm in genital area covered with a few costae, in some species inconspicuous. Intercostal area covered with pavement of irregularly shaped plates, leaving pores between plates for the papulae. Inferomarginal plates occur on every 3–4 adambulacrals plates. One lateral spine and several dorsal-lateral spines form a comb on each side of the arm. Proximal subambulacrals spines with modified end, flattened or capitate. Oral plate fan-shaped from oral view, with expanded proximal wing almost closing the adambulacrals furrow.

Family Brisingidae Sars, 1875.

Included genera: *Astrolirus* Fisher, 1917, *Astrostephane* Fisher, 1917, *Brisinga* Asbjørnsen, 1856, *Brisingenes* Fisher, 1917, *Colpaster* Sladen, 1889, *Freyellaster* Fisher, 1918, *Hymenodiscus* Perrier, 1884, *Midgardia* Downey, 1973, *Lokiella* gen. nov., *Parabrisinga* Hayashi, 1943, *Stegno-brisinga* Fisher, 1916.

Diagnosis. Arms 7–17. The first pair of inferomarginal plates above or insert in the first pair of adambulacrals plates, in contact with the odontophore. A partial fusion between the first and second adambulacrals plates, appearing externally as a suture between the two plates. Papulae absent or restrict to disk margin, one reduced pair on each arm base. One pair of gonads or numerous gonads in series on each arm. Abactinal arm armature in genital region varies, either with costae or pavement of plates. Inferomarginal plates to every or alternate adambulacrals plates. No lateral comb of spines alongside the arm. Oral plate trapezoid shaped from oral view.

Family Freyellidae Downey, 1986

Included genera: *Astrocles* Fisher, 1917, *Freyastera* Downey, 1986, *Freyella* Perrier, 1885.

Diagnosis Arms 6–14. The first pair of inferomarginal plates appear at a certain distance from the disk, not in contact with the odontophore. Articulation between the first and second adambulacrals plates not differentiated from that of the later adambulacrals. Papulae absent. One pair of gonads on each arm. Abactinal arm in genital region covered with pavement of spinate plates, sometimes leaving space or bands in between. Inferomarginal plates to every or alternate adambulacrals plates. No lateral comb of spines alongside the arm. Oral plate trapezoid shaped from oral view.

Lokiella gen. nov. Zhang & Mah

urn:lsid:zoobank.org:act:8B280AF5-2790-4136-88FB-6B4AA369EF6C

Type species: *Lokiella parva* sp. nov. Zhang & Mah

Lokiella parva sp. nov. Zhang & Mah

urn:lsid:zoobank.org:act:7225B945-5CC8-48AF-8BE2-42868DD3FCA2

Diagnosis. Arms 12. Body size small, holotype of typical size, R = 30 mm. Abactinal disk surface densely covered with short spinelets. Genital region of abactinal arm surface with pavement of plates, each with 3–5 sharp, conical spinelets, about 0.3 mm in length. Plates and spinelets on arms aligned transversely, two series corresponding to each adambulacrals plates. Pedicellariae rarely present on the abactinal disk and arm in the genital area. First pair of inferomarginal plates above the first pair of adambulacrals plates. The first pair of adambulacrals plates are adjoining laterally but slightly apart in the distal portion. The first pair of inferomarginal plates small, united only at adoral end. Inferomarginal plates on arm occur at every 2nd adambulacrals plate. Adambulacrals plate with a single elongate furrow spine and one subambulacrals spine. Oral plate with two actinostomal spines, one suboral spine and one aboral furrow spine. Occasionally one additional furrow spine on the

middle or adoral part of the furrow on the oral plate. All adambulacrals and oral spines acicular and bear small pedicellariae.

Etymology. The genus is named for Loki, the god of mischief in Norwegian mythology, who stole Freya's necklace "brisingamen" and hid it in the deep ocean. "parva", from Latin *parvus*, meaning "small". Named by Christopher Mah.

Specimens examined. Holotype, NMV F159236, r = 3 mm, R = 30 mm, Hd (height of disk) = 1 mm, Wb (width of arm base) = 1.5 mm, Lg (length of genital area) = 5 mm, Wg (width of the widest part of genital area) = 2.5 mm. Paratype, NMV F159778, r = 3.5 mm, R = 38 mm, Hd = 2 mm, Wb = 1.5 mm, Lg = 7 mm, Wg = 3 mm. Type specimens are deposited at Museums Victoria, Australia.

Description. Arms 12. Abactinal disk completely covered with short spinelets, approximately 0.3–0.4 mm in length. Spinelets probably 3–5 to a plate. Spinelets with multifid tips (Fig. 9A, D). Madrepore near the margin of the disk, rather large, but concealed by the dense spinelets.

The genital area is about 1/5–1/6 of the total arm length, slightly inflated (Fig. 9A). A pair of gonads on each arm. Abactinal arm in the genital area are covered by pavement of small plates, bearing 3–5 spinelets, about 0.3 mm in length, similar with those on disk. The plates and spinelets are generally well aligned transversely, two lines of plates to each adambulacrals plate (Fig. 9A, E). The plates extend a bit beyond the genital area. Further away the arm is only covered by scattered pedicellariae, not very numerous and more or less in transverse bands.

The first pair of adjacent adambulacrals plates slightly apart distally, with the first inferomarginal plates above them. The first inferomarginal plates small, only united at their adoral end (Fig. 9D). The second inferomarginal plate above the second adambulacrals plate, interradially not in contact with the second inferomarginal plate of the adjacent arm. The third inferomarginal plate occurs at the 5th adambulacrals plate, bearing an inferomarginal spine. The followings occur at every 2nd adambulacrals plate. The first inferomarginal spine about 0.7 mm, the one in the genital region about 1.5 mm in length. Further distally they could be up to 3 mm in length. All the inferomarginal spines sheathed and bear numerous small pedicellariae.

The first adambulacrals plate with two subambulacrals spines and one aboral furrow spine in an oblique line (Fig. 9B, G). The subambulacrals spines about 1 mm and the furrow spine about 0.7 mm in length. The following adambulacrals plate with one adoral furrow spine and one subambulacrals spine. The furrow spine sits just under the subambulacrals spine or slightly adoral to it (Fig. 9G). They are short proximally, but soon becomes long and directing a bit upward, about 0.5–1 mm in the genital region. The subambulacrals spine in the genital region about 1.5 mm. All ambulacrals spines acicular and bear small pedicellariae.

Oral plate with 4–5 spines. Two actinostomal spines, one on the mouth margin towards the actinostome, the other on the adoral furrow margin towards the furrow. One aboral furrow spine and one suboral spine (Fig. 9B, F). The length of the furrow and actinostomal spines about 0.5 mm. The suboral spine up to 1 mm, more robust than the furrow spines. The paratype has an additional furrow spine at the middle of the oral plate. All the oral spines bear a few small pedicellariae.

Crustacean shell was found in the mouth of the paratype.

Distribution. South of Tasmania, southern Pacific. 900–1350 m.

Remarks. This species is characteristic by its small size (R = 30–38 mm), which could be easily interpreted as a juvenile. But fully developed gonads indicate that it is indeed an adult. The presence of the first pair of inferomarginal plates above the first pair of adambulacrals plates confirms its belonging to Brisingidae. It differs from the rest of Brisingidae greatly both in morphology and genetics, forming an independent clade on the phylogenetic tree (Fig. 4, clade 4d). Therefore, it is justified as a new species and genus.

The finding of *Lokiella parva* sp. nov. provided important evidence in understanding the evolution of the abactinal arm armature in Brisingida. This species, together with *Freyellaster* and *Colpaster*, shows the same

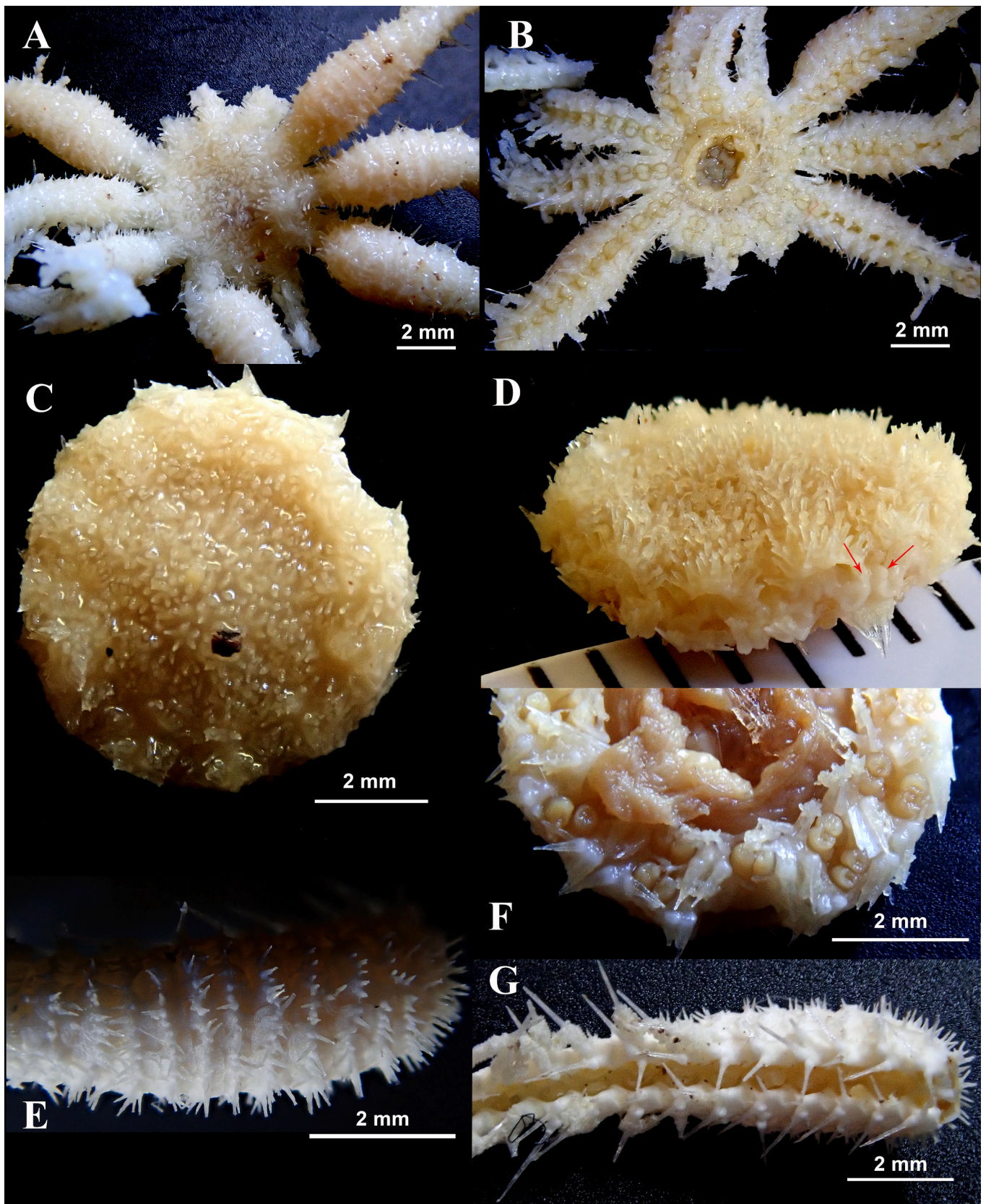


Fig. 9. *Lokiella parva* sp. nov. A, B, E, G. holotype NMV F159236; C, D, F. paratype NMV F159778. A. abactinal view; B. actinal view; C. abactinal view of the disk; D. lateral view of the disk, red arrows show the first inferomarginal plates; E. arm plates; F. mouth plates and spines G. adambulacral spines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

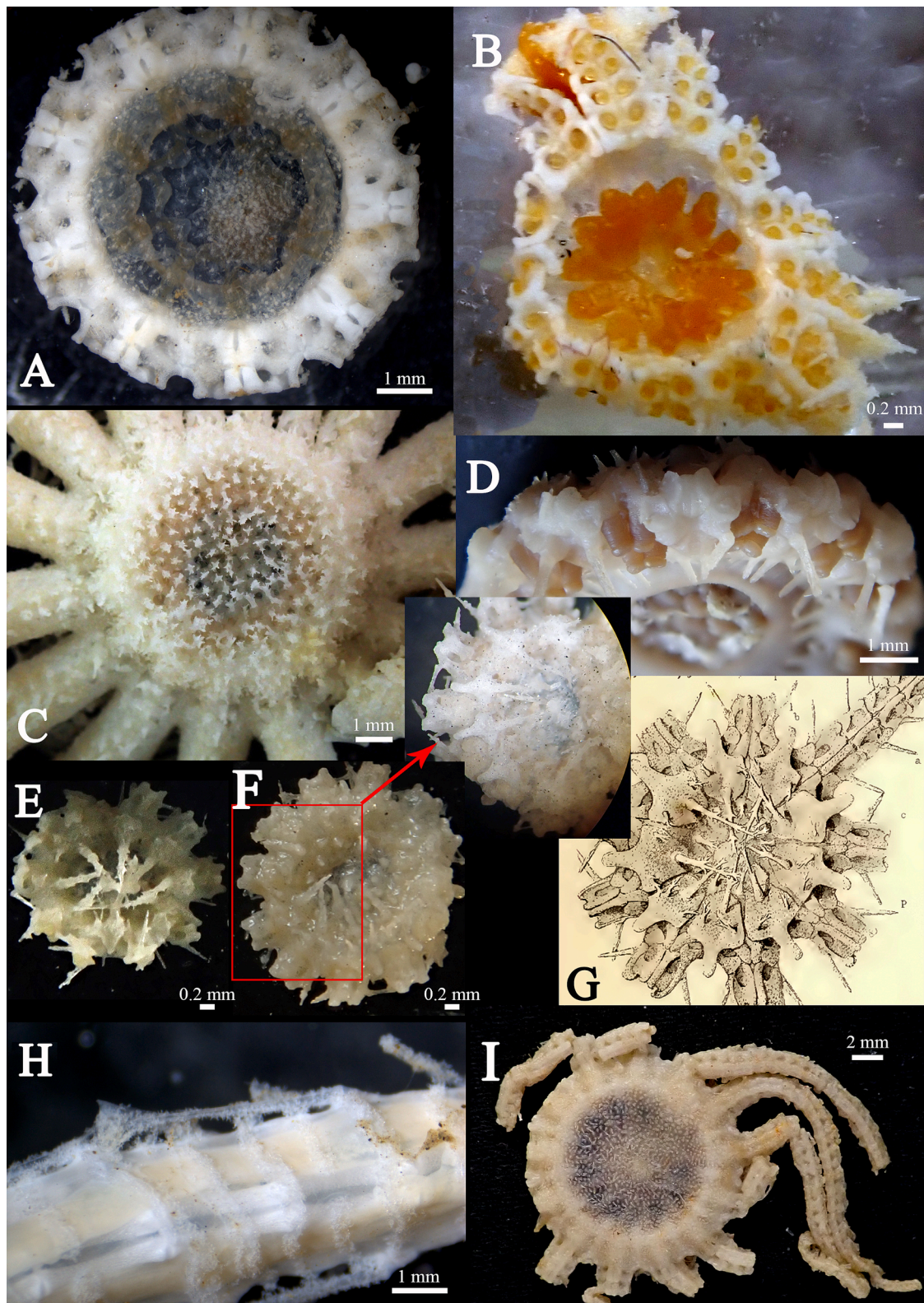


Fig. 10. Juvenile or young specimens examined. A. *Colpaster* sp. 2 MBM287607, $r = 3$ mm, abactinal view of disk; B. *Odinea nutrix* MNHN-IE-2009-7198, $r = 1.1$ mm, a specimen from brooding chamber, actinal view; C. *Novodinia novaezelandiae* NIWA 46635, $r = 4$ mm, abactinal view; D. *Brisingaster helenae* MNHN-IE-2022-2113, $r = 4$ mm, lateral view of disk; E. Brisingidae or Freyellidae, NOC 9754#3, $r = 0.9$ mm, abactinal view; F. Brisingidae or Freyellidae, NOC 9779#1, $r = 1.5$ mm, abactinal view, with inset shows zoom in view of disk plates; G. *Hymenodiscus coronata*, a juvenile specimen drawn by [Perrier \(1894, Pl. Fig. 1\)](#), $r = 1.25$ mm; H. *Colpaster* sp. 2 MBM287607, $r = 3$ mm, abactinal view of proximal part of arm, pedicellariae bands start to form; I. *Brisingenes* sp. 1 MNHN-IE-2007-1309, $r = 7$ mm, abactinal view.

pattern of having pavement of abactinal arm plates resembling Freyellidae, but phylogenetically affinitive to Brisingidae. The fact that these three genera are distantly related indicates that the arm armature might be a convergent character owing to environmental constraints or other unknown causes. The juvenile form of *Lokiella parva* sp. nov. also demonstrated a trend of paedomorphosis also found in other Brisingida genera. Remarks of its paedomorphic characters and phylogenetic implication see Section 4.4.

Freyellaster (Freyellaster) subgen. nov. Zhang

urn:lsid:zoobank.org:act:2D65A280-E4CE-4ADE-B74E-4258DFE35614

Type species: *Freyellaster (Freyellaster) fecundus* Fisher, 1905.

Other species: *Freyellaster (Freyellaster) intermedius* Hayashi, 1943, *Freyellaster (Freyellaster) scalaris* (Clark, 1916), *Freyellaster (Freyellaster) spatulifer* (Fisher, 1916), *Freyellaster (Freyellaster) polycnema* (Sladen, 1889).

Freyellaster (Pseudobrisinga) subgen. nov. Zhang

urn:lsid:zoobank.org:act:9303EB7B-5232-4AA1-B261-6D329038A259

Type species: *Freyellaster (Pseudobrisinga) panopla* (Fisher, 1906) **comb. nov.**,

Other species: *Freyellaster (Pseudobrisinga) hirsuta* (Perrier, 1894) **comb. nov.**, *Freyellaster (Pseudobrisinga) parallela* (Koehler, 1909a) **comb. nov.**, *Freyellaster (Pseudobrisinga) variispina* (Ludwig, 1905) **comb. nov.**

Diagnosis. Arms 8–17. Gonads serial. Papulae absent or a pair of reduced papulae near disk margin at each arm base. The first inferomarginal plates above the first adambulacral plates, all four plates in close contact in fully grown specimens. Inferomarginal plates generally correspond to every adambulacral plates (except in *Freyellaster fecundus* and *Freyellaster polycnema*). Proximal subambulacral spines with truncate end. Suboral spine straight. Large pedicellariae present. *Freyellaster (Freyellaster)* subgen. nov. without costae, a pavement of abactinal plates in arm genital area, bearing spinelets generally well aligned. *Freyellaster (Pseudobrisinga)* subgen. nov. with densely distributed costae, 1–2 to each adambulacral plate, with small discoid plates scattered in the intercostal area, each bear one sharp spinelet.

Remarks. See Section 4.3.

Brisingaster helenae (Rowe, 1989) comb. nov.

Novodinia helenae: Rowe, 1989: 274; Mah in Clark and Mah, 2001: 310.

Brisingaster robillardii pars.: Mah, 1999: 535; Mah in Clark and Mah, 2001: 310; McKnight, 2006: 77; Fau and Villier, 2020; Mah, 2022: 9.

Remarks. This species represents the Pacific individuals of *Brisingaster* based on our molecular analysis. *B. helenae* was first reported as *Novodinia helenae* (Rowe, 1989), but Mah (1999) revealed its morphological affinity with *B. robillardii*. Owing to the polymorphism of *B. robillardii* and a lack of further species delimitation evidence, Mah synonymized the species to *B. robillardii*. In this study, we found the Pacific individuals vary genetically from the Indian Ocean individuals (COI distance > 11 %), thus should be considered as two species. Hence, we restore the species *Novodinia helenae* and replace it in *Brisingaster*, representing the second known *Brisingaster* species. It is to be noted that sound morphological differences were not yet identified between the two species owing to the high degree of polymorphism even within species. The identification of the two species will need to be aided by molecular and distribution information.

Colpaster patricki (Zhang et al., 2020) comb. nov.

Astrolirus patricki: Zhang et al., 2020.

Remarks. This species was first published as *Astrolirus patricki* (Zhang et al., 2020). Upon examination of the type specimen of a rare species *Colpaster scutigerula*, a series of characters were found to link the two species together. These include the first pair of inferomarginal plates inserting in the first pair of adambulacral plates, numerous robust oral spines, a pavement of abactinal arm plates, and inferomarginal spines correspond to every adambulacral plates (Fig. 5F), etc. After comparing both species with *Astrolirus panamensis* (CASIZ 122095), we revised *Astrolirus patricki* to *Colpaster patricki*. It represents the second known species of *Colpaster*, as *Colpaster edwardsi* is found to be mistakenly assigned to the genus (see below).

Freyella edwardsi Perrier, 1882.

Freyella edwardsi: Perrier 1885: 8; 1894: 82; Koehler 1907b: 6; 1909b: 124; Mortensen, 1927: 128; Galkin and Korovchinsky, 1984: 165; Korovchinsky and Galkin, 1984: 1214.

Brisinga edwardsi: Perrier 1882: 61.

Colpaster edwardsi: Downey, 1986: 33; Clark and Downey, 1992: 478; Mah in Clark and Mah, 2001: 318.

Remarks. *Freyella edwardsi* was reported by Perrier in 1882 based on only fragments of arms. Downey (1986) synonymized this species as *Colpaster edwardsi* but probably merely based on an illustration by Koehler (1909b, Pl. 23). In the present study, the type specimen (MNHN-IE-2014–996) and a specimen described by Koehler (MOM 81 0863) of the species were examined. Both specimens fit with the character of *Freyella*, and differ greatly from *Colpaster scutigerula*, especially in lacking the first inferomarginal plates above the first adambulacral plates. Therefore, we restore *C. edwardsi* to *Freyella edwardsi*. The genus *Colpaster* therefore only consists of the type species *Colpaster scutigerula* and *Colpaster patricki* **comb. nov.**

Freyella benthophila Sladen, 1889 comb. nov.

Belgicella racowitzana: Ludwig, 1903: 59; Koehler 1907a: 141; Koehler, 1908: 245; Döderlein, 1928: 293; Fisher, 1928: 6; Fisher, 1940: 75; A.M. Clark, 1962: 68; Jangoux and Massin, 1986: 91; Mah, 1998b: 77; Mah in Clark and Mah, 2001: 317; Moreau et al., 2015: 5; Moreau et al., 2018: 147.

Freyastera benthophila: Downey, 1986: 36; McKnight, 1993: 173; Mah, 1998b: 78; Mah in Clark and Mah, 2001: 318; Dilman, 2014: 38; Moreau et al., 2015: 16; Moreau et al., 2018: 147; Zhang et al., 2019: 7.

Freyella benthophila: Sladen, 1889: 641; Wood-Mason and Alcock 1891: 440; Alcock 1893: 121; Fisher, 1928: 24; Madsen, 1951: 84; Cherbonnier and Sibuet, 1972: 1356; Sibuet, 1975: 292; Galkin and Korovchinsky, 1984: 165; Korovchinsky and Galkin, 1984: 1215.

Freyellidea benthophila: Fisher, 1917: 429

Remarks. *Freyastera benthophila* was classified in genus *Freyastera* based on the presence of six arms (Downey, 1986). However, molecular phylogeny revealed its affinity with *Freyella* (Fig. 4). Character transformation analyses suggest that the larger intervals between inferomarginal plates might be a shared synapomorphy of *Freyella* species and *F. benthophila*. Based on the current phylogenetic results, we move this species to *Freyella*. The boundary between *Freyella* and *Freyastera* will need to be reassessed through a thorough review of morphological characters of both genera.

Furthermore, the sole species in *Belgicella*, *B. racowitzana*, is regarded herein as a junior synonym of *F. benthophila*, after examining the type specimen of *F. benthophila* and photos of type specimen of *B. racowitzana* (see <https://virtualcollections.naturalsciences.be/>, AST.962). *B. racowitzana* is a subantarctic species characterized in having enlarged “primary central plate and interradial plates” which was believed to be unique for this species. However, we found such a character also present in the type specimen of *F. benthophila* as well as several newly examined specimens (Suppl. Fig. 2D). Other characters of *B. racowitzana* also agree

with *F. benthophila*, including the absence of furrow spines, the number of abactinal spines, and the absence of large pedicellariae, etc. Therefore, we synonymize *B. racowitzana* to *F. benthophila* and *Belgicella* to *Freyella*.

4. Discussion

4.1. Families Odiniellidae, Brisingasteridae and Novodiniidae

The three families, Odiniellidae, Brisingasteridae and Novodiniidae, were first proposed by Mah (1998b) to include the three “basal” genera, *Odinella*, *Brisingaster* and *Novodinia*. Later molecular analyses (Mah and Foltz, 2011; Zhang et al., 2019; 2020) and the phylogenetic tree (Fig. 4) also supported these three genera as separate clades from the rest of Brisingida. Several characters from Mah’s matrix were mapped on our new phylogenetic hypothesis and tested in the present study, including the presence of numerous papulae (Char. 3), fan-shaped oral plates (from oral view) (Char. 4), lateral comb of spines (Char. 11), which were found to be synapomorphies for the three genera (Fig. 6, Suppl. Fig. 4). Therefore, the separation of the three genera from Brisingidae is well supported. It is already noticed that the morphological characters of the three genera appear to be more plesiomorphic, i.e., similar to the sister group Forcipulatida, compared with more “derived” Brisingidae (restricted) and Freyellidae (Hayashi, 1943; Mah, 1998b). In particular, the three genera all have numerous papulae, more developed abactinal skeleton and thicker integument and ampullae, which are comparable to Forcipulatida species. Given that the phylogenetic position of Brisingida within Forcipulatida is still unclear yet, with molecular and morphological based phylogenetic hypothesis yielding different results (e.g., Mah and Foltz, 2011; Fau and Villier, 2020), the homology of characters between the three “basal” families with other Forcipulatida families might hold a key to understand the evolution of the order and its phylogenetic relationship with the other Forcipulatida families.

Although the three genera have many shared characters, they also differ with each other greatly. *Odinella* is the most distinctive genus in its reproduction strategy (brooding), its habitat (high-latitude), as well as many unique morphological characters, such as the ovoid sheath covering abactinal and lateral spines, wing-shaped ambulacral plates (Mah, 1998a), an irregular reticulation of abactinal arm skeleton (Fig. 5A). *Brisingaster* and *Novodinia* were more related in morphology, leading to Mah (1999) putting them under one family, the Brisingasteridae. But they also differ in abactinal arm and disk armature, gonads arrangement and the articulation between the first two adambulacral plates. *COI* distances between the three genera were generally larger than the interfamilial differences between Brisingidae (restricted) and Freyellidae (restricted) as well as intergeneric differences within the two families (Suppl. Fig. 11). Given that the classification at higher level, such as family, is usually empirical and incomparable across different taxa (Avisé and Liu, 2011), based on our current knowledge on Brisingida morphology and phylogeny, we choose to put the three genera in three separate families following Mah (1998b) and Clark and Mah (2001), until future evidence suggest otherwise.

The three families currently each composed of one known genera, and *Odinella* and *Brisingaster* are monospecific, but their actual diversity is likely underestimated. Molecular data in the present study revealed that the Pacific *Brisingaster* populations varied significantly from the Indian Ocean *Brisingaster* populations (*COI* distances > 11 %), revealing a diversity not perceived from morphological evidence. To acknowledge the genetic difference between the two populations and to underline such a discrepancy between the molecular-based diversity and morphology-based diversity, we resurrect *Novodinia helenae* and revise it to *Brisingaster helenae* comb. nov. Furthermore, a high genetic diversity among *Odinella nutrix* populations was also noticed. Sixteen individuals collected around the Antarctic from the Antarctic Peninsula, Shag Rocks and Adélie Land clustered into three subclades (Fig. 4 and Supplementary Figs. 1–3), and *COI* distance between individuals is up to 4.39 %,

which could suggest the existence of cryptic species. The true diversity of this peculiar species needs to be further studied using specimens and data collected from more sites around the Antarctic.

4.2. Families Brisingidae and Freyellidae

The Brisingidae and the Freyellidae are shown to be polyphyletic in the present phylogeny (Fig. 4). In addition to *Odinella*, *Brisingaster*, and *Novodinia* being removed to their new, respective families, *Freyellaster* and *Colpaster*, historically interpreted as Freyellidae, were found to be part of the clade formed by the remaining Brisingidae genera. Downey (1986) in her review of Atlantic Brisingida established family Freyellidae to accommodate six genera previously belonging to Brisingidae. The two families were mainly differentiated by the presence/absence of abactinal costation in the genital area. However, since there are no Atlantic *Freyellaster* species, none were examined in Downey’s study. Furthermore, a *Freyella* specimen (USNM E20796) was misidentified as *Colpaster scutigera*, which led to a biased interpretation of the taxonomic position of the two genera.

Freyellaster, encompassing five extant species, was first reported in 1905 by Fisher. Its diagnosis indicated its morphological affinity with both *Freyella* and *Brisinga* (Fisher, 1919). It resembled the former in having a pavement of abactinal plates in the arm genital area, and resembled the latter in having numerous gonads, the first pair of inferomarginal plates above the first pair of adambulacral plates, and a partial fusion between the first and second adambulacral plates. Genus *Colpaster* was established based on a single specimen named *Colpaster scutigera* Sladen, 1889. Sladen argued that it resembled *Freyella* mostly in having a pavement of abactinal plates in the arm genital area, but differed from *Freyella* in the presence of an “azygos interradial plate” visible from the lateral and actinal side of the disk, separating the first pair of adambulacral plates. However, upon re-examination of the type specimen of *Colpaster scutigera* (NHMUK 1890.5.7.1081), this “azygos interradial plate” was actually the first pair of inferomarginal plates of uneven size (Fig. 5F), which is homologous to the first inferomarginal plates in *Freyellaster* and *Brisinga*.

Being morphologically intermediate, molecular phylogeny provide solid evidence of the two genera’s affinity to Brisingidae. *Colpaster patricki* comb. nov. clusters with two *Brisinga* species (Fig. 4, clades 4b, 4c), and *Freyellaster* species form a monophyly with several species used to belong to *Brisinga* (Fig. 4, clades 4a). We therefore remove these two genera from Freyellidae and place them inside Brisingidae. By tracing the transformation of morphological characters, characters defined by Downey to distinguish the two families, such as the presence or absence of costae (Char. 14), whether the first pair of adambulacral plates are united interradially (Char. 7), appear to be homoplastic characters developed independently across phylogenetically distant taxa (Fig. 8, Suppl. Fig. 6). On the other hand, two characters which were first noticed by Fisher (1917), namely, the position of the first inferomarginal plates (Char. 5) and the partial fusion between the first and second adambulacral plates (Char. 10), are found to be synapomorphies distinguishing the “new” Brisingidae and Freyellidae. We hence revise the diagnosis to both families based on the result of character transformation analysis. Furthermore, the new genus *Lokiella* shows intermediate characters, similar to *Freyellaster* and *Colpaster*. The present phylogenetic analysis provides support for attributing this genus to Brisingidae, allowing for further investigation on ontogenetic variation and pedomorphosis.

4.3. Genus-level phylogeny and taxonomic revisions

Within the new Brisingidae family, nine genera fall into two major clades, one consisting of *Freyellaster*, *Brisinga*, *Colpaster*, *Lokiella* gen. nov. and *Hymenodiscus*, the other consisting of *Brisingenes*, *Midgardia*, *Arostephane* and *Astrolirus* (cf.). Morphological characters supporting these inner clades have not been found yet. Comparing with the

morphology-based phylogeny of Mah (1998a) (Fig. 2), a major difference is the relationship between *Hymenodiscus* and *Astrolirus*. The two genera were believed to be closely related and as such were proposed to form a new family Hymenodiscidae (Mah, 1998b). However, the morphological affinity of the two genera were found to be convergence owing to paedomorphosis, and they are here found to be phylogenetically distant (see Section 4.4). Another genus in the proposed “Hymenodiscidae”, *Parabrisinga*, is a monospecific and rarely encountered genus and was not represented in the present molecular analysis. But by examining photos of one of the syntypes (ZIHU-2416) provided by Dr. Toshihiko Fujita and Ms. Mayumi Masuda (NMNS, Japan), this species is found to be closest to *Brisinga* in having the first pair of inferomarginal plates largely inserting in between the first pair of adambulacral plates, having numerous gonads and truncate proximal subambulacral spines. It is potentially a junior synonym of *Brisinga* pending validation. The “Hymenodiscidae” hypothesis is therefore rejected by the present study.

The sister-taxon relationship of *Freyellaster* and *Brisinga* (Fig. 4) is a novel relationship, not observed from prior morphology-based interpretations. Several species previously assigned as *Brisinga* clustered together with *Freyellaster* species, together forming a well-supported clade, though the interspecific relationship is not fully resolved (Fig. 4, Fig. 6A, Suppl. Fig. 1). Another two *Brisinga* species lie outside this clade and cluster with *Colpaster*, thus neither *Brisinga* nor *Freyellaster* is monophyletic. A thorough examination on morphological characters reveals subtle differences between the “*Brisinga panopla* clade” and “*Brisinga chathamica* clade”. The former has more densely distributed inferomarginal plates (at every adambulacral plate) (Suppl. Fig. 5A) and conspicuous large pedicellariae. Its first pair of inferomarginal plates are smaller and usually not inserting in between the first pair of adambulacral plates (in adult specimens) (Suppl. Fig. 8A). Therefore, we reclassify the species of *Brisinga* after reviewing the descriptions of all *Brisinga* species. The type species of *Brisinga*, *B. endecacnemus*, agrees with the characters of the *Brisinga chathamica* clade, thus species in this clade remain in *Brisinga*. Four species with divergent characters, *Brisinga panopla*, *B. hirsuta*, *B. variispina* and *B. parallela* are revised to *Freyellaster*. Given the fact that these species still differ from the original *Freyellaster* species, two new subgenera, *Freyellaster (Freyellaster)* and *Freyellaster (Pseudobrisinga)* are established to accommodate the original *Freyellaster* species and the new comers. The name *Pseudobrisinga* is used to imply the morphological affinity between these species and *Brisinga*. It should be noted that *Freyellaster (Freyellaster)* remains to be paraphyletic in the present phylogenetic hypothesis (Fig. 4).

Within the new Freyellidae family, genus *Freyastera* and genus *Freyella* formed well-supported sister clades, with two species present at outlying position, *Freyastera benthophila* and *Astrocles actinodetus*. The latter species is the type species of genus *Astrocles*, which used to be considered as a junior synonym of *Freyella* (Downey, 1986) but later resurrected by Mah (1998a). In the present study, an *Astrocles actinodetus* specimen (Fig. 5L) was examined and DNA sequences from two *A. actinodetus* specimens previously reported by Mironov et al. (2018) were obtained. The morphological characters of all three specimens clearly agree with the diagnosis of the genus and species. They differ from *Freyella* in having bands of scale-like plates interrupted by bands of bare skin in abactinal genital area and are characterized by “T” shaped proximal furrow spines, with expanded end. The arm plating of *Freyella* species is usually dense and imbricated, but furrow spines with expanded tips were also found in several *Freyella* species, such as in *F. heroina* and *F. remex*. The shape of furrow spines might be of congeneric importance. It is highly possible that *Astrocles*, at least the type species *A. actinodetus*, should be synonymized as *Freyella*. But since none of the type specimens of all three *Astrocles* species were examined, no taxonomic revision was made for *Astrocles* until the types were examined or sequenced.

Freyastera benthophila, on the other hand, was broadly reported from the world ocean and its belonging to *Freyastera* was almost never questioned as it is 6-armed as typical *Freyastera*. A previously reported

Freyastera benthophila was found to cluster with *Freyella* species (Mu et al., 2016), but we re-examined this specimen and recovered that it is a 7-armed *Freyella macropedicellariae*. However, several newly examined *Freyastera benthophila* specimens in this study indeed clustered with *Freyella* (Fig. 4). The assessment of character transformation indicated that the arrangement of inferomarginal plates (Char. 6, Suppl. Fig. 5) might be a key character to delimitate *Freyastera* and *Freyella*, as it was to differentiate *Brisinga* and *Freyellaster*. The evolution and function of such a character is intriguing and requires further investigations. Further characters to delimitate *Freyastera* and *Freyella* were not found in the present study. Based on the robust support by molecular phylogeny, *Freyastera benthophila* is revised to *Freyella benthophila* comb. nov.

4.4. Ontogenetic variation and paedomorphosis

Juvenile specimens from several genera, including *Odinella*, *Brisingaster*, *Novodinia*, *Freyellaster*, *Brisingenes*, *Colpaster* and *Freyella*, at different growth stages were examined and sequenced in the present study, allowing for tracing ontogenetic variation of morphological characters (Fig. 10). Juvenile characters observed from this study and literature (Sars, 1875; Perrier, 1894; Fisher, 1940; Mah, 1998b) generally include 1) small size; 2) gonads not developed; 3) the first pair of inferomarginal plates only joint at adoral end, forming a reversed Y with the odontophore, or partially joint depending on growth stage (Fig. 10A, D, I); 4) the first pair of adambulacral plates not united interradially, or only partially joint depending on growth stage (Fig. 10B, D); 5) abactinal disk spine with spinulated ends (commonly seen in *Novodinia* juveniles but not occurring in all juveniles) (Fig. 10C, E–G); 6) abactinal arm plates or costae not developed. Bands of spinelets or pedicellariae might occur at early stage of growth (Fig. 10H, I); 7) adambulacral plates with only subambulacral spine developed; 8) adambulacral plates much longer than wide. The juvenile characters occurring on the arms can also be found on regenerating arms of adult specimens (e.g., Zhang et al., 2019). The similarity in morphology of *Brisingida* juveniles across genera and families makes morphological identification difficult and often inaccurate, thus needs to be aided by comparison of molecular data.

Furthermore, several of the above-mentioned juvenile characters were notably spotted in adult specimens of *Hymenodiscus*, *Astrolirus* and the new genus *Lokiella*, suggesting paedomorphic development in these genera. These adult specimens are relatively small, with their first inferomarginal plates only joint adorally, forming a reversed Y with the odontophore (Suppl. Fig. 7C). Their first pair of adambulacral plates are separated interradially (Suppl. Fig. 6C), and the adambulacral plates are long and thin in *Hymenodiscus* and *Lokiella*, which coincide with typical juvenile characters of *Brisingida* as mentioned above. Cladistic analysis based on these morphological characters has led to the assumption of *Hymenodiscus* and *Astrolirus* being closely related. However, after reviewing morphological variations through ontogeny, it is clear that in these cases a high degree of convergence is owing to the retention of general juvenile characters. Furthermore, the molecular phylogeny shows that genera with juvenile characters fall on distant lineages (Suppl. Figs. 6A and 7A), suggesting that the trend of paedomorphosis is not indicative of phylogenetic relationship. It is also to be noticed that certain species in Freyellidae (e.g., *Freyastera* species, *Freyella attenuata*, *F. macropedicellaria*, *F. kurilokamachatica*) demonstrate characters that might be related to paedomorphosis, including smaller body size, smaller arm numbers, much longer than wide adambulacral plates (Mah, 1998b) and separated first adambulacral plates. These species often occur at deeper depth zone (mostly > 4000 m) comparing with *Freyella* species without paedomorphic or simplified appearance (e.g., *F. echinata*, *F. elegans*). It is unclear yet why the trend of paedomorphosis appears so frequently in *Brisingida*, but it is not a singleton in deep-sea Echinodermata. Roux and Pawson (1999) noticed a “giant-dwarf” heterochronic gradient between *Hyocrinus* (*Hyocrinida*, *Crinoidea*) species from different environments, suggesting such a pattern might be related

to food supply. Mironov et al. (2016) regarded depth gradient as a main drive of morphological simplification and retention of juvenile characters in Porcellanasteridae (Asteroidea). The fact that paedomorphosis is commonly found in many Echinodermata taxa (e.g., Stöhr and Martynov, 2016; Roux et al., 2013) might also suggest that paedomorphosis “provides a parsimonious framework for selection of favourable adaptation” (Roux et al., 2013). Owing to the many homoplastic characters caused by heterochrony, ontogeny and other unknown reasons, it is important to combine multiple avenues of evidence in phylogenetic interpretation and reconstruction, especially for taxa inhabiting a spectrum of marine habitats like Brisingida.

4.5. Fossil record of Brisingida

The fossil record of Brisingida is exceptionally poor, with only one fossil known to date, *Hymenodiscus* sp., from the Miocene of the Morozaki group, Japan (Yamaoka, 1987). The Morozaki fauna is one of the few bathyal ecosystems preserved (Oji, 2001), and has notably yielded two different species of deep-sea Forcipulatacea starfish, *Hymenodiscus* sp. and a Zoroasteridae *Doraster mizunoi* Kato and Oji, 2013. The present phylogenetic analysis places the genus *Hymenodiscus* in a relatively derived position within the family Brisingidae. Assuming the taxonomic placement of the extinct *Hymenodiscus* sp. within the family is correct, it suggests that all five Brisingida families appeared and diversified by the Miocene. However, due to the uncertainties surrounding the phylogenetic position of the Brisingida within the Forcipulatacea, pinpointing the precise timing of their origin remains challenging. Forcipulataceans have been documented as early as the Early Jurassic (Hettangian, Blake, 1990). Many extinct species, especially Mesozoic species, have been interpreted as members of extant families. However, reexamination of the fossil record has showed that Mesozoic forcipulataceans represent stem groups rather than members of extant families (e.g. Villier et al., 2009; Fau and Villier, 2023). The redefinition of the Asteroidea family, following the analysis by Mah and Foltz (2011) have highlighted the necessity for a thorough reevaluation of the Asteroidea fossil record. Consequently, whether Brisingida holds a more basal position within the Forcipulatacea (Fau and Villier, 2020), suggesting divergence dating back to the Jurassic, or occupies a more derived position (Mah and Foltz, 2011), indicating of a more recent origin, remains uncertain. The comprehension of the evolutionary history of the Brisingida is thus incomplete, awaiting further fossil discoveries for a more comprehensive understanding.

5. Key to Brisingida families and genera after taxonomic revision

<p>1a. Papulae present on abactinal surface of disk and arms; oral plate fan-shaped from oral view; a lateral spine and several dorsal-lateral spines form a comb along each side of the arm; inferomarginal plates occur on every 3–4 adambulacral plates</p>	2	
<p>2a. Genital area specialized with brooding chambers; abactinal plates on arm form a dense reticulation; abactinal and lateral spines enclosed in ovoid sacculi covering with small pedicellariae; ambulacral plates wing-shaped (Mah, 1998a)</p>	3	Odinellidae
<p>2b. No brooding chambers; spines enclosed in a socket sheath covering small pedicellariae ambulacral plates block-shaped (Mah, 1998a)</p>	3	
<p>3a. Gonad numerous, distributed in series on each side of arm. Costae well-developed on arms, sometimes interconnected. Intercostal area skin bare</p>		Brisingasteridae
<p>3b. A pair of gonads on each arm. Intercostal area covered with pavement of irregularly shaped plates, leaving pores between plates for the papulae</p>		Novodiniidae

(continued)

<p>1b. Papulae absent or reduced; oral plate trapezoid; no lateral comb of spines; inferomarginal plates occur on every or alternate adambulacral plates</p>	4	
<p>4a. The first pair of inferomarginal plates above or insert in the first pair of adambulacral plates, in contact with the odontophore. A partial fusion between the first and second adambulacrals, appearing externally as a suture between the two plates</p>		5 Brisingidae
<p>5a. Proximal subambulacral spines with truncate or modified end; when costae present, intercostal area with isolated discoid plates bearing spines</p>	6	
<p>6a. A pair of gonads to each arm; the first pair of inferomarginal plates inserting in and separating the first pair of adambulacral plates, visible from the oral side; abactinal skeleton of arm composed of a dense pavement of plates, costae absent</p>		Colpaster
<p>6b. Numerous gonads in series to each arm</p>	7	
<p>7a. Inferomarginal plates generally correspond to every adambulacral plates beyond the genital area; the first pair of inferomarginal plates small, above the first pair of adambulacral plates, all four plates in close contact</p>		Freyellaster
<p>Costae present; intercostal area with isolated discoid plates bearing spines</p>		Freyellaster (<i>Pseudobrisinga</i>) subgen. nov.
<p>Costae absent; abactinal skeleton of arm composed of a dense pavement of plates</p>		Freyellaster (<i>Freyellaster</i>) subgen. nov.
<p>7b. Inferomarginal plates generally correspond to every 2nd adambulacral plates beyond the genital area; the first pair of inferomarginal plates large, inserting the first pair of adambulacral plates</p>		Brisinga/ <i>Parabrisinga</i>
<p>5b. Proximal subambulacral spines with sharp end; when costae present, intercostal area without plates or with pavement of spineless plates</p>	8	
<p>8a. Suboral spines bent at base; the first pair of inferomarginal plates in full contact in adults</p>	9	
<p>9a. Multiple pairs of gonads to each arm</p>	10	
<p>9b. A pair of gonads to each arm</p>	11	
<p>10a. Intercostal area without plates</p>		Brisingenes
<p>10b. Intercostal area with pavement of spineless plates</p>		Midgardia
<p>11a. Intercostal area without plates</p>		Astrostephane
<p>11b. Intercostal area with pavement of spineless plates</p>		Stegnobrisinga
<p>8b. Paedomorphic adults; suboral spines straight; the first pair of inferomarginal plates in contact only adorally in adults, form a reversed Y with the</p>	12	
<p>12a. Costae present</p>	13	
<p>12b. Costae absent; a pavement of plates in the arm genital area, bearing more or less well-aligned spinelets</p>		Lokiella gen. nov.
<p>13a. Intercostal area with pavement of spineless plates</p>		Astrolirus
<p>13b. Intercostal area without plates</p>		Hymenodiscus
<p>4b. The first pair of inferomarginal plates appear later on arm, not in contact with the odontophore. Articulation between the first and second adambulacral plates not differentiated from that of the later adambulacrals</p>		14 Freyellidae
<p>14a. Arms 6; inferomarginal plates correspond to every adambulacral plates beyond the genital area</p>		Freyastera
<p>14b. Inferomarginal plates correspond to every 2nd adambulacral plates beyond the genital area</p>	15	
<p>15a. Abactinal skeleton of arm composed of a dense pavement of plates</p>		Freyella
<p>15b. Abactinal skeleton of arm composed of transverse lines of flattened plates, separated by intervals devoid of plates</p>		Astrocles

(continued on next column)

6. Conclusions

The present molecular based phylogeny and revised classification have addressed key questions in classification and phylogeny of the extant Brisingida. A novel classification scheme of the order is proposed to encompass 5 families and 17 genera. Molecular phylogenetic trees based on four genes support the monophyly and robustness of the order, 5 families and 14 genera within the new classification scheme (only *Freyella* remains to be polyphyletic). New genus, subgenus and species combinations are described. Ontogenetic variation and paedomorphosis frequently found in Brisingida have added difficulties to taxonomic studies, but suggested intriguing evolutionary history in these deep-sea animals which is in need of future investigation. The new phylogeny of Brisingida, systematic accounts and a DNA dataset reported in this research will support future investigations on Brisingida diversity, ecological functions and evolution, which will serve as the foundation for large-scale biogeographical study and deep-sea ecosystem conservation.

CRedit authorship contribution statement

Ruiyan Zhang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Marine Fau:** Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing. **Christopher Mah:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Marc Eléaume:** Conceptualization, Data curation, Funding acquisition, Resources, Supervision, Writing – review & editing. **Dongsheng Zhang:** Conceptualization, Funding acquisition, Project administration, Resources, Writing – review & editing. **Yadong Zhou:** Investigation, Methodology, Resources, Writing – review & editing. **Bo Lu:** Data curation, Investigation, Resources, Writing – review & editing. **Chunsheng Wang:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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References

- Alcock, A., 1893, XV.—Natural history notes from H.M. Indian marine survey Steamer 'Investigator,' Commander C. F. Oldham, R. N., commanding.—Series II., No. 7. An account of the collection of deep-sea Asteroidea, *Annals and Magazine of Natural History*, Series 6, 11:62, 73–121.
- Améziane, N., Eléaume, M., Roux, M., 2021. Ontogeny of non-muscular brachial articulations in Balanocrininae (Echinodermata, Crinoidea): iterative trajectories or phylogenetic significance? *Zoomorphology* 140, 47–67.
- Amon, D.J., Ziegler, A.F., Kremenetskaia, A., Mah, C.L., Mooi, R., O'Hara, T., Pawson, D. L., Roux, M., Smith, C.R., 2017. Megafauna of the UKSRL exploration contract area and eastern Clarion-Clipperton Zone in the Pacific Ocean: Echinodermata. *Biodivers. Data J.* 5.
- Asbjørnsen, P.C., 1856. Description d'un nouveau genre des Astéries. In: Sars, Koren, Danielsen (Eds.), *Fauna Littoralis Norwegiae*, Vol. 2, pp. 95–101.
- Avise, J.C., Liu, J.X., 2011. On the temporal inconsistencies of Linnean taxonomic ranks. *Biol. J. Linn. Soc.* 102 (4), 707–714.
- Blake, D.B., 1990. Hettangian Asteroidea (Echinodermata: Asteroidea) from southern Germany: taxonomy, phylogeny and life habits. *Paleontol. J.* 64, 103–123.
- Bribiesca-Contreras, G., Dahlgren, T.G., Amon, D.J., Cairns, S., Drennan, R., Durden, J. M., Eléaume, M.P., Hsieh, A.M., Kremenetskaia, A., McQuaid, K. and O'Hara, T.D., 2022. Benthic megafauna of the western Clarion-Clipperton Zone, Pacific Ocean. *ZooKeys*, 1113.
- Brondizio, E., Díaz, S.M., Settle, J., Ngo, H., Guezé, M., Aumeeruddy-Thomas, Y., Bai, X., Geschke, A., Molnár, Z., Niamir, A., Pascual, U., 2019. Chapter 1 Assessing a planet in transformation. *Rationale and Approach of the IPBES Global Assessment on Biodiversity and Ecosystem Services*. Zenodo.
- Campos, L.S., Moura, R.B., Alcântara, P.F., Vasconcelos, R.F., Curbelo-Fernandez, M.P., Veríssimo, I., Cavalcanti, G.H., 2010. On two new records of the Family Brisingidae (Echinodermata: Asteroidea) from the Brazilian continental margin. In: Hampshire, N. (Ed.), *Proceedings of the 12th International Echinoderm Conference*, pp. 139–146.
- Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540–552.
- Cherbonnier, G., Sibuet, M., 1972. Résultats scientifiques de la campagne Noratlante: Astérides et Ophiurides. *Bulletin Du Muséum National D'histoire Naturelle* 3, 1333–1394 [in French].
- Clark, A.H., 1916. One new starfish and five new brittle stars from the Galapagos Islands. *Annals and Magazine of Natural History*. 8 18, 115–122.
- Clark, A.M., Downey, M.E., 1992. *Starfishes of the Atlantic*. Chapman and Hall, London, pp. 1–794.
- Clark, A.M., Mah, C., 2001. An index of names of recent Asteroidea—Part 4: Forcipulatida and Brisingida. *Echinoderm Stud.* 6, 229–347.
- Clark, A.H., 1939. Echinoderms of the Smithsonian-Hartford Expedition, 1937, with other West Indian records. *Proc. U.S. Natl. Mus.* 86, 441–456.
- Clark, A.M., 1962. Asteroidea. Reports (B.A.N.Z. Antarctic Research Expedition, (1929–1931)). *Se. B. Zool. Bot.* IX, 104.

- de Loriol, P., 1883. Catalogue Raisonné des Echinodermes recueillis par M.V. de Robillard à l'île Maurice. Mémoires de la Société de physique et d'histoire naturelle de Genève 28(8), 1–64 [in French].
- Dartnall, A.J., Pawson, D.L., Pope, E.C., 1969. Replacement name for the preoccupied genus name *Odinia* Perrier 1885 (Echinodermata: Asteroidea). Proceedings of the Linnean Society of New South Wales 93 (2), 211.
- Desbruyeres, D., Segonzac, M., Bright, M., 2006. Handbook of deep-sea hydrothermal vent fauna. *Denisia* 18, 1–434.
- Dilman, A.B., 2014. Deep-sea fauna of European seas: an annotated species check-list of benthic invertebrates living deeper than 2000 m in the seas bordering Europe. *Asteroidea. Invertebr. Zool.* 11 (1), 25–42.
- Döderlein, L., 1928. Die Seesterne der Deutschen Sudpolar-Expedition, 1901–1903. Deutsche Sudpolar Expedition XIX. Zoologie XI. 1901–1903. series. 19 (11), 289–301.
- Downey, M.E., 1973. Starfishes from the Caribbean and the Gulf of Mexico. Smithsonian Contributions to Zoology 126, 1–158.
- Downey, M.E., 1986. Revision of the Atlantic Brisingida (Echinodermata: Asteroidea), with description of a new genus and family. *Smithsonian Contrib. Zool.* 435, 1–57.
- Edgar, R.C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797.
- Emsom, R.H., Young, C.M., 1994. Feeding mechanism of the brisingid starfish *Novodinia antillensis*. *Mar. Biol.* 118, 433–442.
- Esteban-Vázquez, B.L., De los Palos-Peña, M., Solís-Marín, F.A., Laguarda-Figueroas, 2021. A. Morphology of endoskeleton and spination in the sea star *Midgardia xandaros* (Brisingida: Brisingidae) from the Gulf of Mexico. *Revista de Biología Tropical* 69(S1), 404–422.
- Fau, M., Villier, L., 2018. Post-metamorphic ontogeny of *Zoroaster fulgens* Thompson, 1873 (Asteroidea, Forcipulatacea). *Journal of Anatomy* 233, 644–665.
- Fau, M., Villier, L., 2020. Comparative anatomy and phylogeny of the Forcipulatacea (Echinodermata: Asteroidea): insights from ossicle morphology. *Zool. J. Linn. Soc.* 189 (3), 921–952.
- Fau, M., Villier, L., 2023. Mesozoic stem-group zoroasterid sea stars imply a delayed radiation of the crown group and adaptation to the deep seas. *J. Syst. Palaeontol.* 21 (1), 2243268.
- Fisher, W.K., 1905. New starfishes from deep water off California and Alaska. *Bull. Bureau Fish.* 24, 291–320.
- Fisher, W.K., 1906. The starfishes of the Hawaiian islands. *Bull. U. S. Fish Comm.* 23, 987–1130.
- Fisher, W.K., 1916. New east Indian starfishes. Proceedings of the Biological Society of Washington 29, 27–36.
- Fisher, W.K., 1917. LL.—New genera and species of Brisingidae. *Ann. Mag. Nat. Hist. Ser.* 8 20 (120), 418–431.
- Fisher, W.K., 1918. Notes on Asteroidea. II. *Annals and Magazine of Natural History* 9 (2), 103–111.
- Fisher, W.K., 1919. Starfishes of the Philippine seas and adjacent waters. *Bull. U. S. Natl. Mus.* 3 (100), 1–547.
- Fisher, W.K., 1928. Asteroidea of the North Pacific and adjacent waters. Part 2. Forcipulata (part). *Bull. U. S. Natl. Mus.* 76, 1–245.
- Fisher, W.K., 1940. Asteroidea. *Discov. Rep.* 20, 69–306.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, A.R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biotech.* 3, 5294–5299.
- Foltz, D.W., Bolton, M.T., Kelley, S.P., Kelley, B.D., Nguyen, A.T., 2007. Combined mitochondrial and nuclear sequences support the monophyly of forcipulatacean sea stars. *Mol. Phylogenet. Evol.* 43 (2), 627–634.
- Gale, K.S., Hamel, J.F., Mercier, A., 2013. Trophic ecology of deep-sea Asteroidea (Echinodermata) from eastern Canada. *Deep Sea Res. Part I* 80, 25–36.
- Galkin, S.V., Korovchinsky, N.M., 1984. Vertical and geographical distribution of the starfishes of the genus *Freyella* (Brisingidae) with some remarks on their ecology and evolution. *Trudy Instituta Okeanologii Akademii Nauk SSSR* 119, 164–178 [in Russian with English abstract].
- Gerdes, K., Kihara, T.C., Arbizu, P.M., Kuhn, T., Schwarz-Schampera, U., Mah, C.L., Norenburg, J.L., Linley, T.D., Shalava, K., Macpherson, E., Gordon, D., 2021. Megafauna of the German exploration licence area for seafloor massive sulphides along the Central and South East Indian Ridge (Indian Ocean). *Biodivers. Data J.* 9.
- Gjerde, K.M., 2006. Ecosystems and biodiversity in deep waters and high seas. *UNEP Regional Seas Reports and Studies* 178, UNEP/IUCN, Gland, Switzerland.
- Glover, A.G., Wiklund, H., Rabone, M., Amon, D.J., Smith, C.R., O'Hara, T., Mah, C.L., Dahlgren, T.G., 2016. Abyssal fauna of the UK-1 polymetallic nodule exploration claim, Clarion-clipperton zone, central Pacific Ocean: Echinodermata. *Biodivers. Data J.* 4 (4), e7251.
- Hayashi, R., 1943. Contributions to the classification of the sea-stars of Japan. II. Forcipulata, with the note on the relationships between the skeletal structure and respiratory organs of the sea stars. *J. Faculty Sci. Hokkaido Univ. (Zool.)* 8, 133–277.
- Hemery, L.G., Eléaume, M., Roussel, V., Améziane, N., Gallut, C., Steinke, D., Craud, C., Couloux, A., Wilson, N.G., 2012. Comprehensive sampling reveals circumpolarity and sympatry in seven mitochondrial lineages of the Southern Ocean crinoid species *Promachocrinus kerguelensis* (Echinodermata). *Mol. Ecol.* 21 (10), 2502–2518.
- Hingsinger, D.D., Debruyne, R., Thomas, M., Denys, G.P., Mennesson, M., Utge, J., Dettai, A., 2015. Fishing for barcodes in the Torrent: from COI to complete mitogenomes on NGS platforms. *DNA Barcodes* 3 (1), 170–186.
- Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35 (2), 518–522.
- Hourdez, S., Jollivet, D., 2019. CHUBACARC cruise, RV L'Atalante, doi: 10.17600/18001111.
- Huelsbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17 (8), 754–755.
- Jangoux, M., Massin, C., 1986. Catalogue commenté des types d'Echinodermes actuels conservés dans les collections nationales belges. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique Biologie* 56, 83–97.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., Von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14 (6), 587–589.
- Kato, M., Oji, T., 2013. A new species of *Doraster* (Echinodermata: Asteroidea) from the lower Miocene of central Japan: implications for its enigmatic paleobiogeography. *Paleontol. Res.* 17 (4), 330–334.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28 (12), 1647–1649.
- Kim, S., Hammerstrom, K., 2012. Hydrothermal vent community zonation along environmental gradients at the Lau back-arc spreading center. *Deep Sea Res. Part I* 62, 10–19.
- Kimura, M., 1980. A simple method of estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111–120.
- Knott, K.E., Wray, G.A., 2000. Controversy and consensus in asteroid systematics: new insights to ordinal and familial relationships. *Am. Zool.* 40 (3), 382–392.
- Koehler, R., 1907a. Astéries, Ophiures et Echinides recueillis dans les mers australes par la "Scotia" (1902–1904). *Zool. Anz.* 32 (6), 140–147 [in French].
- Koehler, R., 1909a. Astéries recueillies par l'Investigator dans l'Océan Indien. I. Les Astéries de Mer Profonde. *Echinoderma of the Indian Museum* 5, 25–143 [in French].
- Koehler, R., 1909b. Echinodermes provenant des campagnes du yacht Princesse-Alice (Astéries, Ophiures, Echinides et Crinoïdes). *Résultats Des Campagnes Scientifiques Accomplies Sur Son Yacht Par Albert Ier Prince Souverain De Monaco* 34, 25–28 [in French].
- Koehler, R., 1907b. Note préliminaire sur quelques astéries et ophiures provenant des campagnes de la "Princesse Alice".
- Koehler, R., 1908. Astéries, ophiures et échinides de l'Expédition Antarctique Nationale Ecosaisse. Vol. 46. No. 22. R. Grant & Son. [in French].
- Korovchinsky, N.M., Galkin, S.V., 1984. New data on the fauna of starfishes of the genus *Freyella* (Brisingidae). *Zool. Zhurnal* 63 (8), 1205–1215 [in Russian with English abstract].
- Lecocq, T., Vereecken, N.J., Michez, D., Dellicour, S., Lhomme, P., Valterova, I., Rasplus, J.Y., Rasmont, P., 2013. Patterns of genetic and reproductive traits differentiation in mainland vs. Corsican populations of bumblebees. *PLoS One* 8 (6), e65642.
- Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* 50 (6), 913–925.
- Ludwig, H., 1903. Seesterne. Résultats du voyage du S.Y. Belgica en 1897–1898–1899. *Rapports Scientifiques* 1–72 [in German].
- Ludwig, H., 1905. Asteroidea. *Memoirs of the Museum of Comparative Zoology at Harvard*, 32, 1–292. [in German].
- Maddison, W.P. and Maddison D.R., 2021. Mesquite: a modular system for evolutionary analysis. *Version 3.70*.
- Madsen, F.J., 1951. Asteroidea. Reports of the Swedish Deep-Sea Expedition 2 (6), 73–92.
- Mah, C.L., 1999. Redescription and taxonomic notes on the South Pacific Brisingidan *Brisingaster robillardii* (Asteroidea) with new ontogenetic and phylogenetic information. *Zoosystema* 21 (3), 535–546.
- Mah, C.L., 2016. A new species of *Brisingenes* from the Hawaii undersea military munitions assessment area with an overview of Hawaiian brisingid in situ video observations and functional morphology of subambulacral spines (Forcipulatacea; Asteroidea). *Deep Sea Res. Part II* 128, 43–52.
- Mah, C.L., 2022. New Genera, Species and Occurrences of Deep-Sea Asteroidea (Valvatata, Forcipulatacea, Echinodermata) collected from the North Pacific Ocean by the CAPSTONE Expedition. *Zootaxa* 5164 (1), 1–75.
- Mah, C.L., Blake, D.B., 2012. Global diversity and phylogeny of the Asteroidea (Echinodermata). *PLoS One* 7 (4), e35644.
- Mah, C., Foltz, D., 2011. Molecular phylogeny of the Forcipulatacea (Asteroidea: Echinodermata): systematics and biogeography. *Zool. J. Linn. Soc.* 162 (3), 646–660.
- Mah, C.L., 1998a. Preliminary phylogeny and taxonomic revision of the Brisingida. In: Mooi, R.; Telford, M. (Eds.), Proceedings of the Ninth Echinoderm Conference, San Francisco, 1996. A.A. Balkema, Rotterdam, pp. 273–277.
- Mah, C.L., 1998b. A phylogeny, taxonomic revision, and natural history of the order Brisingida (Asteroidea). Unpublished Master's Thesis, San Francisco State University, pp. 1–95.
- Mah, C.L., 2023. World Asteroidea Database. Brisingida. Accessed through: World Register of Marine Species at: <https://www.marinespecies.org/aphia.php?p=taxdata&iid=123085>.
- McKnight, D.G., 1973. Additions to the asteroid fauna of New Zealand: families Benthoptectinidae, Odontasteridae, Asteridae and Brisingidae; with notes on *Porcellanaster caeruleus* Wyville Thomson (Family Porcellanasteridae). *New Zealand Oceanographic Institute Records* 1 (16), 220–240.
- McKnight, D.G., 1993. Records of echinoderms (excluding holothurians) from the Norfolk Ridge and Three Kings Rise north of New Zealand. *N. Z. J. Zool.* 20 (3), 165–190.
- McKnight, D.G., 2006. The marine fauna of New Zealand, Echinodermata: Asteroidea (Sea-stars). 3. Orders Velatida, Spinulosida, Forcipulata, Brisingida with addenda to Paxillosida, Valvatida. NIWA Biodiversity Memoir 120, 1–187.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, O., Woodhams, M.D., Von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* 37 (5), 1530–1534.

- Mironov, A.N., Dilman, A.B., Vladychenskaya, I.P., Petrov, N.B., 2016. Adaptive strategy of the porcellanasterid sea stars. *Biol. Bull.* 43 (6), 503–516.
- Mironov, A.N., Minin, K.V., Dilman, A.B., Smirnov, I.S., 2018. Deep-sea echinoderms of the Sea of Okhotsk. *Deep Sea Res. Part II* 154, 342–357.
- Moreau, C.V., Agüera, A., Jossart, Q., Danis, B., 2015. Southern Ocean Asteroidea: a proposed update for the Register of Antarctic Marine Species. *Biodivers. Data J.* 3, e7062.
- Moreau, C., Mah, C., Agüera, A., Améziane, N., Barnes, D., Crokaert, G., Eléaume, M., Griffiths, H., Guillaumot, C., Hemery, L.G., Jazdzewska, A., 2018. Antarctic and Sub-Antarctic asteroidea database. *Zookeys* 747, 141–156.
- Mortensen, T., 1927. *Handbook of the Echinoderms of the British Isles*. Oxford University Press.
- Mu, W., Liu, J., Zhang, H., 2018. The first complete mitochondrial genome of the Mariana Trench *Freyastera* benthophila (Asteroidea: Brisingida: Brisingidae) allows insights into the deep-sea adaptive evolution of Brisingida. *Ecol. Evol.* 8 (22), 10673–10686.
- Oji, T., 2001. Deep-sea communities. In: Briggs, D.E.G., Crowther, P.R. (Eds.), *Palaeobiology II*. Blackwell Science, Oxford, pp. 444–447.
- Palumbi, S., Martin, A., Roman, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The simple fool's guide to PCR. Special Publication, Department of Zoology, University of Hawaii, Honolulu.
- Parker, S.J., Penney, A.J., Clark, M.R., 2009. Detection criteria for managing trawl impacts on vulnerable marine ecosystems in high seas fisheries of the south Pacific ocean. *Marine Ecology Progress* 397 (397), 309–317.
- Perrier, E., 1882. Note sur les Brisinga. *C. R. Hebd. Seances Acad. Sci.* 95, 61–63 [in French].
- Perrier, E., 1884. Mémoire sur les étoiles de mer recueillies dans la mer. In: des Antilles et le golfe du Mexique : durant les expéditions de dragage faites sous la direction de M. Alexandre Agassiz, 2. *Archives Muséum national d'histoire naturelle, France*, pp. 127–276.
- Perrier, E., 1885. Première note Préliminaire des les Echinodermes, recueillis durant les campagnes de dragages sous-marines du Travailleur et du Talisman. *Annales Des Sciences Naturelles, Zoologie* 22 (8), 1–72 [in French].
- Perrier, E., 1894. Stellérides. *Expéditions Scientifiques Du Travailleur Et Du Talisman* 3, 1–431 [in French].
- Piepenburg, D., Buschmann, A., Driemel, A., Grobe, H., Gutt, J., Schumacher, S., Segelken-Voigt, A., Sieger, R., 2017. Seabed images from Southern Ocean shelf regions off the northern Antarctic Peninsula and in the southeastern Weddell Sea. *Earth System Science Data* 9 (2), 461–469.
- Piepenburg, D., Segelken-Voigt, A., Gutt, J., 2013. Seabed photographs taken along OFOS profile PS81/225-6 during POLARSTERN cruise ANT-XXIX/3. Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, PANGAEA, p. 818500. <https://doi.org/10.1594/PANGAEA>.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* 67 (5), 901–904.
- Roux, M., Eléaume, M., Hemery, L.G., Améziane, N., 2013. When morphology meets molecular data in crinoid phylogeny: a challenge. *Cahiers de Biologie Marine* 54 (4), 541–548.
- Roux, M., Messing, C.G., Améziane, N., 2002. Artificial keys to the genera of living stalked crinoids (Echinodermata). *Bulletin of Marine Science* 70 (3), 799–830.
- Roux, M., Pawson, D.L., 1999. Two new Pacific Ocean species of hyocrinid crinoids (Echinodermata), with comments on presumed giant-dwarf gradients related to seamounts and abyssal plains. *Pac. Sci.* 53 (3), 289–298.
- Rowe, F.W.E., 1989. Nine New Deep-Water Species of Echinodermata from Norfolk Island and Wanganella Bank, northeastern Tasman Sea, with a Checklist of the Echinoderm Fauna. *Proc. Linnean Soc. NSW* 111 (1–4), 257–292.
- Sars, G.O., 1875. On Some Remarkable Forms of Animal Life from the Great Deeps off the Norwegian Coast. II. Researches on the Structure and Affinity of the genus *Brisinga* Based on the study of a New Species, *Brisinga coronata*. Christiania University, Christiania.
- Sibuet, M., 1975. Astérides abyssales de l'Atlantique sud. (Résultats de la campagne Walda, juin-juillet-août 1971). *Bulletin Du Muséum National D'histoire Naturelle, Zoologie* 3 (289), 281–297 [in French].
- Sladen, W.P., 1889. Report on the Asteroidea. Report on the Scientific Results of the Voyage of H.M.S. Challenger during the years 1873-1876. *Zoology* 30 (51), 125–174.
- Stöhr, S., Martynov, A., 2016. Paedomorphosis as an evolutionary driving force: insights from deep-sea brittle stars. *PLoS One* 11 (11), e0164562.
- Sun, S.E., Xiao, N., Sha, Z., 2022. Mitogenomics provides new insights into the phylogenetic relationships and evolutionary history of deep-sea sea stars (Asteroidea). *Scientific reports* 12 (1), 1–14.
- Swofford, D.L., Maddison, W.P., 1987. Reconstructing ancestral character states under Wagner parsimony. *Math. Biosci.* 87 (2), 199–229.
- Tortonese, E., 1958. Euclasteroidea: nuovo ordine di asteroidi (Echinodermi). *Doriana* 2 (88), 1–3.
- Tracey, D.M., Parker, S.J., Mackay, E., Anderson, O., Ramm, K., 2008. Classification guide for potentially vulnerable invertebrate taxa in the SPRFMO Area. New Zealand Ministry of Fisheries, Wellington, New Zealand.
- Villier, L., Charbonnier, S., Riou, B., 2009. Sea stars from the Middle Jurassic Lagerstätte of La Voulte-sur-Rhône (Ardèche, France). *J. Paleol.* 83, 389–398.
- Ward, R.D., Holmes, B.H., O'Hara, T.D., 2008. DNA barcoding discriminates echinoderm species. *Mol. Ecol. Resour.* 8 (6), 1202–1211.
- Wood-Mason, J., Alcock, A., 1891. LII. Natural history notes from H. M. Indian marine survey Steamer "Investigator". Series II, No. 1. On the results of deep-sea dredging during the season 1890–91. *Ann. Mag. Nat. Hist.* 6, 427–452.
- Woods, D., Cheadle, M.J., John, B.E., German, C.R., Van Dover, C.L., 2022. Making Use of Relicts: Brisingid Seastars Aggregate on Hydrothermally Inactive Sulfide Chimneys Near Black Smokers. *Front. Mar. Sci.* 9, 142.
- Xia, X., 2018. DAMBE7: new and improved tools for data analysis in molecular biology and evolution. *Mol. Biol. Evol.* 35, 1550–1552.
- Yamaoka, M., 1987. Fossil asteroids from the Miocene Morozaki group, Aichi Prefecture, Central Japan. *Kaseki No Tomo* 31, 5–23 [in Japanese with English abstract].
- Zhang, R., Zhou, Y., Xiao, N. and Wang, C., 2020. A new sponge-associated starfish, *Astrolirus patricki* sp. nov. (Asteroidea: Brisingida: Brisingidae), from the northwestern Pacific seamounts. *PeerJ*, 8, e9071.
- Zhang, R., Wang, C., Zhou, Y., Zhang, H., 2019. Morphology and molecular phylogeny of two new species in genus *Freyastera* (Asteroidea: Brisingida: Freyellidae), with a revised key to close species and ecological remarks. *Deep Sea Res. Part I* 154, 103163.
- Zulliger, D.E., Lessios, H.A., 2010. Phylogenetic relationships in the genus *Astropecten* Gray (Paxilloidea: Astropectinidae) on a global scale: molecular evidence for morphological convergence, species-complexes and possible cryptic speciation. *Zootaxa* 2504, 1–19.