Description of two species of the genus *Astrodia* Verrill, 1899 (Ophiuroidea, Euryalida, Asteronychidae), including a new species from seamounts in the West Pacific

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Abstract
Five specimens of Ophiuroidea from deep-sea seamounts in the West Pacific were collected and identified as two species, *Astrodia duospina* sp. nov. and *Astrodia abyssicola*. The new species, *Astrodia duospina* sp. nov., can be distinguished from its congeners by having indistinct or underdeveloped oral papillae, relatively short genital slits, crescent-shaped lateral arm plates, and plate-shaped external ossicles on the aboral surface of the disc. One specimen was identified as *Astrodia abyssicola*, which has been reported in the north-western Pacific and the north-eastern coast of Japan. The most recent tabular key of *Astrodia* was revised with two more key characteristics added, the shape and presence of oral papillae and the number of arm spines. The phylogenetic relationship of *Astrodia* and *Asteronyx* was analyzed based on 16S and COI sequences. The discovery of the two species further expanded the geographical distribution of the genus *Astrodia*.
Keywords
Deep sea, molecular phylogeny, morphology, ophiuroids, taxonomy

Introduction

Class Ophiuroidea, as the largest group among echinoderms, with 2126 valid species (Stöhr et al. 2022), are widely distributed from the tropics to polar seas, and from the intertidal to the deep ocean. The Indo-Pacific, North Pacific, and South Pacific regions are reported to have relatively high ophiuroid species richness (Stöhr et al. 2012). Due to the technical limitations of deep sea exploration, the deep-sea ophiuroid fauna remains poorly known (Rodrigues et al. 2011). Seamounts are often of volcanic origin, with elevated topography from the deep-sea floor, which alters the flow of ocean currents and provides highly heterogeneous habitats serving as “hotspots” for deep-sea animals, especially for suspension-feeding epibenthic organisms (e.g. corals, sponges, and ophiuroids) (Yesson et al. 2011). Understanding the biodiversity of ophiuroids from seamounts will provide key information for the protection of this vulnerable ecosystem.

The order Euryalida Lamarck, 1816 comprises about 200 species from three families, Euryalidae Gray, 1840, Asteronychidae Ljungman, 1867, and Gorgonocephalidae Ljungman, 1867 (Stöhr et al. 2022). Among these, Asteronychidae is the smallest family with only 12 extant species from four genera (Asteronyx Müller & Troshel, 1842, Astrodia Verrill, 1899, Astronebris Downey, 1967 and Ophiocreas H.L. Clark, 1911). The genus Astrodia was erected by Verrill, in 1899 and currently comprises four species, Astrodia abyssicola (Lyman, 1879), Astrodia excavata (Lütken & Mortensen, 1899), Astrodia plana (Lütken & Mortensen, 1899) and Astrodia tenuispina (Verrill, 1884). Astrodia tenuispina was first described by Verrill (1884) under the name Asteronyx tenuispina, and was transferred to Astrodia by Verrill (1899). Koehler (1922) described a new species, Astrodia bispinosa, which was later regarded as a junior synonym of Astrodia tenuispina (Baker 1980). The most recent description of Astrodia plana was published by Döderlein (1927). Recently, Okanishi and Fujita (2014) reviewed this genus and transferred Ophiocreas abyssicola Lyman, 1879 to Astrodia. In their review, Okanishi and Fujita (2014) provided interspecific distinguishing characteristics including the shape and arrangements of external ossicles on the aboral surface of the disc, length of genital slits in relation to the height of the disc, the shape of the lateral arm plates, presence or absence of a projection of the lateral arm plates on the middle to the distal portion of the arms. Additionally, the geographical distribution of the four species was summarized (Okanishi and Fujita 2014).

In this study, we describe a new species, Astrodia duospina sp. nov., and redescribe Astrodia abyssicola, from seamounts of the West Pacific. New interspecific diagnostic characteristics were identified, and the tabular key of Okanishi and Fujita (2014) for the genus Astrodia was updated. DNA sequences were used to infer the phylogenetic relationship of the two species with their congeners.
Materials and methods

Sample collection

Five specimens of *Astrodia* were collected by ROV *HAILONG III*, ROV *HAILONG IV*, and HOV *JIAOLONG*, from seamounts in the Philippine Sea and the Northwest Pacific, during several COMRA’s cruises in 2013, 2020, and 2021 (Fig. 1). All specimens were preserved in 95% ethanol on board the vessels and photographed using a digital camera (Canon EOS 5D), then deposited in the repository of the Second Institute of Oceanography, Hangzhou, China (RSIO).

Morphological analysis

Morphological characters were examined and photographed using a stereoscopic microscope (Zeiss Axio Zoom V16). Arm skeletons were examined with a Hitachi TM1000 scanning electron microscope. Skeletal elements were prepared by submerging in commercial bleach (2.5% NaOCl). Washed in distilled water and ethanol, air-dried, and mounted on a stub using dissolved carbon tapes.

Figure 1. Sampling sites of two species in the Philippine Sea and the Northwest Pacific (red circles represent the sampling sites of *Astrodia duospina* sp. nov., the blue circle represents the sampling site of *Astrodia abyssicola*).
The following literature was used as references for the morphological analysis: Okanishi and Fujita (2014), Okanishi et al. (2018), Manso (2010), Baker (1980), and Martynov (2019).

**Molecular analysis**

Several arm segments were dissected from each individual for genomic DNA extraction using DNeasy Blood & Tissue Kit (QIAGEN) following the manufacturer’s protocols. The COI sequences and 16S rRNA sequences were amplified with primers listed in Table 1. The PCR procedures were as follows: an initial denaturation step at 95 °C for 4 min followed by 35 cycles of 94 °C for 15 s, 50 °C for 30 s, and 72 °C for 1 min, and a final extension step at 72 °C for 10 min, for COI; an initial denaturation at 95 °C for 4 min, followed by 35 cycles of 94 °C for 15 s, 50 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 7 min, for 16S. PCR reactions were performed using 25 µL volumes containing: 1 µL of DNA template, 1 µL of each primer, 9.5 µL of dd H2O, and 12.5 µL of 2 × Phanta Max Master Mix (Vazyme, China). PCR products were purified with a QIAquick PCR purification kit (QIAGEN) following the protocol supplied by the manufacturer. Sequencing was performed by Sangon Biotech (Shanghai, China) on an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). Forward and reverse sequences were de novo assembled and edited using Geneious Prime 2021 (https://www.geneious.com), deposited in GenBank (COI: OP328780–OP328783; 16S: OP325290–OP325293).

Seventy-two 16S sequences and 28 COI sequences of Asteronychidae were downloaded from the NCBI. In total, 78 16S sequences and 34 COI sequences (Table 2), including four new 16S sequences and four new COI sequences were used for phylogenetic analysis, with two species of *Asteroschema* as the outgroup. COI and 16S were aligned using Clustal Omega (Sievers and Higgins 2014) as a plug-in in Geneious with default settings, respectively. Maximum likelihood trees were inferred based on a concatenated alignment of 16S and COI, as well as an alignment of 16S and COI respectively. IQ-TREE was used to perform the maximum likelihood bootstrap method (http://iqtree.cibiv.univie.ac.at/) (Nguyen et al. 2015), with the substitution model GTR+I+G, bootstrap support values determined by the ultrafast bootstrap algorithm for 100,000 replicates (Hoang et al. 2018). The best substitution model was selected by ModelFinder as a plug-in in IQ-TREE websites. (Kalyaanamoorthy et al. 2017).

**Table 1.** Information on primers used for PCR programs.

| Primer  | Sequence                      |
|---------|-------------------------------|
| Oph-COI-F | TTTCACTAATCAAGGAYATWGG       |
| Oph-COI-R | CTTCAGGRTGWWCCRAARAYCA       |
| 16Sar    | CGCCTGTTTATCAAAAAACAT        |
| 16Sbr    | CGGGTCTGAACCTCAGATCACGT      |
Table 2. Voucher specimens and accession numbers of COI and 16S sequence data used in the phylogenetic analysis (IDSSE, Institute of Deep-sea Science and Engineering, China; MV, Museums Victoria, Australia; NSMT, National Museum of Nature and Science, Japan; RSIO, Second Institute of Oceanology, China; SIO, Scripps Institution of Oceanography, USA).

| Species               | Locality                    | Voucher number | COI          | 16S          | Code from Okanishi et al. (2018) |
|-----------------------|-----------------------------|----------------|--------------|--------------|----------------------------------|
| *Asteronyx longissimus* | Monterey, California        | SIO: BIC: E6108 | -            | KM014337     |                                   |
| *Asteronyx loveni*     | South China Sea             | IDSSE-EEB-SW0002 | MZ198756     | MZ203264     |                                  |
| *Asteronyx loveni*     | New Zealand                 | MV F188855     | KU1895061    |              |                                  |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-A  | -            | LC276316     | OK-226                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-B  | -            | LC276354     | OK-315                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-C  | LC276289     | LC276330     | OK-256                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-G  | LC276290     | LC276331     | OK-257                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-H  | LC276282     | LC276317     | OK-227                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-I  | -            | LC276359     | OK-339                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-J  | -            | LC276350     | OK-295                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-K  | -            | LC276332     | OK-258                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-L  | -            | LC276358     | OK-337                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-R  | -            | LC276334     | OK-262                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-S  | -            | LC276353     | OK-314                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6904-T  | -            | LC276333     | OK-261                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6951-B  | -            | LC276343     | OK-281                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6951-C  | LC276292     | LC276337     | OK-269                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6951-D  | -            | LC276344     | OK-284                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6951-F  | -            | LC276336     | OK-268                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6951-G  | -            | LC276341     | OK-279                           |
| *Asteronyx loveni*     | Off Abashiri, Hokkaido      | NSMT E-6951-H  | LC276291     | LC276335     | OK-267                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-6943-A  | LC276288     | LC276329     | PT-253                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-6256    | AB578757     | AB605076     | PT-41                            |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5641-A  | LC276284     | LC276320     | PT-238                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5641-B  | LC276285     | LC276321     | PT-239                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5641-C  | -            | LC276322     | PT-240                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5641-D  | LC276286     | LC276323     | PT-241                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5641-E  | -            | LC276324     | PT-242                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5638-A  | LC276278     | LC276308     | PT-213                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5638-B  | -            | LC276352     | PT-306                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5638-C  | -            | LC276357     | PT-323                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5638-D  | -            | LC276356     | PT-320                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5637-A  | -            | LC276310     | PT-215                           |
| *Asteronyx loveni*     | Off Miyako, Iwate           | NSMT E-5637-B  | LC276281     | LC276314     | PT-220                           |
| *Asteronyx loveni*     | Shima Spur, Mie             | NSMT E-6360    | -            | LC276302     | PM-199                           |
| *Asteronyx loveni*     | Shima Spur, Mie             | NSMT E-6983    | LC276280     | LC276312     | PM-218                           |
| *Asteronyx loveni*     | Shima Spur, Mie             | NSMT E-6983    | -            | LC276347     | PM-290                           |
| *Asteronyx loveni*     | Shima Spur, Mie             | NSMT E-6982    | -            | LC276309     | PM-214                           |
| *Asteronyx loveni*     | Off Tosa, Kochi             | NSMT E-1143-A  | -            | LC276318     | PK-231                           |
| *Asteronyx loveni*     | East China Sea, west of Japan| NSMT E-6986-A  | LC276273     | LC276298     | ECS-195                          |
| *Asteronyx loveni*     | East China Sea, west of Japan| NSMT E-6986-C  | LC276272     | LC276297     | ECS-194                          |
| *Asteronyx reticulata* | East of Hiraji Bank, Nagasaki| NSMT E-6912    | -            | LC276355     | ECS-316                          |
| *Asteronyx reticulata* | East of Hiraji Bank, Nagasaki| NSMT E-6915    | -            | LC276358     | ECS-272                          |
| *Asteronyx reticulata* | East of Hiraji Bank, Nagasaki| NSMT E-7016    | -            | LC276301     | ECS-198                          |
| *Asteronyx reticulata* | East of Naka-Kasayama Bank, Nagasaki | NSMT E-6908-C | -            | LC276293     | ECS-190                          |
| *Asteronyx reticulata* | East of Naka-Kasayama Bank, Nagasaki | NSMT E-6908-D | LC276271     | LC276296     | ECS-193                          |
| *Asteronyx reticulata* | East of Naka-Kasayama Bank, Nagasaki | NSMT E-6931    | LC276279     | LC276311     | ECS-217                          |
| *Asteronyx reticulata* | West of Gajajima Isl, Kagoshima | NSMT E-6354    | -            | LC276305     | ECS-204                          |
| *Asteronyx reticulata* | East of Hiraji Bank, Nagasaki | NSMT E-6910    | -            | LC276300     | ECS-197                          |
| *Asteronyx reticulata* | East of Hiraji Bank, Nagasaki | NSMT E-6911    | -            | LC276294     | ECS-191                          |
| *Asteronyx reticulata* | East of Hiraji Bank, Kagoshima | NSMT E-6926    | -            | LC276342     | ECS-280                          |
Species | Locality | Voucher number | CO1 | 16S | Code from Okanishi et al. (2018)
--- | --- | --- | --- | --- | ---
*Asteronyx reticulata* | East of Hiraji Bank, Kagoshima | NSMT E-6929 | - | LC276304 | ECS-203
*Asteronyx reticulata* | West off Takarajima Isl. | NSMT E-6355 | LC276274 | LC276299 | ECS-196
*Asteronyx reticulata* | West of Amami Ohshima Isl., Kagoshima | NSMT E-6351-A | - | LC276325 | ECS-243
*Asteronyx reticulata* | West of Amami Ohshima Isl., Kagoshima | NSMT E-6942-B | - | LC276339 | ECS-274
*Asteronyx reticulata* | West of Ensei Knoll, Kagoshima | NSMT E-6921 | - | LC276349 | ECS-294
*Asteronyx reticulata* | West of Ensei Knoll, Kagoshima | NSMT E-6922-A | LC276287 | LC276328 | ECS-249
*Asteronyx reticulata* | West of Ensei Knoll, Kagoshima | NSMT E-6925-A | - | LC276326 | ECS-247
*Asteronyx reticulata* | West of Ensei Knoll, Kagoshima | NSMT E-6925-B | - | LC276327 | ECS-248
*Asteronyx reticulata* | East China Sea, west of Japan | NSMT E-7001 | LC276276 | LC276306 | ECS-205
*Asteronyx reticulata* | East China Sea, west of Japan | NSMT E-7002 | LC276277 | LC276307 | ECS-206
*Asteronyx reticulata* | Off Amami Ohshima Isl. Kagoshima | NSMT E-692 | - | LC276315 | ECS-223
*Asteronyx reticulata* | West of Minami-Ensei Knoll, Kagoshima | NSMT E-6916 | - | LC276345 | ECS-286
*Asteronyx reticulata* | West of Minami-Ensei Knoll, Kagoshima | NSMT E-6923-A | - | LC276340 | ECS-278
*Asteronyx reticulata* | West of Minami-Ensei Knoll, Kagoshima | NSMT E-6923-B | LC276275 | LC276303 | ECS-202
*Asteronyx reticulata* | East China Sea, west of Japan | NSMT E-7003-A | - | LC276351 | ECS-303
*Asteronyx reticulata* | East China Sea, west of Japan | NSMT E-7003-B | - | LC276346 | ECS-288
*Asteronyx reticulata* | East China Sea, west of Japan | NSMT E-7000-A | - | LC276348 | ECS-291
*Asteronyx reticulata* | Off Iejima Isl., Okinawa | NSMT E-6987 | - | LC276313 | ECS-219
*Asteronyx sp.* | Between Yakushima Isl and Tanegashima Isl., Kagoshima | NSMT E-3157-B | LC276283 | LC276339 | PSW-237
*Asteronyx luzonicus* | South China Sea | IDSSE-EEB-SW0003 | MZ198757 | MZ203265 | -
*Astrodia abyssicola* | Miyagi, off Onahama | NSMT E-6257 | AB758828 | AB605077 | -
*Astrodia abyssicola* | Philippine Sea, KPR Seamount | RSIO68002 | OP328783 | OP325293 | -
*Astrodia duospina* sp. nov. | Philippine Sea, KPR Seamount | RSIO59012 | OP328780 | OP325290 | -
*Astrodia duospina* sp. nov. | Northwest Pacific, Ko-Hakacho-Guyout Seamount | RSIO61068 | OP328781 | OP325291 | -
*Astrodia duospina* sp. nov. | Northwest Pacific, RB Seamount | RSIO61069 | OP328782 | OP325292 | -
*Asterochema ajax* | Off Lord Howe Isl. | MV F99759 | AB758762 | AB605078 | -
*Asterochema clavigerum* | North Atlantic | haplotype 1 | HM587850 | HM587828 | -

Results and discussion

Systematics

Class Ophiuroidea Gray, 1840
Order Euryalida Lamarck, 1816
Family Asteronychidae Ljungman, 1867
Genus *Astrodia* Verrill, 1899

*Astrodia duospina* sp. nov.
https://zoobank.org/FC14B3BB-E9BB-4E61-A959-266A0CA733C8
Figs 2–7

Material examined. **Holotype:** CHINA • 1 specimen; Northwest Pacific, Nazimov Guyot; 15°11.34’N, 162°49.26’E; depth 2713 m; 16 September 2020; collected by ROV HAILONG III; preserved in alcohol; RSIO61068. **Paratypes:** CHINA • 1 specimen; Northwest Pacific, Nazimov Guyot; 15°11.34’N, 162°49.26’E; depth 2713 m; 16
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September 2020; collected by ROV HAILONG III; preserved in alcohol; RSIO61069 • 1 specimen; Northwest Pacific, Caiwei Guyot; 15°40.61'N, 154°53.77'E; depth 2744 m; 7 September 2013; collected by HOV JIAOLONG; preserved in alcohol; RSIO31004 • 1 specimen; the Philippine Sea, Kyushu-Palau Ridge, Roischesar Peak; 13°20.85'N, 134°32.81'E; depth 1900–2000 m; 2 August 2020; collected by ROV HAILONG IV; preserved in alcohol; RSIO59012.

**Diagnosis.** Disc raised high above the arm. Aboral disc with plate-shaped external ossicles in the center and on the periphery. Radial shield narrow, longer than wide. Teeth triangular, oral papillae indistinct or underdeveloped. Genital slits short, approximately one-fourth of the height of the disc. Lateral arm plates crescent and not projecting on arms. Arm spines no more than two.

**Description of holotype.** Disc pentagonal, notched interradial edges, 14 mm in diameter, 4.7 mm in height. Aboral surface almost flat, slightly depressed in the center, entirely covered by thickened skin with plate-shaped external ossicles in the center, about 220 µm long (Fig. 3A). Peripheral disc covered with a few plate-shaped external ossicles, similar to those in the center but larger, approximately twice in length. Radial

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**Figure 2.** In situ and on-board photos of *Astrodia duospina* sp. nov. A photo in situ (RSIO61068: the individual below, RSIO61069: the individual above, attached to an unidentified sea pen species) B photo on board (RSIO61068: the individual on the left, RSIO61069: the individual on the right) C, D photos on board (RSIO31004), aboral side (C), oral side (D). Scale bars: 10 mm (B); 20 mm (C, D).
shields narrow, tumid, bar-like, without granules or spines, and almost reach center of disc (Fig. 3A, B). Approximately 7.2 mm long and 550 µm wide in the center and 1.1 mm wide at periphery.
Oral surface flat, covered by thickened skin. Oral shield small to invisible, one madreporite. Adoral shield obscured by skin (Fig. 3C). Oral interradial surface covered with several plate-shaped external ossicles (Fig. 3C). Six teeth, triangular, forming vertical row on dental plate, each jaw covered by a pair of conical oral tentacles (Fig. 3C). Oral papillae invisible or underdeveloped. Two genital slits, small, about 1/4 as long of Figure 4.

Figure 4. Vertebrae in basal arm of *Astrodia duospina* sp. nov. (holotype: RSIO61068) A proximal view B distal view C oral view D aboral view E, F lateral view. Abbreviations: PB podial basin; LF lateral furrow. Scale bars: 200 µm (A–F).
disc height (1.3 mm long and 260 µm wide), present on oral side of each interradius (Fig. 3D). Gonads visible on each interradius (Fig. 3C, D).

Five arms, long and slender, about eight to nine times as long as disc diameter, no abrupt change in width basally (Fig. 3E). Proximal segments 2.5 mm wide and 1.7 mm high, with arched aboral surface and flattened oral surface (Fig. 3E), gradually tapering

**Figure 5.** Vertebrae in distal arm of *Astrodia duospina* sp. nov. (holotype: RSIO61068) A proximal view B distal view C oral view D aboral view E, F lateral view. Abbreviations: PB podial basin; LF lateral furrow. Scale bars: 100 µm (A–F).
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toward tip. Arm spines only present on ventral side. First to fourth tentacle pores with one arm spine and following tentacle pores with two arm spines. Outer arm spines slightly shorter than inner ones at proximal segments, but only three-fifths as long as inner spines on middle and distal segments (Fig. 3F).

Figure 6. Lateral arm plates and arm spines of Astrodia duospina sp. nov. (holotype: RSIO61068) A, B lateral arm plates from proximal arm, outer view (A), inner view (B) C, D lateral arm plates from distal arm, outer view (C), inner view (D) E, F arm spines from proximal (E) and distal arm (F). Abbreviations: MO muscle opening; NO nerve opening; R ridge. Scale bars: 200 µm (E); 100 µm (F); 90 µm (A, B); 60 µm (C, D).
Color. Pink in situ, white in alcohol (Fig. 2).

Ossicle morphology of holotype. Vertebrae articulation streptospondylyous, wider than long in proximal segments (Fig. 4A, B), longer than wide in distal segments (Fig. 5A, B). Oral side of each vertebra with longitudinal groove along midline.
deeply depressed, and no oral bridge (Figs 4C, 5C). Pair of podial basins on oral side moderate in size (Figs 4C, 5C). Aboral side of each arm vertebra with longitudinal aboral groove, moderately depressed (Figs 4D, 5D). Lateral furrow of vertebrae declining obliquely from aboral to oral side (Figs 4E–F, 5E–F). Lateral arm plates crescent-shaped, each associated with one or two arm spines and spine articulations with nerve and muscle opening separated. Spine articulation bulges outward (Fig. 6A, C). A ridge on inner side of lateral arm plate, parallel to proximal edge (Fig. 6B, D). Arm spines cylindrical, never hooked, bearing fine thorns at tip throughout arms (Figs 3F, 6E–F).

**Description of paratypes.** Two paratypes (RSIO31004, RSIO61069) share the same morphological characteristics as the holotype, disc diameter 10.17 and 13.94 mm, about 1/10 and 1/9 as wide as the length of the arms, respectively. However, the radial shields of RSIO31004 are shorter than the radial shields of the holotype and of RSIO61069 (Fig. 7F). Three arm spines exceptionally occurred only once in both paratypes (RSIO31004 and RSIO61069), the innermost arm spine of RSIO61069 is the longest and the stoutest, while the middle arm spine of RSIO31004 is the stoutest. (Fig. 7A, B). The other paratype (RSIO59012) is smaller, only 6 mm in disc diameter, about 1/3 as wide as the length of the arms and may be a juvenile of this species. The radial shields and the genital silts are much shorter than in the other three specimens (Fig. 7C, D). Likewise, the arm spines are shorter than one segment (Fig. 7E).

**Etymology.** The species name *duo* is derived from the Latin numeral word, meaning two, and Latin feminine noun, *spina*, meaning spine, referring to the presence of no more than two arm spines throughout the arm.

**Remarks.** This new species falls within the genus *Astrodia* by only possessing cylindrical unhooked arm spines. The new species resembles *Astrodia abyslicola* mostly by having plate-shaped external ossicles on the aboral disc and crescent-shaped lateral arm plates. However, the oral papillae are indistinct or underdeveloped in *Astrodia duospina*, which can be used to distinguish the two species from each other (Fig. 3D). Moreover, the genital slits are very short in *Astrodia abyslicola*, which are only one-fifth of the height of the disc, while *Astrodia duospina* has larger genital slits, being longer than one-fourth the height of the disc (Fig. 3C). *Astrodia duospina* can easily be distinguished from *A. plana* and *A. excavata* by external ossicles and lateral arm plates. External ossicles are plate-shaped on the aboral surface of the disc in *Astrodia duospina* (Fig. 3A, B), but are absent in *A. plana*. Lateral arm plates are not projecting in the new species (Fig. 3E), but are distinctly projecting from the oral surface of the arm in *A. excavata*. Additionally, the new species differs from *A. tenuispina* by having distinctly smaller genital slits (Figs 3C, 7C).

*Astrodia tenuispina* is a widely distributed species and was characterized by having slender unhooked arm spines, small and short oral papillae, separated genital slits (Verrill 1884). Baker (1980) compared specimens from south of Australia and the northwest Atlantic, described this species with 2 or 3 arm spines, and imbricating punctate scales on the disc surface. Okanishi and Fujita (2014) redescribed this species as with plate-shaped external ossicles on the periphery of the aboral disc, granule-shaped on the central disc, genital slits half of the height of the disc, lateral arm plates
not projecting. According to these descriptions, *A. duospina* sp. nov. can be differentiated from *A. tenuispina* by having smaller genital slits and indistinct oral papillae. Furthermore, in two of the three large specimens of the new species, three arm spines were observed exceptionally at one arm segment (Fig. 7A, B), while the other three species possess three arm spines at several successive segments in the middle part of the arms. Since only a small number of specimens were examined, this characteristic was not used to distinguish the new species from its congeners, and more specimens should be examined before a robust result can be achieved.

*Astrodia abyssicola* (Lyman, 1879)
Figs 8–12

*Ophiocreas abyssicola* Lyman, 1879: 64–65, plate 17, figs 470–473.
*Astrodia abyssicola*: Okanishi and Fujita 2014: 188–192, figs 2–4.

**Material examined.** China • 1 specimen; Philippine Sea, Kyushu-Palau Ridge, Mugi-boshi Seamount; 16.57.14’N, 134.52.7’E; depth 3225 m; 11 August 2021; collected by an HOV JIAOLONG; preserved in alcohol; RSIO68002.

**Description.** Disc pentagonal and almost flat, 10 mm in diameter, 3.2 mm in height, skin wrinkled under dry conditions (Fig. 9A, B). Aboral surface of disc lacks external ossicles (Fig. 9A, B). Radial shields narrow, slightly tumid, bar-like, with-
out granules or spines, and almost reaching center of disc. (Fig. 9A). Approximately 3.8 mm long and 0.6 mm wide in center and 0.8 mm wide on periphery (Fig. 9A).

Oral surface flat, covered by thin skin, and lacking external ossicles (Fig. 9C). Oral shield triangular, one madreporite (Fig. 9D). Adoral shield big and thick, quadrangular, and longer than wide (Fig. 9D). Teeth spearhead-shaped, vertically on dental plate;
each jaw bears a pair of short, conical oral tentacles (Fig. 9C). Oral papillae indistinct or underdeveloped (Fig. 9C). Two genital slits very short, 560 µm long and 110 µm wide, present on oral side of each interradius. Gonads visible in each interradius (Fig. 9D).

Five arms, long and slender, about nine to ten times as long as disc diameter, no abrupt change in width basally (Fig. 9E). Proximal portion of arm 1.8 mm wide and 420 µm high, with arched aboral surface and flattened oral surface. Arms tapering
Description of two species of the genus *Astrodia*

Figure 11. Vertebrae in distal arm of *Astrodia abyssicola* holotype: RSIO68002) A proximal view B distal view C oral view D aboral view E, F lateral view. Abbreviations: PB podial basin; LF lateral furrow. Scale bars: 150 µm (C, D, E); 90 µm (A, B, F).

Gradually toward tip. Arm spines only present in ventral part of arm. First to third tentacle pores without arm spines, fourth tentacle pores with one arm spine and following tentacle pores with two arm spines. Inner arm spines longer than outer arm spines. On middle and distal part of arm, outer arm spines three-fourths as long as inner spines (Fig. 9F). Three arm spines occurred once in two of the five arms. Lateral arm plates not projecting on arms.
Figure 12. Lateral arm plates and arm spines of Astrodia abyssicola (RSIO68002) A, B lateral arm plates from the proximal arm, outer view (A), and inner view (B) C, D lateral arm plates from the distal arm, outer view (C), and inner view (D) E, F arm spines from proximal (E) and distal (F). Abbreviations: MO muscle opening; NO nerve opening; R ridge. Scale bars: 90 µm (A, B, F); 60 µm (C, D, E).

**Color.** Bright pink in situ, entirely white in alcohol (Fig. 8B, C).

**Ossicle morphology.** Vertebrae articulation streptospondylos, wider than long in proximal segments (Fig. 10A, B), longer than wide in distal segments (Fig. 11A, B). Oral side of each vertebra with longitudinal groove along midline, deeply depressed, and no oral bridge (Figs 10C, 11C). Pair of podial basins on oral side moderate
in size (Figs 10C, 11C). Aboral side of each arm vertebra with longitudinal aboral groove, moderately depressed (Figs 10D, 11D). Lateral furrow of vertebrae declining obliquely from aboral to oral side (Figs 10E, F, 11E, F). Lateral arm plates crescent-shaped, each associated with one or two arm spines. Spine articulations with separated nerve and muscle openings, bulging outwards (Fig. 12A, C). A ridge on inner side of lateral arm plate (Fig. 12D). Arm spines cylindrical, never hooked, bearing fine thorns at apex throughout arms (Fig. 12E, F).

Remarks. Ophiocreas abyssicola was first described by Lyman (1879). Okanishi and Fujita (2014) transferred O. abyssicola to the genus Astrodia and redescribed it. This specimen (RSIO68002) was identical to Astrodia abyssicola by having 0–2 arm spines, rather short genital slits and crescent-shaped lateral arm plates. However, this specimen lacks external ossicles on the disc and arms, which is different from previous descriptions of Astrodia abyssicola by Okanishi and Fujita (2014) as having plate-shaped external ossicles on the periphery. Nevertheless, the genetic distance of COI and 16S (2.9% and 1.9%) between the new collected specimen and A. abyssicola are too small to justify two different species. Therefore, this specimen was identified as A. abyssicola, thus the external ossicles on the aboral surface of the disc could be plate-shaped or absent in this species.

Key morphological characters to the species of Astrodia

The key morphological characters among the five species from the genus Astrodia based on Okanishi and Fujita (2014) were revised in this study (Table 3). Three diagnostic characteristics were proposed by Okanishi and Fujita (2014) in their key for Astrodia: the length of the genital slits related to the height of the disc, external ossicles on the aboral disc surface, and shape and existence of projections of lateral arm plates. All three characteristics were useful to distinguish the new species from its congeners. The external ossicle, being absent in the A. abyssicola specimen examined in the present study but present and plate-shaped in the previous descriptions (Okanishi and Fujita 2014), might be an intraspecific variation. Additionally, we added two morphological characters, the number of arm spines and the shape of oral papillae, as key characters for interspecific discrimination of Astrodia. Astrodia abyssicola is the only species that possesses no more than two arm spines along their arms, whereas the other four species possess up to three arm spines or occasionally four. Furthermore, oral papillae are indistinct or underdeveloped in A. duospina sp. nov., but are domed granule-shaped in the four known species. Thus, we consider the number of arm spines and the shape and existence of oral papillae important characteristics for interspecific discrimination within Astrodia (Table 3).

Molecular phylogenetic analysis

Based on the COI (583–1511 bp) and 16S (431–539 bp) sequences, the phylogenetic relationship of the two genera, Astrodia and Asteronyx, was inferred. The ML tree based on the concatenated 16S and COI sequences suggested that both Astrodia and Asteronyx were monophyletic with high bootstrap values (Fig. 13, Suppl. material 1: Fig. S1). The ML tree based on COI sequences was consistent with the tree generated
Table 3. Comparison of key morphological characters among species in the genus *Astrodia*.

| Species                  | Arm spines | Genital slits | External ossicles | Lateral arm plates on middle to distal portion of arms | Oral papillae | Reference                                                                 |
|--------------------------|------------|---------------|-------------------|-------------------------------------------------------|--------------|---------------------------------------------------------------------------|
| *Astrodia abys-sicola* (Lyman, 1879) | 0–2        | very short, -1/5 (height of disc) | plate-shaped on periphery | shapes: crescent; projections: absent | domed granule-shaped | Lyman (1879), Okanishi and Fujita (2014), This study |
| *Astrodia excavata* (Lütken & Mortensen, 1899) | 0–3        | large, -2/3 (height of disc) | granule-shaped near radial shields and genital slits | shapes: bar-like; projections: present | domed granule-shaped | Lütken and Mortensen (1899), Okanishi and Fujita (2014) |
| *Astrodia plana* (Lütken & Mortensen, 1899) | 0–3        | short, -1/4 (height of disc) | absent | shapes: oblong; projections: absent | domed granule-shaped | Lütken and Mortensen (1899), Döderlein (1927), Okanishi and Fujita (2014) |
| *Astrodia tenuispina* (Verrill, 1884) | 0–3, occasionally 4 | short, -1/2 (height of disc) | plate-shaped on periphery, granule-shaped in center | shapes: unknown; projections: absent | domed granule-shaped, small and short | Verrill (1884), Koehler (1906), Koehler (1922), Baker (1980), Gage et al. (1983), Manso (2010), Okanishi and Fujita (2014) |
| *Astrodia duospina* sp. nov. | 0–2, occasionally 3 | short, -1/4 (height of disc) | plate-shaped on periphery and in center | shapes: crescent; projections: absent | indistinct or underdeveloped | This study |

Figure 13. Maximum likelihood tree of the genus *Astrodia* based on concatenated sequences of COI and 16S (clades of Lin 1A, Lin 1B, Lin 2A, Lin 2B, Lin 2C and Lin 3 are from Okanishi et al. (2018), more detailed information about these clades showed in Suppl.materials. Values of each clade: SH-aLRT support (%) / ultrafast bootstrap support (%)).
from two genes (Suppl. material 1: Fig. S2), while in the ML tree based on 16S sequences, *Astrodia abyssicola* clustered with *Asteronyx*, with a low bootstrap value (Suppl. material 3: Fig. S3). Okanishi et al. (2018) suggested that the relationship of the two genera was unclear based on COI and 16S sequences. With newly sequenced DNA data added, our results indicated that the two genera are probably monophyletic. Additionally, the genetic distances of COI and 16S between *A. duospina* sp. nov. and *A. abyssicola* were 9.0% and 9.1%, respectively, supporting the morphological identification results. Molecular analysis also supported that the three specimens identified as *Astrodia duospina* sp. nov. are the same species, and the specimen identified as *Astrodia abyssicola* is closely related to the published sequence of this species with very small genetic distances (2.9% for COI and 1.9% for 16S) that fall into the intra-species genetic distance of Euryalida (Okanishi et al. 2018; Nethupul et al. 2022).

**Conclusion**

In this study, we described a new species of the genus *Astrodia* collected from seamounts in the West Pacific, and another species (*Astrodia abyssicola*) was redescribed. Through comparing the five species of *Astrodia*, the tabular key of Okanishi and Fujita (2014) was revised and two additional key characteristics, the number of arm spines and the shape of the oral papillae, were identified for interspecific discrimination of *Astrodia*. Maximum likelihood trees supported our morphological results and suggested that both *Astrodia* and *Asteronyx* were monophyletic. This study provided both morphological and molecular information of the two *Astrodia* species, and the specimens reported further expanded the known geological distribution of the genus.

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**Supplementary material 1**

**Figure S1**

Authors: Xiaojun Xie, Dongsheng Zhang

Data type: Image.

Explanation note: Maximum likelihood tree of the genus *Astrodia* and *Asteronyx* based on concatenated sequences of COI and 16S.

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**Supplementary material 2**

**Figure S2**

Authors: Xiaojun Xie, Dongsheng Zhang

Data type: Image.

Explanation note: Maximum likelihood tree of the genus *Astrodia* and *Asteronyx* based on COI sequences.

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Supplementary material 3

Figure S3
Authors: Xiaojun Xie, Dongsheng Zhang
Data type: Image.
Explanation note: Maximum likelihood tree of the genus *Astrodia* and *Asteronyx* based on 16S sequences.
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