Description of three species of Ophioplinthacids, including a new species, from a deep seamount in the Northwest Pacific Ocean (#55728)

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Description of three species of Ophioplinthacids, including a new species, from a deep seamount in the Northwest Pacific Ocean

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Five specimens of brittle star were collected from a deep-sea seamount in the Northwest Pacific, and identified into three species. One of which is new to science, *Ophioplinthaca grandisquama* n. sp., can be easily distinguished from its congeners by the distinctly elongated and stout tentacle scales, stout and long disc spines, capitate with typically elongate to flaring head bearing numerous distinct thorns, radial shields roughly triangular and contiguous distally. One specimen was identified as *Ophioplinthaca semele* (A.H. Clark, 1949), which had been reported in Hawaii seamounts, is a new record of this species in the Northwest Pacific. The remaining specimen was an unknown species of *Ophioplinthaca*, with some different characteristics from other species of *Ophioplinthaca*. However, we, herein, prefer not to attach a name to this specimen until more morphological characteristics are available. The finding of this new species and two new records further enriches the distribution of *Ophioplinthaca* in the seamount of Northwest Pacific, providing useful information for marine protection in the cobalt-rich area.

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3 Northwest Pacific Ocean

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14 **Abstract**

- 15 Five specimens of brittle star were collected from a deep-sea seamount in the Northwest Pacific,
- and identified into three species. One of which is new to science, *Ophioplinthaca grandisquama*
- 17 n. sp., can be easily distinguished from its congeners by the distinctly elongated and stout
- 18 tentacle scales, stout and long disc spines, capitate with typically elongate to flaring head bearing
- 19 numerous distinct thorns, radial shields roughly triangular and contiguous distally. One specimen
- 20 was identified as Ophioplinthaca semele (A.H. Clark, 1949), which had been reported in Hawaii
- 21 seamounts, is a new record of this species in the Northwest Pacific. The remaining specimen was
- an unknown species of *Ophioplinthaca*, with some different characteristics from other species of
- 23 *Ophioplinthaca*. However, we, herein, prefer not to attach a name to this specimen until more
- 24 morphological characteristics are available. The finding of this new species and two new records
- 25 further enriches the distribution of *Ophioplinthaca* in the seamount of Northwest Pacific,
- 26 providing useful information for marine protection in the cobalt-rich area.

27 28 **[**]

Introduction

Ophioplinthaca Verrill, 1899 is a genus in the family Ophiacanthidae Ljungman, 1867

- 30 which is distinguished from other Ophiacanthid genera by the deep interradial incisions into the
- 31 disc which are lined distally by enlarged disc plates (O'Hara & Stöhr, 2006). Ophioplinthaca is a
- 32 widely distributed genus, and thirty-one valid species are known around the world. Among
- 33 which, twenty-one species have been found occurring in the Indo-Pacific Ocean, six in the West
- 34 Indian Ocean, and seven in the Atlantic Ocean. Recently, it was suggested that *Ophioplinthaca* is
- one of the dominant groups of megafauna in seamounts (O'Hara, Rowden & Williams, 2008;



36	Ch 008). The northwest Pacific region has the highest number of seamounts globally (Yesson
37	et al., 2011), and many of the seamounts are covered with cobalt-rich ferromanganese crusts,
38	which is a valuable mineral (Hein et al., 2009). However, few studies of Ophiuroid in this area
39	were reported (Litvinova, 1981; Zhang et al., 2018; Na et al., 2019).
40	In 2019, several <i>Ophioplinthaca</i> specimens were collected from a sear nt in the Northwest
41	Pacific by a Remotely Operated Vehicle (ROV). Three specimens were determined to be a new
42	species of genus Ophioplinthaca which we described herein. The other two specimens, identifed
43	as Ophioplinthaca semele and an unknown species, were described here as new records of
44	Ophioplinthacids in the Northwest Pacific Ocean. This study provides biodiversity information
45	of seamounts in the cobalt-rich area, which may be useful for marine protection from future
46	deep-sea mining.
47	
48	Materials & Methods
49	Ophiuroid specimens from a seamount in the Northwest Pacific Ocean were collected
50	during cruise DY56 using an ROV HAILONG III. Sampling sites are shown in Fig. 1. Specimens
51	were fixed in 90% ethanol on board and deposited in the sample Repository of the Second
52	Institute of Oceanography (RSIO), Ministry of Natural Resources, Hangzhou, China.
53	Specimens were examined and photographed using a stereoscopic microscope (Zeiss Axio
54	Zoom.V16). Arm skeletal elements were obtained after submerging in commercial bleach (2.5% $$
55	NaOCl), until all soft issue dissolved, washed in distilled water, air-dried and then mounted on
56	stubs, imaged using a Hitachi TM1000 scanning electron microscope.
57	Genomic DNA was extracted from arm tissue using DNeasy® Blood & Tissue Kit
58	(QIAGEN) following the manufacturers' protocols. The mitochondrial COI sequences were
59	amplified with primers listed in Table 1. PCR reactions were performed using 50 μL volumes
60	containing: 5 μ L 10 x Buffer (containing Mg ²⁺), 10 mM of each dNTP, 0.1 mM of each primer,
61	$37.5~\mu L$ of ddH $_2O$, $2.5~U$ of Taq DNA Polymerase (Vazyme, China), and $2~\mu L$ of DNA template.
62	PCR products were purified with QIAquick PCR purification kit (QIAGEN) following the
63	protocol supplied by the manufacturer. Sequencing was performed by Sangon Biotech
64	(Shanghai, China) on an ABI 3730XL DNA analyzer (Applied Biosystems).
65	To date, only 10 COI sequences of Ophioplinthaca are available from the Genbank and
66	BOLD database (Table 2). In this study, we included another two COI sequences of O. defensor



from a recent study (Na et al., in press). Totally, 19 COI sequences (Table 2), including 5 new 67 68 sequences and 2 sequences from *Ophiacantha* as outgroup, were used for phylogenetic analysis. 69 COI sequences were aligned using Geneious Prime 2019 with default settings. Phylogenetic 70 analysis was conducted by RAxML (Stamatakis, 2014), with a 1000-replicate bootstrap support value for each node and a GTR+I+T substitution model. Pairwised genetic distance (K2P) were 71 72 calculated for COI sequences in MEGA6 (Tamura et al., 2013). The Automatic Barcode Gap Discovery (ABGD) analysis (Puillandre et al., 2012) was carried out on the web interface 73 74 (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) to establish molecular operational 75 taxonomic units (MOTUs) from COI gene sequence data. The Kimura (K80) model (Kimura, 1980) with a TS/TV of 2.0 (K2P), Pmin = 0.001, Pmax = 0.1, 10 steps and a relative gap width 76 of 1.0. 77 78 Nomenclatural acts 79 The electronic version of this article in Portable Document Format (PDF) will represent a 80 published work according to the International Commission on Zoological Nomenclature (ICZN), 81 and hence the new names contained in the electronic version are effectively published under that 82 Code from the electronic edition alone. This published work and the nomenclatural acts it 83 contains have been registered in ZooBank, the online registration system for the ICZN. The 84 ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed 85 through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The 86 LSID for this publication is: urn:lsid:zoobank.org:pub:A48B7301-0D4B-4280-BF81-87 639689F923F6. The online version of this work is archived and available from the following 88 digital repositories: PeerJ, PubMed Central and CLOCKSS. 89 Results 90 91 **Systematics** 92 Class Ophiuroidea Gray, 1840 93 Order Ophiacanthida O'Hara, Hugall, Thuy, Stöhr and Martynov, 2017 94 Family Ophiacanthidae Ljungman, 1867 95 Genus Ophioplinthaca Verrill, 1899 96 Ophioplinthaca grandisquama n. sp. (Fig. 2-5) 97 urn:lsid:zoobank.org:act:8509E6DB-E902-4A71-9339-EA40725DD688



99 specimens (RSIO56013, RSIO56014, RSIO56060). 100 **Habitat.** All three specimens of the new species were attached to a *Calyptrophora* (Fig. 2). 101 **Etymology.** The specific name alludes to the large and long tentacle scales. 102 **Description of the holotype.** Disc 7 mm d.d., mod rapely high, five arms-and at least six times of the disk diameter in length. Disc incised interradially more than 1/5 d.d. creating five wedge-103 104 shaped divisions which in contrast to the sunken centre and interradii of the disc (Fig. 3A). Each 105 division covered by a pair of large radial shields and a number of irregular plates. Radial shields 106 naked, triangular, about 1/4 d.d. in length, one and a half times as long as wide with a truncate distal edge and a sharp proximal angle, broadly contiguous distally (Fig. 3A). Disc plates 107 overlapping, covered with distinctly elongated disc spines, up to 0.8 mm in length, 4-6 times as 108 109 high as wide. Disc spines stout, bearing numerous distinct thorns on both lateral side and apex, 110 some bifurcated into two prongs at the top, one of the two prongs elongated and inflated. (Fig. 3B). Ventral disc surface covered in small and overlapped plates, few of which bear spines 111 112 thinner than those on the dorsal surface. Genital slits wide, extending from the oral shields to the 113 dorsal disc surface (Fig. 3C). 114 Oral shields arrow-head-like shape, with an obtuse proximal angle, rounded laterals and a 115 small obtuse distal lobed, 2 times as wide as long, one of which is expanded as madreporite. 116 Adoral plates quadrilateral, 2 times as long as wide, not separating the oral shields from the 117 lateral arm plate. Jaw triangular, wider than long with 1-2 blunt and serrated apical papillae, and 118 3-4 conical lateral oral papillae longer than wide with pointed tip (Fig. 3C). One oral tentacle scale situated between the gap of two jaws, slightly larger than oral papillae, often longer than 119 120 high with a rounded free edge and covered by distal oral papillae. 121 Five arms, wide and slightly moniliform. Dorsal arm plates trapezoid to triangular with 122 slightly convex distal edge on proximal segments, contiguous to each other; on distal segments dorsal arm plates change to fan-shaped and just contiguous (Fig. 3D). Basal ventral arm plate 123 124 trapezoid much wider than long with a short proximal edge, concave and diverging lateral edges, distal margin much wider. The following plates become pentagonal, slightly wider than long, 125 126 with a sharp proximal angle, diverging lateral sides which are widely excavated by the 127 corresponding tentacle scales, distal margin board and convex, all separated from each other 128 (Fig. 3F). Tentacle pores covered on the first segments with one or two leaf-shaped scales, and a

Material examined. — St. RC-ROV05, 161.78°E, 15.54°N, 1049m, September 17, 2019, 3



129	tentacle; one fusiform or conical tentacle scale from the second segments, elongated and thorny
130	with a trunk base tapering into a blunt point, slightly longer than one arm segment; tentacles long
131	and glassy, longer than the ventral arm plates, absence from the seventh arm segments (Fig. 3C,
132	F). Arm spines seven, up to three arm segments in length on proximal arm segments, dorsally
133	four arm spines are thin with distinct lateral thorns, tapering into a sharp point, the second dorsal-
134	most arm spines longest; ventral arm spines shorter and blunt, finely rugose (Fig. 3D). Color in
135	life orange-brown.
136	Description of the paratype. The two paratypes (RSIO56013, RSIO56014) share the same
137	morphological characteristics with the holotype (Fig. 4A-D, 5A-E). For one of the two paratypes
138	(RSIO56014) (Fig. 4A, B), the oral structure is incomplete with one of five oral plate sets is
139	missing, which may be due to the malfaction or predation. The remaining oral shields are
140	relatively smaller than the holotype, adoral plates are wider. The other paratype (RSIO56013)
141	with stronger disc spines, elongate to flaring head bearing numerous distinct thorns, up to 1.4
142	mm in length, 4-6 times as high as wide (Fig. 4C, D). Tentacle pores covered, on the proximal
143	arm segments one elongated with a rounded base tapering to a blunt point scales, and on
144	tentacle.
145	Description of the skeletal elements (Paratype: RSIO56014): Oral plates longer than high, only
146	slightly lower in central part than at proximal end, adradial muscle fossa large with finer mesh
147	stereom than remaining ossicle, conspicuous s-curved suture line crosses foot basin (Fig. 5F). A
148	row of three papillae sockets and pores near lower edge of adradial proximal oral plate as
149	articulations of oral papillae (Fig. 5G). Dental plate entire with single column of wide sockets,
150	dorsal and ventral beer below the level of middle part of the dental plate, not penetrating (Fig.
151	5H). Adradial genital plate long, articulation surface with slightly elevated elongated condyle
152	(Fig. 5I, K); abradial genital plate slightly smaller in size than adradial plate and articulating
153	below level of the adradial plate condyle (Fig. 5J). Radial shield longer than wide, with abradial
154	projection and convex radial edge (Fig. 5L). Internally, radial shield with one distal domed
155	condyle and one groove (Fig. 5M).
156	The vertebrae articulation zygospondylous, wider than long in proximal segments and gradually
157	change to longer than wide from the middle to distal segments (Fig. 5N-R). The aboral groove on
158	the dorsal side is moderately expressed without extension (Fig. 5N). The podial basins on the
159	ventral side are moderate in size (Fig. 50). Lateral arm plates (LAP) with constriction in



160 proximal part leading to raised distal portion (Fig. 5S). The external surface of the LAP consisted of regularly meshed stereom, mesh size gradually decreasing from the middle to the proximal 161 162 margin, while in the distal part, mesh size is mostly small but larger in the distal margin (Fig. 5S). Arm spine articulations well developed, volute-shaped, dorsal and ventral lobes merged at 163 their proximal tips, sigmoidal fold present (Fig. 5S). The muscle opening is larger than the nerve 164 165 opening (Fig. 5S). On the internal side₃ a row of perforations on the distal part, parallel to the arrangement of spine articulation (Fig. 5T). A short ridge continuous, obliquely stretching from 166 the ventral perforations to the middle of proximal edges. A prominent knob close to the ventral 167 edge, separated from the ridge (Fig. 5T). 168 **Remark.** Ophioplinthaca grandisquama n. sp. is characterized by the stout disc spines, capitate 169 with typically elongate to flaring head bearing numerous distinct thorns, radial shields roughly 170 triangular, more than 1/5 d.d. in length and contiguous distally, the tentacle scales elongated and 171 stout. The thick tentacle scales in O. grandisquama n. sp. are elongated with a rounded base 172 tapering to a blunt point and covered in irregular thorns similar to arm spines, which is distinctly 173 174 distinguished from its congeners and most of them bearing oval or leaf-shaped tentacle scales 175 (Thomson C.W., 1877; Lyman, 1878; Lyman, 1883; Clark H.L., 1900; Koehler, 1904; Clark H.L., 1911; Koehler, 1922; Mortensen, 1933; Clark H.L., 1939; Koehler, 1930; Clark A.H., 176 177 1949; John & A.M. Clark, 1954; Cherbonnier & Sibuet, 1972; Guille, 1981; O'Hara and Stöhr, 2006). 178 179 The sizes and shapes of radial shields and disc spines have been suggested to be the primary criteria for delimiting species (O'Hara & Stöhr, 2006). O. hastata Koehler, 1922 and O. globata 180 181 Koehler, 1922, which resemble the new species mostly, also have stout and capitate disc spines. However, in O. grandisquama n. sp., the disc spines are more elongated, 4-6 times as high as 182 183 wide, bearing numerous distinct thorns all over the whole spine except the basal trunk, whereas the disc spines are only 2-3 times as high as wide, capitate with a convex to flaring head bearing 184 numerous small thorns in O. hastata, and are cylindrical to conical with obvious thorns only in 185 the upper half in O. globata with similar height-width ratio to O. hastata (O'Hara & Stöhr, 186 187 2006). Radial shields are relatively small, in O. hastata and O. globata, with 1/6 d.d. and 1/5 to 188 1/8 d.d. in length, respectively, and contiguous distally or separate, instead of 1/4 d.d. in length, broadly contiguous distally in the new species. Additionally, dorsal arm plates are also different 189 190 between the new species and O. hastata and O. globata. Dorsal arm plates are a little longer than



wide or as wide as long, separated from the basal arm segments, instead of contiguous at least on 192 proximal segments in O. grandisquama n. sp. 193 Another species, O. amezianeae O'Hara & Stöhr, 2006 and O. rudis (Koehler, 1897), were 194 described with elongated spines, greater than 3 times as high as wide in this genus. The former is clearly different from O.grandisquama n. sp. in having slender disc spines, with a rounded base 195 196 tapering to a sharp point or terminating in 2-3 small thorns, radial shields separate, oral shields as long as wide. The latter can be distinguished by having needle-like disc spines, long and slender, 197 up to 1.3 mm in length, smooth to finely serrate, pentagonal oral shields, and bottle-shaped to 198 pointed tentacle scales, half as long as the ventral arm plate. 199 200 Ophioplinthaca semele (A.H. Clark, 1949) (Fig. 6-8) 201 Material examined. — St. RC-ROV08, 161.81°E, 15.53°N, 1024m, September 20, 2019, 1 202 specimen (RSIO56057). 203 204 **Habitat and Distribution.** This specimen was found attaching on a blade-like glass sponge together with a sea lilly (Fig. 6). The holotype and other specimens were collected near Hawaii 205 (537-1250m); this is the first record of this species from a seamount in the Northwest Pacific 206 207 (1024m). **Description of morphological characteristics.** Disc 11.2 mm d.d., moderately high, arms at 208 209 least six times disk diameter. Disc almost incised interradially 1/3 d.d., creating five wedge-210 shaped divisions covered by a pair of large, naked radial shields and a number of irregular plates (Fig. 7A). Radial shields triangular, about 1/3 d.d. in length, 1.5-2 times as long as wide with a 211 212 truncate distal edge and blunt proximal angles, contiguous for 1/3-1/2 of the length (Fig. 7B). 213 Disc plates overlapping, bearing cylindrical swollen stumps, up to 0.5mm high, covered in 214 obvious thorns on the upper half (Fig. 7C, 8A). Disc spines at the distal margin and between radial shields are thinner with less thorns (Fig. 7C). Ventral disc surface covered in small and 215 216 overlapped plates, without spines. Genital slits long and wide (Fig. 7D). 217 Oral shields diamond-shape, with an obtuse proximal angle, rounded laterals and an obtuse to lobed distal angle, 2 times as wide as long, one of which expanded as madreporite (Fig. 7D). 218 219 Adoral plates quadrilateral, large and broad, two times as long as wide, not separating the oral 220 shields from the first lateral arm plate. Jaw triangular, as long as wide with 2-3 thin and long 221 apical papillae. Lateral oral papillae 4-5, pointed, up to 3 times longer than wide, and the distal



223 elongated oral tentacle scale (Fig. 7D). 224 Five arms, wide and slightly moniliform. First dorsal arm plate wider than long with obtuse 225 proximal angle and straight distal border. Succeeding plates triangular to scallop-shaped with convex distal edge, slightly wider than longer, separated from each other (Fig. 7B, E, 8B). 226 Ventral arm plates pentagonal with a sharp proximal angle, diverging lateral sides which are very 227 228 widely excavated by the corresponding tentacle scales, distal side convex, widely separated from 229 each other (Fig. 7F, 8C). Tentacle pores on the first arm segments, covered with one or two 230 scales leaf-like, pointed and spiniform, more than half length of ventral arm plates, decreasing to one scale thereafter until nearly the end of the arm; One glassy tentacle on each tentacle pore. 231 longer than the ventral arm plates, absent fructhe tenth arm segments (Fig. 7D, F). The 232 233 proximal arm segments bearing up to eight arm spines, with sharp tip and distinct teeth, almost 234 meeting each other on the dorsal mid-line on the fourth segments (Fig. 7E). The third dorsalmost 235 arm spines are the longest, up to three segments in length, lowermost shortest, one segment in length. As the arm segments reduced distally, arm spines reduced to five. Color in life orange-236 237 white. **Description of the skeletal elements**. The vertebrae articulation zygospondylous, wider than 238 239 long in proximal segments and gradually change to longer than wide from the middle to distal segments (Fig. 8D-H). The aboral groove on the dorsal side is moderately expressed without 240 extension (Fig. 8E). The podial basins on the ventral side are moderate in size (Fig. 8F). LAP 241 242 with constriction in proximal part leading to raised distal portion. Arm spine articulations well 243 developed, volute-shaped, dorsal and ventral lobes merged at their proximal tips, sigmoidal fold present (Fig. 8I). The muscle opening is larger than the nerve opening. On the internal side, a 244 245 group of small, irregular perforations near kink between central oblique and short ventral ridges 246 (Fig. 8J). A prominent knob close to the ventral edge, separated from the ridge (Fig. 8T). **Remark.** This specimen was identified as O. semele (A.H. Clark, 1949) based on the multiple 247 apical papillae, large radial shields contiguous for 1/3-1/2 of the length distally, cylindrical disc 248 249 stumps with obvious thorns on the top and upper half. It also has some slight differences, having 250 two or three shaped and leaf-shaped tentacle scales on the first tentacle pore instead of three or more broad and spoon-shaped scales in the holotype, adoral plates complete instead of divided 251 252 into two or more plates in the holotype. Clark (1949) suggested the difference of tentacle scales

two oral papillae slightly broadened and leaf-shaped, standing vertical by the squeezing of an



253 and lateral oral papillae between two smaller specimens and holotype. Furthermore, tentacle 254 scale is not reliable for species delimitating in the genus *Ophioplinthaca* (O'Hara & Stöhr, 255 2006). Therefore, with only one specimen of this species in our collection, these differences are 256 possibly inter-species variation rather than the characteristics for taxonomic delimitation. The cylindrical disc granules with a flaring top of a few thorns are reminiscent of 257 258 Ophioplinthaca citata Koehler, 1904 from the New Caledonia, which can be differed by having narrower radial shields, contiguous dorsal and ventral arm plates and single ventral-most teeth 259 (O'Hara & Stöhr, 2006). Besides, several other species are also close to O. semele in the shape of 260 disc spines. *lobata* also has cylindrical to conical granules, the upper half covered in obvious 261 thorns, but can be differed in having single ventral-most tooth, togethre with some other 262 263 differences such as size and shape of radial shields and jaws, number of arm spines. On clothilde A.H. Clark, 1949 has stumps terminating in flaring irregular crown of a dozen or more spines, 264 and O. lithosora (H.L. Clark, 1911) has low cylindrical stumps with two to six tiny thorns near 265 266 the apex. But they can be distinctly distinguished by size of radial shields and the number of 267 apical papillae. 268 *Ophioplinthaca* sp. (Fig. 9-11) 269 Material examined. — St. RC-ROV08, 161.80°E, 15.52°N, 1146m, September 20, 2019, 1 270 271 specimen (RSIO56058). **Habitat.** This specimen was found attaching on a *Narella* (Fig. 9). 272 273 **Description of morphological characteristics.** Disc 10.4 mm d.d., mode by high, arms at 274 least seven times of disk diameter. Disc incised interradially 1/3 d.d., creating a wedge over each arm base, wedges tumid in contrast to the sunken centre and interradii of disc (Fig. 10A). Radial 275 shields naked, triangular, more than 1/4 d.d. in length, 1.5~2 times as long as wide with a round 276 277 distal margin and a sharp proximal angle, distally contiguous more than half of the length, and 278 separated proximally by a triangular plate (Fig. 10A). The center of the disc is occupied by small irregular plates, bearing small granules up to 0.25 mm high, 1~1.5 times as high as wide, 279 280 cylindrical to capitate with a terminal crown of thorns (Fig. 10B). Ventral disc surface covered in small and uneven plates without granules (Fig. 10C). Genital slits long and wide. 281 Oral shields diamond-shaped, with an obtuse proximal angle, rounded laterals and an obtuse 282 283 to lobed distal angle, 2 times as wide as long, one of which is expanded as madreporite (Fig.



284	10C). Adoral plates quadrilateral, 3 times as long as wide, not separating the oral shields from
285	the first lateral arm plate. Jaw triangular, wider than long with 1 blunt apical papilla, and 3 lateral
286	oral papillae swollen and conical, gradually decrease from inside to outside. One large oral
287	tentacle scale situated under the distal oral papillae, conical and atropic, or elongate, up to 2
288	mm long (Fig. 10C).
289	Five arms, wide and slightly moniliform. Dorsal arm plates triangular to scallop-shaped
290	with convex distal edge, separated with each other (Fig. 10D). Ventral arm plates pentagonal,
291	separated from each other, with a small proximal angle, diverging lateral sides which are
292	excavated by the corresponding tentacle scales, and distal side convex (Fig. 10C, E). Tentacle
293	pores on the first arm segments, covered with one or two scales, decreasing to one scale
294	thereafter to the end of the arm, and one tentacle. Tentacle scales thick and smooth on the basal
295	segments, change to smaller, leaf-like and thorny on the following segments, almost half length
296	of the ventral arm plates; tentacles long and glassy, longer than the ventral arm plates,
297	disappeared from the seventh arm segments (Fig. 10C, E). The proximal arm segments with up
298	to seven spines, of which the dorsally second or third are the longest, three segments in length,
299	lowermost shortest, one segment in length (Fig. 10D). The number of arm spines reduced to four
300	on distall segments.
301	Description of the skeletal elements. The vertebrae articulation zygospondylous, wider than
302	long in proximal segments and gradually change to longer than wide from the middle to distal
303	segments (Fig. 11D-H). The aboral groove on the dorsal side is moderately expressed without
304	extension (Fig. 11E). The podial basins on the ventral side are normal rate in size (Fig. 11F). LAPs
305	with constriction in proximal part leading to raised distal portion (Fig. 11I). Arm spine
306	articulations well developed, volute-shaped, dorsal and ventral lobes merged at their proximal
307	tips, sigmoidal fold present (Fig. 11I). The muscle opening is larger than the nerve opening (Fig.
308	11I). On the internal side, a group of small, irregular perforations near kink between central
309	oblique and short ventral ridge (Fig. 11J).
310	Remark. This specimen is characterized by the deep interradial incisions, radial shields twice as
311	long as wide, 1/4 d.d. in length, contiguous for most of their length, the disc spines cylindrical to
312	capitate with a terminal crown of thorns, and jaw wider than long with 1 blunt apical papilla and
313	3 small lateral oral papillae in each side, gradually decrease from inside to outside.
314	Ophioplinthaca pulchra Koehler, 1904 is similar to our specimen in the shape of disc spines, but



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it differs in having some spherical and smooth disc granules, large radial shields, up to 1/3 mm d.d., only contiguous distally, oral shields much longer than wide, and nine pointed to square-shaped oral papillae in each jaw. *Ophioplinthaca pulchra* is quite similar to *Ophioplinthaca plicata* (Lyman, 1878), and can be difficult to distinguish. Θ_{λ} plicata is highly variable, particularly in the shape of the disc stumps, the position of the radial shields, and the shape of oral shields (O'Hara & Stöhr, 2006). Some features of this specimen fall within the range of variation, such as the broadly contiguous radial shield and small oral shield, but the capitate disc spines and only three small lateral oral papillae in each side of jaws can be distinguished from *O.plicata*. However, the limits of species in genus *Ophioplinthaca* are obscure (O'Hara & Stöhr, 2006) and with only one specimen, it is impossible to provide a full description of the range of variation and stable characteristics for diagnosis, therefore, we prefer not to attach a name to this single specimen.

Phylogeny

The phylogenetic analysis (Fig. 12) supported that O. grandisquama n. sp. is clearly distinguished from other species of *Ophioplinthaca*, furthermore, the genetic distance among the three specimens of O. grandisquama n. sp. is 0.2%-0.9% (Table 3), supporting that they belong to the same species (Ward et al. 2008). The maximum likelihood tree showed that O. semele was clustered with O. rudis, whereas Ophioplinthaca sp. was clustered with O. globata. The genetic distances between O. semele and O rudis was 3.2%, between O. sp. and O. globata was 6.3%, which confirm that they are different species. Although O. semele was closely related to O. rudis, they can be easily differed from each other based on the morphological characteristics, especially in the shape of disc spines, which are needle-like, long and slender in O.rudis instead of cylindrical with obvious thorns on the upper half in O. semele. O_1 globata differs from O. sp. in having variable disc spines, many cylindrical to conical, others with only 3 terminal thorns or trifid with bifurcated tips, and radial shields 1/5-1/8 d.d. in length, only contiguous distally or completely separated, whereas in O.sp., radial shields 1/4 d.d. in length, contiguous for most of their length, and the disc spines cylindrical to capitate with a terminal crown of thorns. The genetic distances within the other two species, O. defensor and O. plicata, were very small, 0.1%-2.8% and 0.4%-1.2%, respectively. The genetic distances between the two species in this study and their closest congeners are higher than the intraspecies genetic distances, supporting the results that they are different species. In addition, we used molecular species delimitation



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- method of ABGD delimit species of Ophioplinthaca. In total, the ABGD method identified 10 346 putative species from the COI dataset in this study (Fig. 12). The three O, grandisquama n. sp. 347 specimens are identified as one MOTU, the Ophioplinthaca sp. and Ophioplinthaca semele are 348 349 delimited as distinct MOTUs from their closely related congeners, which supported the result of
- morphological identification. 350

Conclusions

Three species of the genus Ophioplinthaca were recorded and described, including a new 352 species, *Ophioplinthaca grandisquama* n. sp., which can be easily distinguished from its congeners 353 by the shape and size of tentacle scales and disc spines, as well as radial shields. Morphological 354 characteristics of internal skeleton were also described, providing significant information for 355 356 future taxonomic study of this genus. Phylogenetic study based on COI supported the delimitation of the three species in this study. These findings further enrich the distribution of *Ophioplinthaca* 357 358 from the seamount in the Northwest Pacific Ocean, filling the knowledge gap of benthic invertebrate in the cobalt-rich area. 359

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References

- 367 Cherbonnier G, Sibuet M. 1972. Resultats Scientifique de la compagne Noratlante: Asterides et
- 368 Ophiures. Bulletin du Museum National d'Histoire Naturell Paris 3e serie Zoologie No. 76. 102: 369
- 370 Cho WW, 2008. Faunal Biogeography Community Structure and Genetic Connectivity of North Atlantic Seamounts. No. MIT/WHOI-20(5)5. WOODS HOLE OCEANOGRAPHIC INSTITUTION MA 371
- 372 DOI: 10.1575/1912/2633.
- 373 Christodoulou M, O'Hara TD, Hugall AF & Arbizu PM. 2019. Dark Ophiuroid Biodiversity in a
- 374 Prospective Abyssal Mine Field. Current Biology. 29(22): 1–4 DOI: 10.1016/j.cub.2019.09.012.
- 375 Clark AH. 1949. Ophiuroidea of the Hawaiian Islands. Bulletin of the Bernice P. Bishop Museum. 195: 3-376 133.
- 377 Clark HL. 1900. The Echinoderms of Porto Rico. Bulletin of the U.S. Fisheries Commission. 20(2): 233-378 263.
- 379 Clark HL. 1911. North Pacific Ophiurans in the collection of the United States National
- 380 Museum. Smithsonian Institution United States National Museum Bulletin. 75: 1-302.



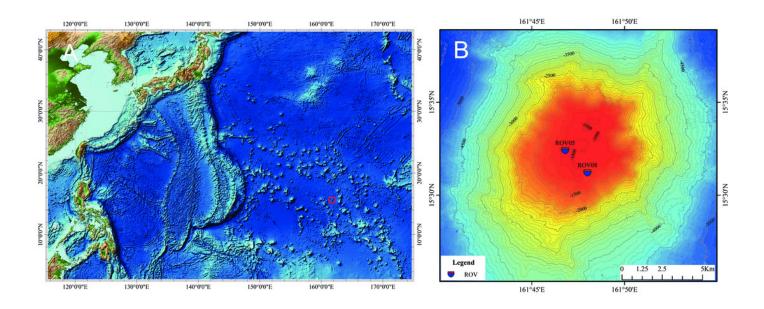
- 381 Clark HL. 1939. Ophiuroidea. Scient. Rep. Murray Exped. 6: 29-136.
- Guille A. 1981. Echinodermes: Ophiurides. in: Forest, J. (Ed.) Résultats des campagnes MUSORSTOM:
- Philippines (18-28 Mars 1976). Mémoires du Muséum national d'Histoire naturelle. Série A,
 Zoologie. 91: 413-456.
- Hein JR, Conrad TA, Dunham RE. 2009. Seamount Characteristics and Mine-Site Model Applied to
 Exploration- and Mining-Lease-Block Selection for Cobalt-Rich Ferromanganese Crusts. *Marine Georesources and Geotechnology*. 27: 160-176 DOI: 10.1080/10641190902852485.
- John DD & Clark AM. 1954. The "Rosaura" expedition. 3. The echinodermata. *Bulletin of the British Museum (Natural History) Zoology*. 2: 139-162, pl. 6.
- Kimura M. 1980. A simple method for estimating evolutionaryrates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*. 16: 111 120.
- Koehler R. 1897. Echinodermes recueillis par "l'Investigator" dans l'Ocean Indien. I. *Les Ophiures de mer profonde. Annales des Sciences Naturelles Zoologie, series 8.* 4: 277-372, pl. 5-9.
- Koehler R. 1904. Ophiures de l'expédition du Siboga. Part 1. Ophiures de mer profonde. In: Weber,
 Siboga Expeditie. M. E.J. Brill, Leiden. 45a: 1-176.
- Koehler R.1922. Ophiurans of the Philippine Seas and adjacent waters. *Smithsonian Institution United* States National Museum Bulletin. 100(5): 1-486.
- Koehler R. 1930. Ophiures recueillies par le Docteur Th. Mortensen dans les Mers d'Australie et dans
 l'Archipel Malais. Papers from Dr. Th. Mortensen's Pacific Expedition 1914-16. LIV.
 Videnskabelige Meddelelser fra Dansk naturhistorisk Forening. 89: 1-295, 22 pl.
- 401 Litvinova NM. 1981. Brittle-stars (Ophiuroidea). Institute of Oceanology P.P. Shirshov oh the Russian
 402 Academy of Science. Moscow: 113-131.
- 403 Ljungman A. 1867. Ophiuroidea viventia huc usque cognita enumerat. *Öfversigt af Kgl. Vetenskaps-*404 *Akademiens Förhandlingar 1866.* 23(9): 303-336.
- Lyman T. 1869. Preliminary report on the Ophiuridae and Astrophytidae dredged in deep water between Cuba and Florida Reef. *Bulletin of the Museum of Comparative Zoology*. 1: 309-354.
- Lyman T. 1878. Ophiuridae and Astrophytidae of the exploring voyage of H.M.S. Challenger, under Prof.
 Sir Wyville Thomson, F.R.S. Part 1. *Bulletin of the Museum of Comparative Zoology, Harvard* University. 5: 65-168, pls 1-10.
- Lyman T. 1883. Reports on the results of dredging, under the supervision of Alexander Agassiz, in the
 Carribbean Sea (1878-79), and on the east coast of the United States, during the summer of 1880, by
 the U.S. coast survey steamer "Blake", commander J.R. Bartlett, U.S.N., commanding. XX.- Report
 on the Ophiuroidea. *Bulletin of the Museum of Comparative Zoology at Harvard*. 10(6): 227-287.
- Mortensen T. 1933. Echinoderms of South Africa (Asteroidea and Ophiuroidea) Papers from Dr. Th.
 Mortensen's Pacific Expedition 1914-16. Videnskabelige Meddelelser fra Dansk naturhistorisk
 Forening 93. 65: 215-400.
- Na J, Zhang D, Cheng H, Yang J, Zhang R, Chen W & Wang C. 2019. The complete mitochondrial
 genome of a deep sea ophiuroid of the genus *Amphiura* (Ophiuroidea: Amphiuridae). *Mitochondrial* DNA Part B. 4(2): 3709-3710 DOI: 10.1080/23802359.2019.1679047.
- Na J, Chen W, Zhang D, et al. In press. Morphological description and population structure of an
 ophiuroid species from cobalt-rich crust seamounts in the northwest Pacific: implications for marine
 protection under deep-sea mining. *Acta Oceanologica Sinica*.



- 423 O'Hara TD & Stöhr S. 2006. Deep water Ophiuroidea (Echinodermata) of New Caledonia:
- 424 Ophiacanthidae and Hemieuryalidae. *Tropical Deep Sea Benthos (Mémoires du Muséum national d'Histoire naturelle 193*). 24: 33–141.
- O'Hara TD, Rowden AA, Williams A. 2008. Cold-water coral habitats on seamounts: do they have a specialist fauna?. *Diversity and distributions*. 14(6): 925-934 DOI: 10.1111/j.1472-4642.2008.00495.x.
- O'Hara TD, Hugall AF, Thuy B, Stöhr S & Martynov AV. 2017. Restructuring higher taxonomy using broad-scale phylogenomics: The living Ophiuroidea. *Molecular Phylogenetics and Evolution*. 107: 415–430 DOI: 10.1016/j.ympev.2016.12.006.
- Puillandre N, Lambert A, Brouillet S, Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*. 21 (8): 1864–1877 DOI: 10.1111/j.1365-294X.2011.05239.x.
- Stamatakis A. 2014. RaxmL version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313 DOI: 10.1093/bioinformatics/btu033.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. Mega6: molecular evolutionary genetics
 analysis version 6.0. *Molecular Biology & Evolution*. 30(12):2725-2729 DOI:
 10.1093/molbev/mst197.
- Thomson CW. 1877. The voyage of the "Challenger." The Atlantic; a preliminary account of the general results of the exploring voyage of H.M.S. "Challenger" during the year 1873 and the early part of the year 1876, Volume 1 xxix+424 pp. (Macmillan and Co.: London).
- Verrill AE. 1899. Report on the Ophiuroidea collected by the Bahama expedition in 1893. *Bulletin of the Laboratories of Natural History of the State of Iowa*. 5: 1-88, pls 1-8.
- Ward RD, Holmes BH & O'Hara TD. 2008. DNA barcoding discriminates echinoderm species.
 Molecular Ecology Resources. 8 (6): 1202–1211 DOI: 10.1111/j.1755-0998.2008.02332.x.
- Yesson C, Clark M R, Taylor M & Rogers AD. 2011. The global distribution of seamounts based on 30-second bathymetry data. *Deep-Sea Research Part I*. 58: 442–453 DOI: 10.1016/j.dsr.2011.02.004.
- Zhang D, Lu B, Wang C, O'Hara TD. 2018. The first record of *Ophioleila elegans* (Echinodermata:
 Ophiuroidea) from a deep-sea seamount in the Northwest Pacific Ocean. *Acta Oceanologica Sinica*.
 37 (10): 180-184 DOI: 10.1007/s13131-018-1323-0.

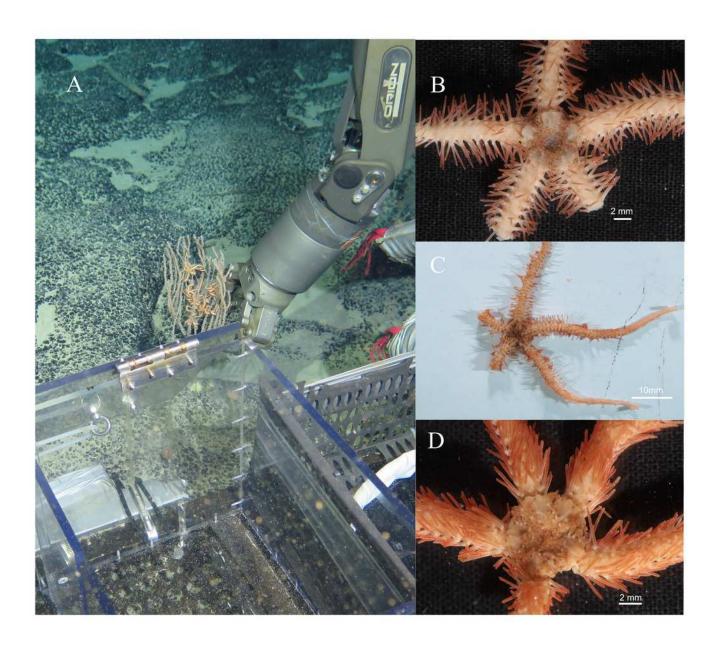


Map of the study seamont (indicated by the small red block) in the northwest Pacific (A) and sampling sites of specimens of Ophioplinthacids (B).



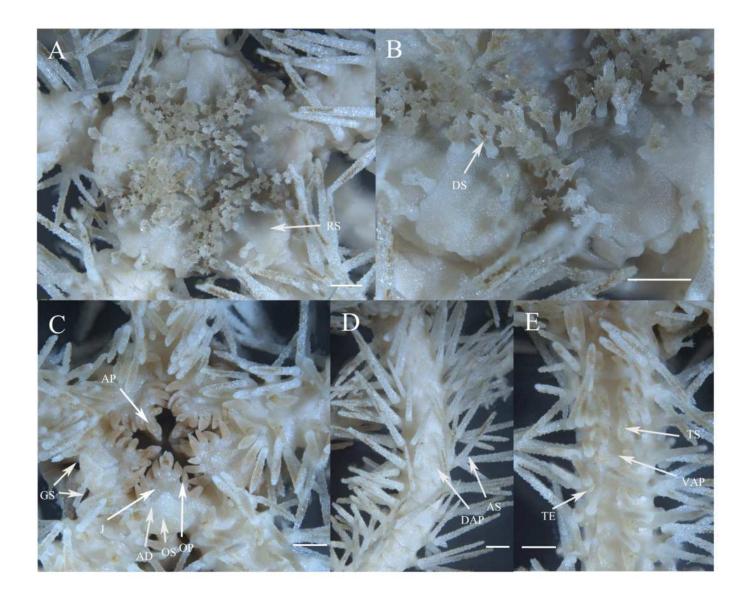
In situ and on board photos of Ophioplinthaca grandisquama n. sp.

- (A) in situ observations, several specimens attached on a Primnoid. (B-D) photos on board.
- (B) holotype (RSIO56060). (C) paratype (RSIO56014). (D) paratype (RSIO56013).



Morphological characters of Ophioplinthaca grandisquama n. sp. (Holotype: RSIO56060).

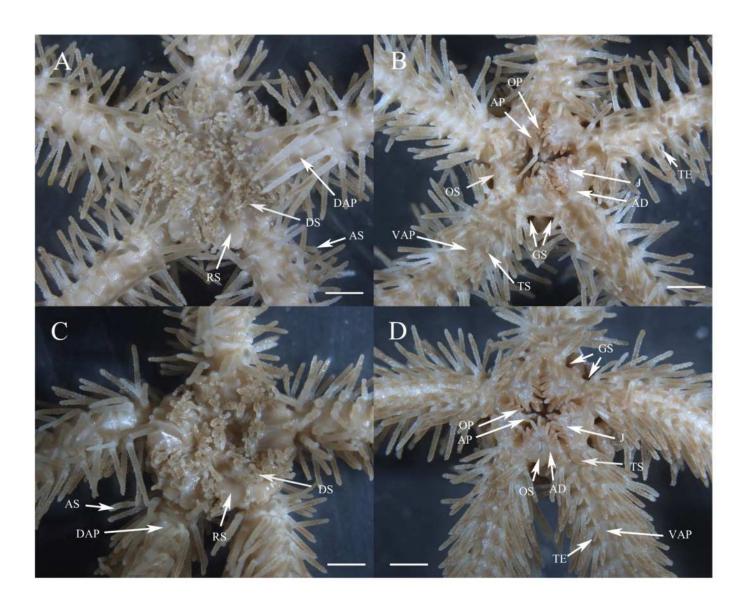
(A) dorsal view of disc. (B) enlarged disc spines. (C) ventral view of disc. (D) dorsal view of arm, proximal part. (E) ventral view of arm, proximal part. Abbreviations: AD, adoral plate; AP, apica pillae; AS, arm spine; DAP, dorsal arm plate; DS, disc spine; GS, genital slits; J, jaw; OP, oral papilla; OS, oral shield; RS, radial shield; VAP, ventral arm plate; TE, tentacle; TS, tentacle scale. Scale bars: 1 mm.





Morphological characters of *Ophioplinthaca grandisquama* n. sp. (Paratype: RSIO56013, RSIO56014).

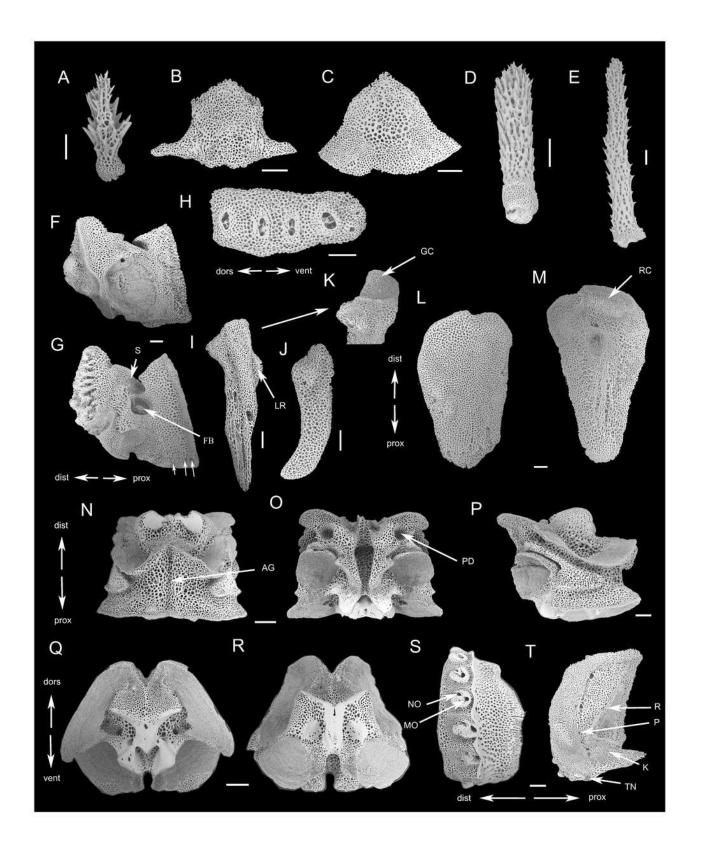
(A-B) Morphological characters of paratype RSIO56014. (A) dorsal view of disc. (B) ventral view of disc. (C-D) Morphological characters of paratype RSIO56013. (C) dorsal view of disc. (D) ventral view of disc. Abbreviations: AD, adoral plate; AP, apical papillae; AS, arm spine; DAP, dorsal arm plate; DS, disc spine; GS, genital slits; J, jaw; OP, oral papilla; OS, oral shield; RS, radial shield; VAP, ventral arm plate; TE, tentacle; TS, tentacle scale. Scale bars: 2 mm.



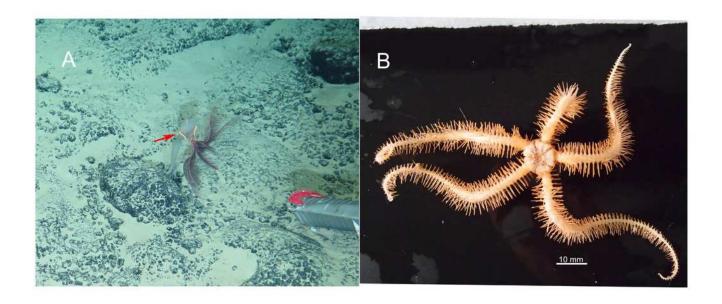


SEM photographs of skeletons of *Ophioplinthaca grandisquama* n. sp. (Paratype: RSIO56014).

(A) disc spine. (B) ventral arm plate from proximal segment, external view. (C) dorsal arm plate from proximal segment, external view. (D) ventral-most arm spine. (E) dorsal-most arm spine. (F) oral plate, abradial face. (G) oral plate, adradial face, white arrows point to oral papillae sockets and pores. (H) dental plate. (I) adradial genital plate. (J) abradial genital plate. (K) adradial genital plate, distal end. (L) radial shield, external aspect. (M) radial shield, internal aspect. (N-R) vertebrae from proximal portion of arm. (N) dorsal view. (O) ventral view. (P) lateral view. (Q) distal view. (R) proximal view. (S) external view of LAP. (T) internal view of LAP. Abbreviations: AG, aboral groove; dors, dorsal; dist, distal; FB, foot basin; GC, adradial genital plate condyle; K, knob; LR, lateral ridge of the adradial genital plate, attachment area of the abradial genital plate; MO, muscle opening; NO, nerve opening; P, perforations; PD, podial basins; prox, proximal; R, ridge; RC, radial shield condyle; S, suture line; TN, tentacle notch; vent, ventral. Scale bars: 200 μm.



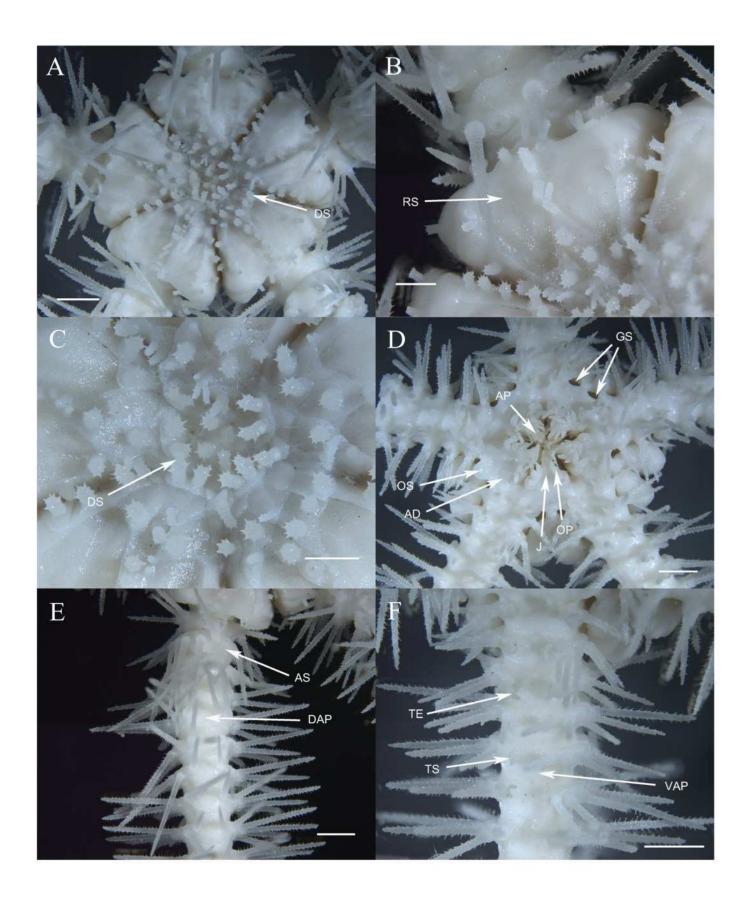
In situ (A) and on board (B) photos of Ophioplinthaca semele.





Morphological characters of *Ophioplinthaca semele* (RSIO56057).

(A) dorsal view of disc. (B) radial shields. (C) disc spines. (D) ventral view of disc. (E) dorsal view of arm, proximal part. (F) ventral view of arm, proximal part. Abbreviations: AD, adoral plate; AP, apical papillae; AS, arm spine; DAP, dorsal arm plate; DS, disc spine; GS, genital slits; J, jaw; OP, oral papilla; OS, oral shield; RS, radial shield; VAP, ventral arm plate; TE, tentacle; TS, tentacle scale. Scale bars: 1 mm (B, C), 2 mm (A, D-F).

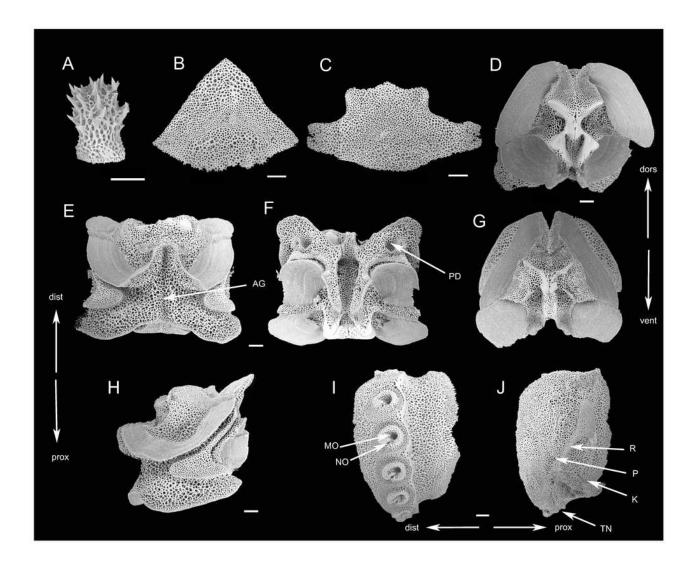




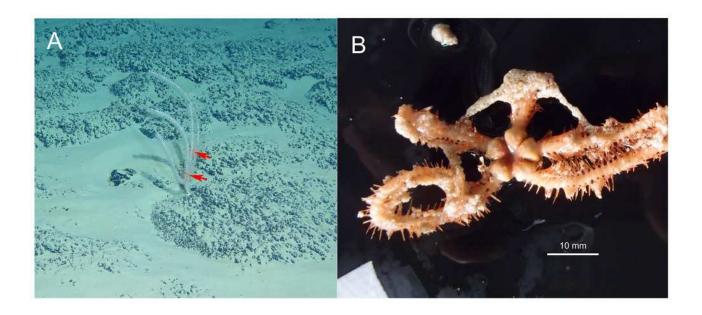
SEM photographs of Ophioplinthaca semele (RSIO56057).

(A) disc spine. (B) dorsal arm plate from proximal segment, external view. (C) ventral arm plate from proximal segment, external view. (D-H) vertebrae from proximal portion of arm. (D) proximal view. (E) dorsal view. (F) ventral view. (G) proximal view. (H) lateral view. (I) external view of lateral arm plate. (J) internal view of lateral arm plate. Abbreviations: AG, aboral groove; dors, dorsal; dist, distal; K, knob; MO, muscle opening; NO, nerve opening; P, perforations; PD, podial basins; prox, proximal; R, ridge; TN, tentacle notch; vent, ventral. Scale bars: 200 μm.



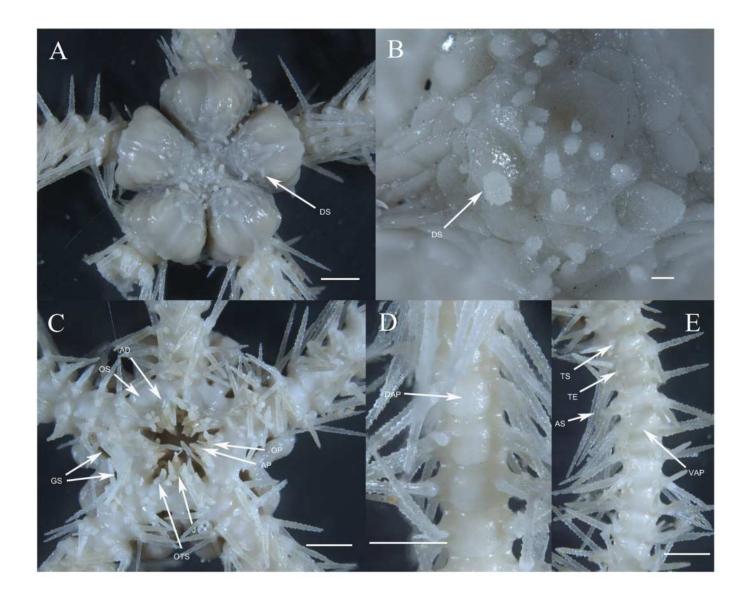


In situ (A) and on board (B) photos of Ophioplinthaca sp.



Morphological characters of Ophioplinthaca sp. (RSIO56058).

(A) dorsal view of disc. (B) disc spines. (C) ventral view of disc. (D) dorsal view of arm, proximal part. (E) ventral view of arm, proximal part. Abbreviations: AD, adoral plate; AP, apical papillae; AS, arm spine; DAP, dorsal arm plate; DS, disc spine; GS, genital slits; J, jaw; OP, oral papilla; OS, oral shield; OTS, oral tentacle scale; RS, radial shield; VAP, ventral arm plate; TE, tentacle; TS, tentacle scale. Scale bars: 2 mm (A, C-E), 0.2mm (B).

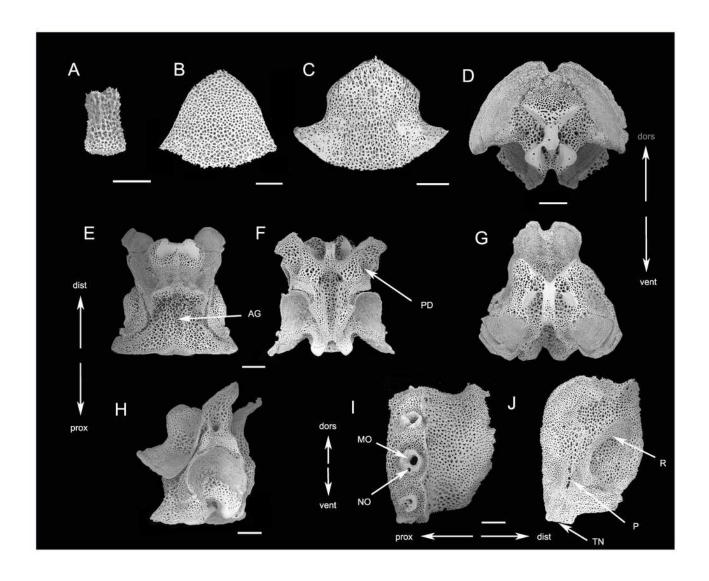




SEM photographs of Ophioplinthaca sp. (RSIO56058).

(A) disc spine. (B) dorsal arm plate from proximal segment, external view. (C) ventral arm plate from proximal segment, external view. (D-H) vertebrae from proximal portion of arm. (D) proximal view. (E) dorsal view. (F) ventral view. (G) distal view. (H) lateral view. (I) external view of lateral arm plate. (J) internal view of lateral arm plate. Abbreviations: AG, aboral groove; dors, dorsal; dist, distal; MO, muscle opening; NO, nerve opening; P, perforations; PD, podial basins; prox, proximal; R, ridge; TN, tentacle notch; vent, ventral. Scale bars: 200 µm.







Maximum likelihood tree of the genus Ophioplinthaca based on COI sequences.

Colored bars in red refer to MOTUs in ABGD.

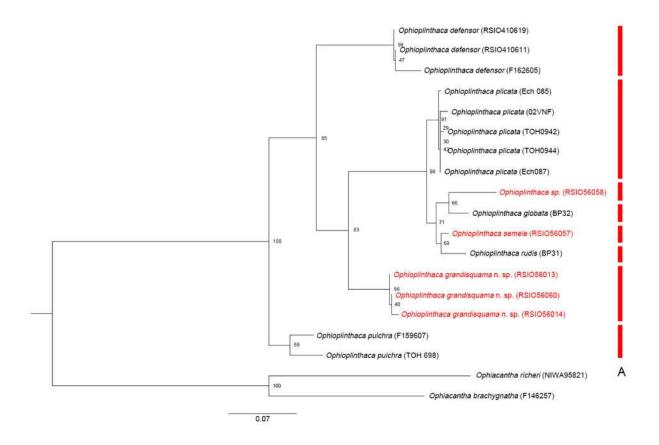




Table 1(on next page)

Information of primers used for PCR programs.



Prime	Sequence
Oph-COI-F	TTTCAACTAATCAYAAGGAYATWGG
Oph-COI-R	CTTCAGGRTGWCCRAARAAYCA
LCO1490	GGTCAACAAATCATAAAGATATTGG
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA



Table 2(on next page)

Information of COI sequences used in phylogenetic analysis.



Taxa	Voucher/isolate	GenBank accession number/						
		BOLD sequence ID						
Ophioplinthaca grandisquama n. sp.	RSIO56060	MW284982						
Ophioplinthaca grandisquama n. sp.	RSIO56013	MW284978						
Ophioplinthaca grandisquama n. sp.	RSIO56014	MW284979						
Ophioplinthaca semele	RSIO56057	MW284980						
Ophioplinthaca sp.	RSIO56058	MW284981						
Ophioplinthaca pulchra	TOH_698	HM400467						
Ophioplinthaca pulchra	F159607	KU895136						
Ophioplinthaca defensor	TOH_0941	ECHOZ371-10.COI-5P						
Ophioplinthaca defensor	RSIO410611	MT025802						
Ophioplinthaca defensor	RSIO410619	MT025808						
Ophioplinthaca globata	BP32	KU895134						
Ophioplinthaca rudis	BP31	KU895135						
Ophioplinthaca plicata	Ech085	EU869990						
Ophioplinthaca plicata	Ech087	EU869989						
Ophioplinthaca plicata	02VNF	KU895133						
Ophioplinthaca plicata	TOH_0942	ECHOZ372-10.COI-5P						
Ophioplinthaca plicata	TOH_0944	ECHOZ374-10.COI-5P						
Ophiacantha richeri	NIWA9 <u>5</u> 821	KU895387						
Ophiacantha brachygnatha	F146257	KU895386						



Table 3(on next page)

The genetic distance of COI gene (K2P) of Ophioplinthaca.

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 Ophioplinthaca pulchra (F159607)																		
2 Ophioplinthaca pulchra (TOH 698)	0.049																	
3 Ophioplinthaca sp. (RSIO56058)	0.157	0.184																
4 Ophioplinthaca rudis (BP31)	0.160	0.176	0.078															
5 Ophioplinthaca semele (RSIO56057)	0.160	0.156	0.060	0.032														
6 Ophioplinthaca plicata (Ech085)	0.140	0.148	0.084	0.051	0.036													
7 Ophioplinthaca plicata (02VNF)	0.153	0.160	0.085	0.055	0.042	0.012												
8 Ophioplinthaca plicata (TOH0942)	0.134	0.145	0.086	0.050	0.030	0.008	0.009											
9 Ophioplinthaca plicata (Ech087)	0.137	0.155	0.084	0.050	0.036	0.005	0.008	0.004										
10 Ophioplinthaca globata (BP32)	0.146	0.150	0.063	0.057	0.050	0.057	0.051	0.032	0.053									
11 Ophioplinthaca plicata (TOH0944)	0.125	0.149	0.092	0.054	0.031	0.005	0.003	0.003	0.000	0.000								
12 O. grandisquama n. sp. (RSIO56014)	0.123	0.144	0.136	0.125	0.108	0.110	0.115	0.110	0.110	0.107	0.103							
13 O. grandisquama n. sp. (RSIO56013)	0.112	0.135	0.141	0.129	0.111	0.114	0.116	0.117	0.114	0.127	0.107	0.009						
14 O. grandisquama n. sp. (RSIO56060)	0.119	0.148	0.152	0.127	0.108	0.113	0.118	0.120	0.115	0.115	0.117	0.007	0.002					
15 Ophioplinthaca defensor (F162605)	0.111	0.147	0.180	0.174	0.138	0.149	0.152	0.153	0.153	0.097	0.152	0.096	0.091	0.119				
16 Ophioplinthaca defensor (RSIO410611)	0.122	0.131	0.176	0.159	0.140	0.148	0.153	0.137	0.146	0.151	0.137	0.104	0.103	0.121	0.025			
17 Ophioplinthaca defensor (RSIO410619)	0.119	0.129	0.174	0.156	0.137	0.146	0.151	0.135	0.144	0.151	0.134	0.106	0.106	0.123	0.028	0.001		
18 Ophiacantha richeri (NIMA95821)	0.270	0.339	0.393	0.357	0.368	0.387	0.350	0.423	0.399	0.300	0.434	0.366	0.349	0.373	0.369	0.350	0.353	
19 Ophiacantha brachygnatha (F146257)	0.278	0.286	0.346	0.311	0.316	0.316	0.304	0.335	0.312	0.280	0.339	0.307	0.309	0.309	0.324	0.303	0.308	0.202