Acta Oceanol. Sin., 2021, Vol. 40, No. 12, P. 79–89
https://doi.org/10.1007/s13131-020-1666-1
http://www.aosocean.com
E-mail: ocean2@hyxb.org.cn

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

Jieying Na[1, 3], Wanying Chen[1], Dongsheng Zhang[1, 2, 4*], Ruiyan Zhang[1, 4], Bo Lu[1], Chengcheng Shen[1], Yadong Zhou[1], Chunsheng Wang[1, 2, 4, 5]

[1] Key Laboratory of Marine Ecosystem Dynamics, Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China

[2] Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519080, China

[3] School of Marine Science, China University of Geosciences (Beijing), Beijing 100083, China

[4] School of Oceanography, Shanghai Jiao Tong University, Shanghai 200230, China

[5] State Key Laboratory of Satellite Ocean Environment Dynamics, Hangzhou 310012, China

Received 4 March 2020; accepted 30 July 2020

Abstract

Many seamounts are covered with cobalt-rich ferromanganese crusts, and are increasingly attracting interest for the potential extraction of valuable mineral resources from deep seabed environments. However, the impacts of potential mining activities on the vulnerable seamount ecosystem remain unclear. To enhance the understanding of connectivity in benthic invertebrate populations in the Northwest Pacific, several seamounts were surveyed and biological samples collected. In the present study, the ophiuroid species *Ophioplinthaca defensor* is reported for the first time from four deep seamounts in the Northwest Pacific, and described, providing more detailed morphological diagnosis characters. To assess the population structure of the species between and within seamounts, two mitochondrial markers (*COI* and *16S*) were sequenced. In total, 20 haplotypes from 32 *COI* sequences and 8 haplotypes from 37 *16S* sequences were recovered. The star-shaped TCS networks and non-significant pairwise population differences reveal the absence of distinct population structures between and within seamounts. In addition, the *O. defensor* population seemed to have undergone a demographic expansion in history. This is the first study on the genetic population structure of a benthic invertebrate from seamounts in the Northwest Pacific, and this results suggest a potentially high, long distance dispersal capacity in *O. defensor* between seamounts, which could inform the development of the Regional Environmental Management Plans for the cobalt-rich crust seamounts in the area.

Key words: Ophiuroidea, connectivity, deep-sea, cobalt-rich crust seamount, Northwest Pacific

Citation: Na Jieying, Chen Wanying, Zhang Dongsheng, Zhang Ruiyan, Lu Bo, Shen Chengcheng, Zhou Yadong, Wang Chunsheng. 2021. 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, 40(12): 79–89, doi: 10.1007/s13131-020-1666-1

1 Introduction

Seamounts are isolated, topographic elevations with summit heights of at least 100 m above the seafloor. Large seamounts (heights >1 000 m from the seabed) are estimated to range from 33 000 to 100 000 in number, covering approximately 4.7% of the ocean floor area (Yesson et al., 2011). Seamounts are considered biodiversity hotspots, “stepping stones” for dispersal, and oases of species abundance and biomass (Shank, 2010). Due to the relatively high levels of productivity on seamounts, they support abundant fish stocks (O’Driscoll and Clark, 2005), which makes them key targets of deep-sea bottom trawling fishing activities. Furthermore, numerous seamounts that are covered with cobalt-rich ferromanganese crusts are considered potential sources of cobalt, tellurium, and other valuable metals through mining. Human activities, whether fishing or potential seabed mining, could have severe negative impacts on the vulnerable biological communities on seamounts (Clark et al., 2010).

Population connectivity refers to the exchange of individuals among geographically isolated populations. Population connectivity influences gene flow, regulates population size and function, and influences the capacity of an ecosystem to recover after natural or anthropogenic disturbance (Hilário et al., 2015). Consequently, population connectivity is often considered a key criterion during the design of marine protect areas (MPAs) (Bal-

---

Foundation item: The National Natural Science Foundation of China under contract No. 42076135; the Foundation of China Ocean Mineral Resources R & D Association under contract Nos DY135-E2-2-06 and DY135-E2-2-03; the Project of State Key Laboratory of Satellite Ocean Environment Dynamics, Second Institute of Oceanography, Ministry of Natural Resources, under contract No. SOEDZZ2002; the Scientific Research Fund of the Second Institute of Oceanography, Ministry of Natural Resources, under contract No. JG1528.

*Corresponding author, E-mail: dszhang@sio.org.cn

bar and Metaxas, 2019). To date, numerous studies have explored the genetic connectivity of both commercially fished species and non-commercial invertebrates from seamounts (Samadi et al., 2006; Cho and Shank, 2010; Miller and Gunasekera, 2017; Zeng et al., 2019). Different scales of connectivity have been observed among seamounts for different taxonomic groups, which were either highly connected or genetically structured, depending on their dispersal ability or the existence of physical barriers (Baco et al., 2016; Taylor and Roterman, 2017).

Ophiuroids commonly associate with key benthic structural features such as corals and sponges, acting as sources of critical information on biodiversity, basic evolutionary processes, community diversity, and biogeographical structures of seamounts (O'Hara, 2007). Recently, different studies have investigated genetic connectivity in ophiuroids in deep-sea environments in the Atlantic, Pacific, Antarctic and Mediterranean (Hunter and Halanych, 2008, 2010; Cho and Shank, 2010; Boissin et al., 2011; Pérez-Portela et al., 2012; Boschen et al., 2013; O'Hara et al., 2013, 2014; Sands et al., 2015; Taboada and Pérez-Portela, 2016; Galaska et al., 2016, 2017). However, the studies reported inconsistent connectivity patterns and genetic structures under different geological scales, which is attributable to the varied reproductive strategies, dispersal abilities, and life histories of different ophiuroid taxa. Although understanding the degrees of genetic connectivity either between or within seamounts facilitates the design and establishment of MPAs (Palumbi, 2003), due to sampling challenges, understanding the connectivity patterns among deep-sea fauna remains limited, not only among regional seamounts but also within single seamounts (Taylor and Roterman, 2017).

The Northwest Pacific region has the highest number of seamounts globally (Yesson et al., 2011), and many of the seamounts are covered with cobalt-rich ferromanganese crusts, which is a valuable mineral (Hein et al., 1992). The International Seabed Authority (ISA) has issued four contracts for the exploration of cobalt-rich crusts in the region (https://www.isa.org.jm/exploration-contracts/cobalt-rich-ferromanganese). However, the potential impacts of the mining activities on the vulnerable seamount ecosystem remain unclear. Only a few studies have explored the benthic megafauna community structure and diversity focusing on cobalt-rich crust (CRC) seamounts (Schlacher et al., 2014; Morgan et al., 2015), while their resilience and recovery have been reported to be weak due to slow growth rates of the dominant cold coral reef communities (Clark et al., 2019).

The growing pressure on seabed ecosystems due to the associated mineral resources call for the formulation of robust environmental management plans for areas that could be affected. Connectivity is a critical criterion to consider during the design and establishment of MPAs, and it is also considered a scientific design principle of the Regional Environmental Management Plan (REMP) for CRC seamount areas in the Northwest Pacific (referred as "Triangle Area" in the ISA Technical Study No. 23 (ISA, 2018)). Furthermore, population structures could provide key insights that could inform the design of impact reference zone and preservation reference zone in CRC seamounts (ISBA/19/LTC/8, 2013).

To address the knowledge gap on the connectivity and population structure in the Northwest Pacific, several seamounts, including the Caiwei Guyot and Weijia Guyot, where the China Ocean Mineral Resources Research and Development Association (COMRA)'s contract area located, were surveyed in the course of several cruises, and ophiuroid specimens collected using a HOV and ROVs. In the present study, for the first time, reporting the population structure of a common benthic megafaunal species, *Ophioplinthaca defensor* (Koehler, 1930), from seamounts in the Northwest Pacific. Two mitochondrial genes were examined to investigate the genetic diversity and population structure of the species among seamounts and within Weijia Guyot. The results of the present study could enhance the understanding of population connectivity among and within seamounts, and, in turn, facilitate the establishment of REMPs in the region.

2 Material and methods

2.1 *Specimen collection and morphology description*

A total of 37 *O. defensor* individuals were collected from four seamounts in the Northwest Pacific during COMRA DY35, DY41B, and DY56 cruises. A HOV (*Jiaolong*) and ROVs (*Haima* and *Hailong III*) were used to obtain high quality specimens of benthic organisms. Data on the sampling sites are presented in Table 1 and Fig. 1. Ophiuroids were preserved in 90% ethanol and deposited in the Repository of the Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou, China.

Specimens used for morphological identification were examined and photographed using a dissection-microscope (AXIO Zoom V16, Zeiss Microscopy GmbH, Jena, Germany) equipped with a camera (Axiocam 506 color, Zeiss). Arm skeletal elements from proximal segments of all specimens were submerged in commercial bleach (2.5% NaOCl) until all soft issue dissolved, and then washed in tap water, air-dried, mounted on stubs, and images obtained using a TM1000 scanning electron microscope (Hitachi Technologies, Tokyo, Japan). For each specimen, a disc spine was removed for SEM imaging, with the same protocol as used for arm skeletal elements.

2.2 *DNA extraction, amplification, and sequencing*

DNA was extracted from a piece of arm using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to

Table 1. *Ophioplinthaca defensor* sampling data in the Northwest Pacific

Cruise	Site	Seamount	Date	Latitude	Longitude	Depth/m	Individuals
DY35	CW-DV81	Caiwei	2014.7.24	15.683 0°N	154.923 0°E	1 660	1
DY41B	WJ-ROV01	Weijia	2017.9.28	12.694 8°N	156.538 1°E	1 670	8
DY41B	WJ-ROV04	Weijia	2017.9.18	13.024 2°N	156.884 5°E	1 571	4
DY41B	WJ-ROV05	Weijia	2017.9.19	12.884 5°N	157.029 3°E	1 643	1
DY41B	WJ-ROV06	Weijia	2017.9.21	12.789 5°N	156.687 7°E	1 935	19
DY56	BG-ROV04	Batiza	2019.9.11	20.015 7°N	156.540 4°E	1 699	1
DY56	RC-ROV06	RC*	2019.9.17	15.529 0°N	161.753 5°E	1 482	3
DY56	RC-ROV07	RC*	2019.9.19	15.495 5°N	161.794 0°E	1 660	1

Note: * represents this seamount is an unnamed seamount; RC was used as a temporary code.

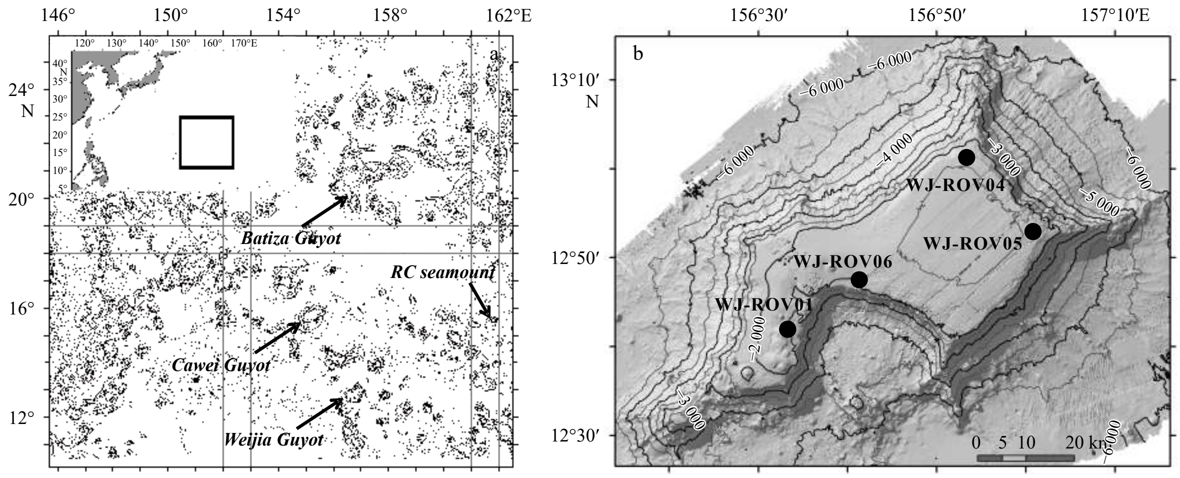

Fig. 1. Map of the study seamounts in the Northwest Pacific (a) and sampling sites in the Weijia Guyot (b).

the manufacturer's protocol. Partial *COI* and *16S* sequences were amplified using specific (O'Hara et al., 2014) and universal primers (Palumbi, 1996), respectively. PCR reactions were carried out in 25-μL volumes containing 2.5 μL 10× Buffer (Mg^{2+} plus), 10 mmol/L of each dNTP, 0.1 mmol/L of each primer, 18.75 μL of ddH$_2$O, 5 U/μL of Taq DNA polymerase (Vazyme, Nanjing, China), and 1 μL of DNA template. The thermal cycling profile consisted of 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 products were purified using a QIAquick PCR purification Kit (QIAGEN) and bidirectionally sequenced by Sangon Biotech (Shanghai, China) on an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequences are available in GenBank (*COI*: MT025778-MT025808, *16S*: MT031925-MT03 1961).

2.3 *Data analysis*

Forward and reverse sequences were de novo assembled and edited using Geneious Prime 2020 (https://www.geneious.com), generating 531-bp *COI* and 496-bp *16S* datasets. *COI* and *16S* alignments were performed using MUSCLE (Edgar, 2004) as a plug-in in Geneious with default settings. Haplotype diversity (H_d) and nucleotide diversity (π) were calculated using DnaSP v6.0 (Rozas et al., 2017) based on *COI* and *16S* datasets, respectively. To illustrate the evolutionary relationships among haplotypes, TCS networks were generated from the *COI* and *16S* alignments using Popart v1.7 (Leigh and Bryant, 2015). To assess population differentiation among sampling sites between and within seamounts, pairwise fixation index ($\mathrm{F_{st}}$) and genetic differentiation ($\mathrm{G_{st}}$) were calculated using DnaSP v6.0.

Population size histories were investigated based on mismatch distributions using DnaSP v6.0, as well as the Harpending's raggedness index (r) (Harpending, 1994) to quantify the smoothness of the observed pairwise difference distributions. Neutrality tests, including Tajima (1989)'s D-test and Fu's Fs statistic (Fu, 1997), were applied in Arlequin v3.5 (Excoffier and Lischer, 2010) with default settings to evaluate the assumption of selective neutrality of mt DNA sequences.

3 Results

3.1 *Morphological description of Ophioplinthaca defensor*

Systematics

Class Ophiuroidea Gray, 1840

Order Ophiacanthida O'Hara, Hugall, Thuy, Stöhr and Martynov, 2017

Family Ophiacanthidae Ljungman, 1867

Genus *Ophioplinthaca* Verrill, 1899

Ophioplinthaca defensor Koehler, 1930

Material examined: the Caiwei Guyot, St. CW-DV81, 1 specimen (RSIO358101); the Weijia Guyot, St. WJ-ROV01, 8 specimens (RSIO410102–RSIO410109); the Weijia Guyot, St. WJ-ROV04, 4 specimens (RSIO410407, RSIO410408, RSIO410411, RSIO410412); the Weijia Guyot, St. WJ-ROV05, 1 specimen (RSIO410502); the Weijia Guyot, St. WJ-ROV06, 19 specimens (RSIO410601–RSIO410619); the Batiza Guyot, St. BG-ROV04, 1 specimen (RSIO560406); the RC seamount, St. RC-ROV06, 3 specimens (RSIO560601, RSIO560605, RSIO560607); the RC seamount, St. RC-ROV07, 1 specimen (RSIO560705). Information of sampling sites are shown in Table 1.

Description: discs of adult specimens 6–12 mm disc diameter (d.d.), arms >4 times d.d. in length, disc incised interradially to almost 1/2 d.d., creating five wedge-shaped divisions covered by a pair of large naked radial shields and numerous irregular plates. Radial shields triangular, about 1/3 d.d. in length, contiguous distally, and separated proximally by a triangular plate. The center of the disc is occupied by small irregular plates, bearing large cylindrical granules or stumps up to 0.7 mm long, thorny at the top (Figs 2a and 3g). Granules at disc margin are smaller and with less thorns. The ventral side of the disc is covered by some uneven plates. Genital slits long and narrow (Figs 2b and c).

Oral shields arrow-shaped, wider than long, with a triangular proximal angle, blunt lateral angles, and an elongated distal lobe, grooved (Fig. 2b). Adoral shields quadrilateral, 2–2.5 times wider than long, not separating the oral shields from first lateral arm plates (Fig. 2b). Jaw triangular, as long as wide, with 4–5 lateral oral papillae, conical and pointed, 2–3 times longer than wide, in an irregular arrangement, with the distal one or two slightly broadened; one apical papilla slightly enlarged (Fig. 2b).

Five arms, wide, and slightly moniliform. The first dorsal arm plates are trapezoidal, with a small proximal edge, straight and divergent lateral edges, and a rounded distal margin, contiguous

Fig. 2. Morphological characters of *Ophioplinthaca defensor* (RSIO410602). a. Dorsal view; b. ventral view of disc; c. the interradial characters of ventral side; d. dorsal view of arms, proximal part; e. ventral view of arm, proximal part; f. dorsal view of arms, distal part. AD: adoral shield; AS: arm spines; DAP: dorsal arm plate; DS: disc spines; GS: genital slits; J: jaw; OP: oral papillae; OS: oral shield; RS: radial shield; VAP: ventral arm plate. Scale bars: 2 mm.

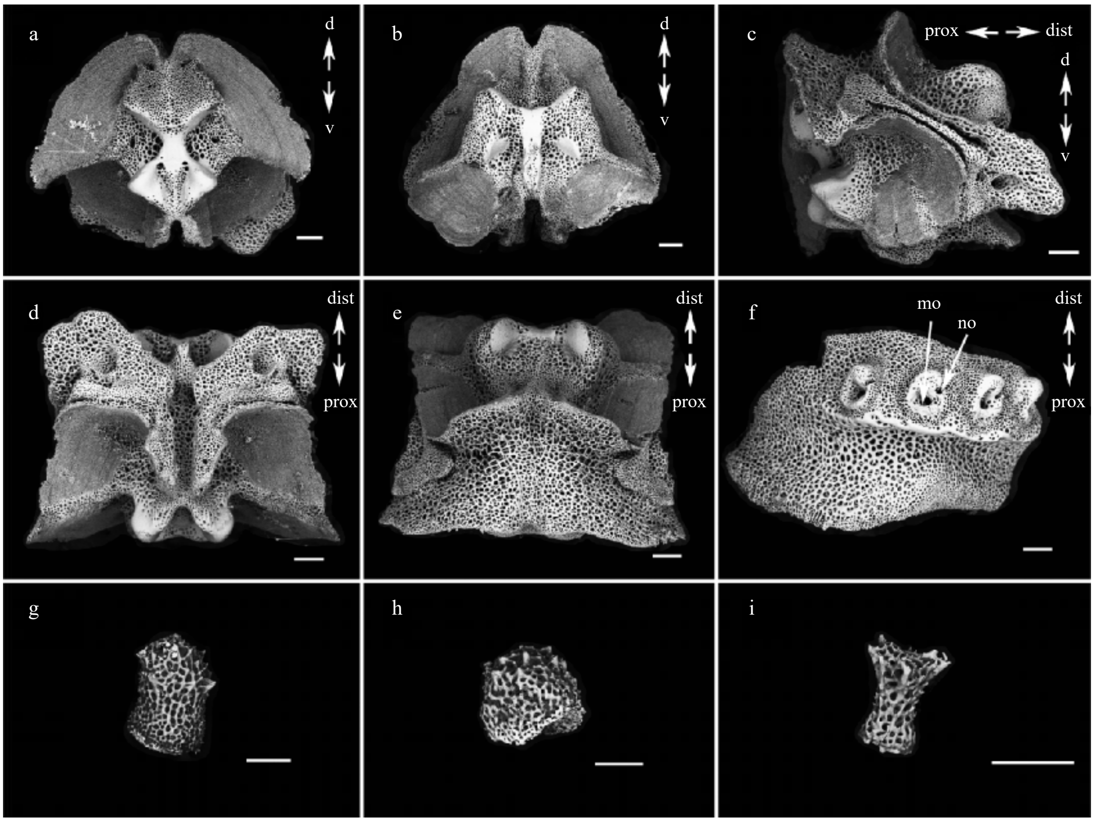

Fig. 3. Scanning electron microscope photographs of arm skeletal characters and disc spines of *Ophioplinthaca defensor*. a–f. Arm skeletal characters from adult paratype (RSIO410602): proximal view of vertebrae; distal view of vertebrae; lateral view of vertebrae; ventral view of vertebrae; dorsal view of vertebrae; external view of lateral arm plate. g–i. Different types of disc spines, large cylindrical granules from adult paratype RSIO410602; conical or round granules from adult paratype RSIO410617; disc spines cylindrical with flaring thorny apex from juvenile paratype RSIO410502. mo: muscle opening; no: nerve opening. Arrows: d. dorsal side; v. ventral side; dist. distal side; prox. proximal side. Scale bars: 200 μm.

with the second arm plates (Fig. 2d). The following dorsal arm plates gradually changed from trapezoidal to sub-triangular and distinctly separated from each other (Fig. 2d). First ventral arm plates triangular, with a blunt proximal angle, diverged lateral edges, and convex distal edge. The following plates pentagonal, with a very small proximal angle, the lateral edges diverged and indented by tentacle scales, distal edge broad and slightly rounded, widely separated from the succeeding ventral arm plates (Fig. 2e). The lateral arm plates slightly prominent, meeting each other both above and below, with a low ridge bearing up to five arm spines, widely separated dorsally. The proximal arm segments with five spines, of which the dorsally second or third are the longest, up to two segments long, lowermost shortest, one segment in length. As the segments reduced distally, the number of arm spines reduced to four, while the longest arm spines became approximately five segments long. One oval tentacle scale, almost as long as one ventral arm plate.

The arm spine articulations well developed, positioned at a small angle in relation to the lateral plate. There is a well-defined volute-shape perforate lobe, occupying the distal part of the lateral arm plate, with a rounded larger muscle opening and a smaller nerve opening. The proximal edge of the spine articulation is entire and does not connect with the main part of the lateral arm plate. The vertebrae zygospondylous articulation not keeled, with a distinct suture line in both dorsal and ventral view (Figs 3a–f).

Variation: one specimen (RISO410617) collected from the Weijia Guyot, with larger radial shields, more than 1/3 d.d. in length, completely occupied each division of the disc, largely contiguous, only proximally separated by a small triangle plate (Fig. 4a). The central region of the disc sunken, with a certain number of large conical or round granules (Fig. 3h), tightly arranged, covering the plates below (Fig. 4b). Jaw wider than long with 4 lateral oral papillae and 1–2 apical papillae, oral papillae small, conical as long as wide, irregular arrangement (Fig. 4c).

One specimen (RISO358101) collected from the Caiwei Guyot with disc flattened, large radial shields, 2/5 d.d. long, contiguous more than half of their length. Two pairs of radial shields abnormal, each composed of more than two irregular plates, one of the two pairs of radial shields are separated by a long narrow plate between them (Fig. 4d). The central region bears a few round granules, slightly thorny or smooth at the top (Fig. 4e), similar to those on the specimen mentioned above (RISO410617). Jaw as long as wide with 3–4 lateral oral papillae and 1–2 apical papillae, oral papillae thinner, two to three times taller than wide, distal ones not broadened (Fig. 4f).

Four small specimens (2.5 mm d.d.) from the Weijia Guyot (RISO410502 and RISO410412) and the RC seamount (RISO 560605 and RISO560705) are likely to be the juveniles, incised interradially to 1/4 d.d. with small triangular radial shields, less than 1/3 times d.d. in length, contiguous distally, less than half their length and separated inward by a triangular plate (Fig. 4g), disc spines cylindrical with flaring thorny apex (Fig. 4h), restric-

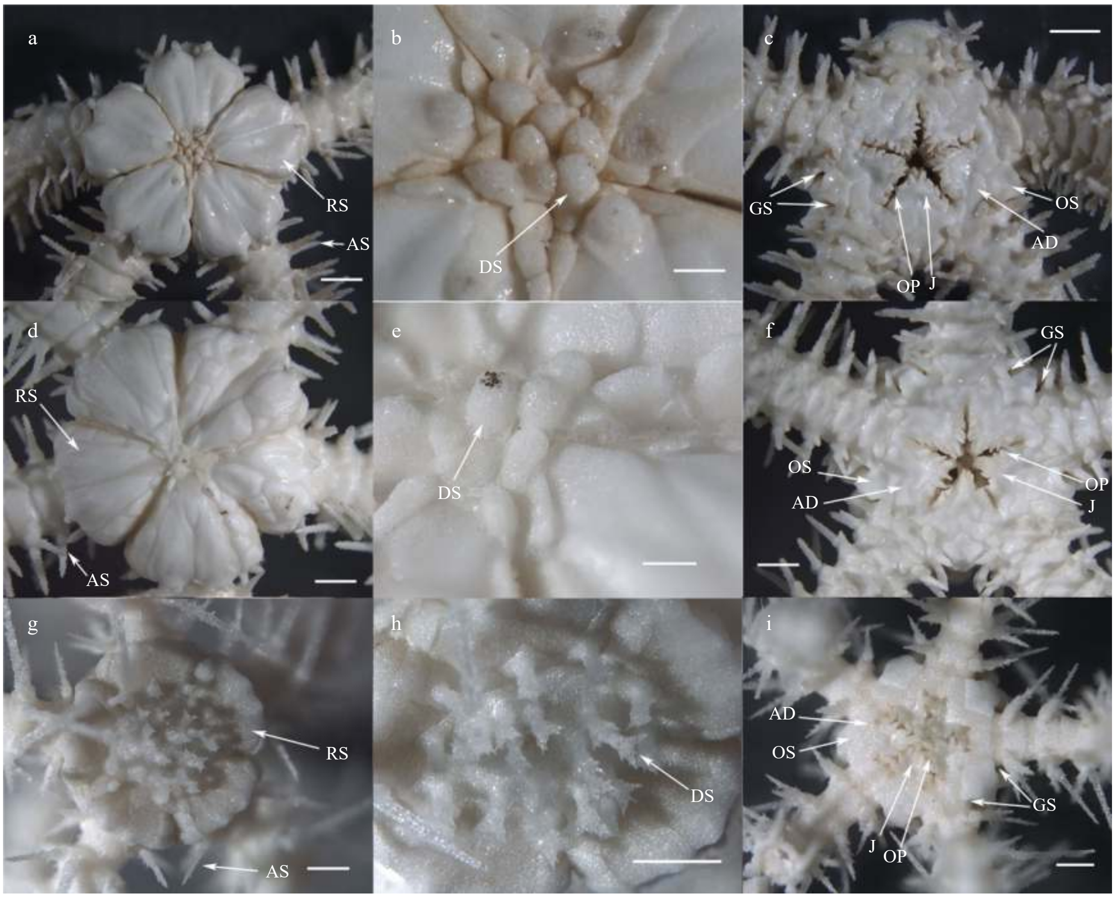

Fig. 4. Variation in morphological characters of *Ophioplinthaca defensor*. a–c. Adult (RSIO410617) from the Weiija Guyot: dorsal view of disc; disc granules; ventral view of disc. d–f. Adult (RSIO358101) from the Caiwei Guyot: dorsal view of disc; disc granules; ventral view of disc. g–i. Juvenile (RSIO410502) from the Weijia Guyot: dorsal view of disc; disc granules; ventral view of disc. Scale bars: 2 mm (a, c, d, f), 500 μm (b, e, g–i).

ted to the central region of the disc. One tentacle scale, oval to conical, shorter than the ventral arm plates. Jaws triangular, longer than wide, with three lateral conical oral papillae and one apical papilla. Oral shields pentagonal, similar to the adults but not grooved or slightly grooved in the distal margin, just separated from the first lateral arm plates by the adoral shields (Fig. 4i). Both dorsal arm plates and ventral arm plates are distinctly separated, four arm spines up to three segments long (Figs 4g and i).

3.2 *Haplotype diversity and population structure*

Specimens successfully sequenced were showed in Table S1. Thirty-one *COI* sequences were obtained with 25 variable nucleotide sites detected from the alignment. A total of 20 haplotypes were recovered from the study area, with 18 haplotypes recovered from the Weijia Guyot, one from the Batiza Guyot, and 3 from the RC seamount. Both the H_d and π were higher in the Weijia Guyot than those in the RC seamount. Within the Weijia Guyot, the highest number of haplotypes was obtained from WJ-ROV06, but H_d and π recovered from WJ-ROV01 were highest (Table 2). Thirty-seven *16S* sequences were obtained with 9 variable nucleotide sites detected from the alignment. Eight haplotypes were recovered from *16S* rRNA sequences, all of which were recovered from the Weijia Guyot, while only one and two haplotypes were recovered from the Batiza Guyot and the RC seamount, respectively. Similar to the *COI* distribution patterns, higher *16S* H_d and π were observed in the Weijia Guyot than in the RC seamount. Within the Weijia Guyot, the number of haplotypes, H_d, and π were highest at WJ-ROV06 (Table 2).

Both the TCS networks based on *COI* and *16S* are star-shaped (Fig. 5). In the *COI* network, the dominant haplotype (Hap_2-COI) was recovered from four sampling sites of the three seamounts, while the other two shared haplotypes (Hap_14-COI and Hap_19-COI) were restricted to single sampling site in the Weijia Guyot and the RC seamount, respectively. The other 17 haplotypes were singletons (Fig. 5a). With regard to the *16S* network, the dominant haplotype (Hap_3-16S) was recovered from the Weijia Guyot and the RC seamount, in addition to another shared haplotype (Hap_1-16S), while Hap_2-16S was recovered from Weijia Guyot and the Batiza Guyot (Fig. 5b). The star-like haplotype networks revealed no significant geographical structures among seamounts or sampling sites within the Weijia Guyot. The F_{st} and G_{st} values of *COI* and *16S* were very low and statistically non-significant, both among sampling sites and between seamounts (Table 3).

3.3 *Demographic history*

According to the results of the neutrality tests, Tajima’s D values were all negative for both *COI* and *16S*, with significant *p*-values being observed only in the WJ-ROV01 and WJ populations. Fu’s Fs values were negative in most populations, excluding WJ-ROV04, although there were no significant *p*-values (Table 4). In addition, the Harpending’s raggedness values were low (Table 4) and the mismatch distribution for all sequence data from the Weijia Guyot displayed a unimodal pattern for both *COI* and *16S* (Fig. 6), suggesting that the *O. defensor* population in Weijia Guyot may have experienced a demographic expansion in history.

4 Discussion

Ophioplinthacids, which have the greatest diversity and are abundant in the Indo-Pacific region (O’Hara and Stöhr, 2006), have been suggested to be a dominant group in seamount ecosystems (O’Hara, 2007). In the Weijia Guyot and the other two seamounts studied, large number of *O. defensor* were tightly associated to sponges (Fig. 7), making it a potential useful indicator species for deep-sea mining environmental impact assessment.

4.1 *Morphology diagnostic characters*

Numerous characters that are often used to diagnose species within the Ophiacanthidae tend to be variable in *Ophioplinthaca*. The sizes and shapes of radial shields and disc spines were used as the primary criteria for delimiting species (O’Hara and Stöhr, 2006). In the original description of *O. defensor*, the round smooth disc spines were used to distinguish it from other congeners (Koehler, 1930). Among the specimens examined in the present study, disc spines were consistent with the unique holotype in shape and size; however, sometimes tiny thorns existed at the apex in the center region. The two juveniles had different disc spines with long flaring thorns at the apex. This is potentially due to the variations in the shapes and sizes of the disc spines during ontogeny (O’Hara and Stöhr, 2006). The radial shield also varied in size; however, compared to its congeneric species, occupying a large proportion of the disc surface and the long continuity of paired radial shields can be used as distinguishable characters. The Caiwei specimen had two abnormal pairs of radial shields, which could be a type of congenital malformation, or caused by predation or some other type damage.

Ophioplinthaca defensor resembles *O. pulchra* Koehler, 1904, and *O. plicata* Lyman, 1878 the most. Radial shields are less than 1/3 d.d., only distally contiguous in *O. pulchra*, 1/3–1/4 d.d., and separated or distally contiguous in *O. plicata*. Disc spines are cylindrical or clavate with a few longer thorns, 0.3–0.4 mm high in *O.*

Table 2. *Ophioplinthaca defensor* genetic diversity based on *COI* and *16S*

Site	*COI*				*16S*			
	N	Hap	H_d	π	*N*	Hap	H_d	π
WJ-ROV01	8	7	0.964 3	0.006 7	8	2	0.250 0	0.000 5
WJ-ROV04	4	3	0.833 3	0.003 9	4	2	0.500 0	0.001 0
WJ-ROV05	1	1	–	–	1	1	–	–
WJ-ROV06	13	8	0.807 7	0.004 6	19	7	0.666 7	0.002 3
BG-ROV04	1	1	–	–	1	1	–	–
RC-ROV06	3	2	0.666 7	0.003 7	3	1	–	–
RC-ROV07	1	1	–	–	1	1	–	–
Weijia Guyot	26	18	0.910 8	0.005 6	32	8	0.562 5	0.001 7
Batiza Guyot	1	1	–	–	1	1	–	–
RC seamount	4	3	0.833 3	0.003 1	4	2	0.500 0	0.001 0

Note: *N*, sample size; Hap, number of haplotypes; H_d, haplotype diversity; π, nucleotide diversity; – represents no data.

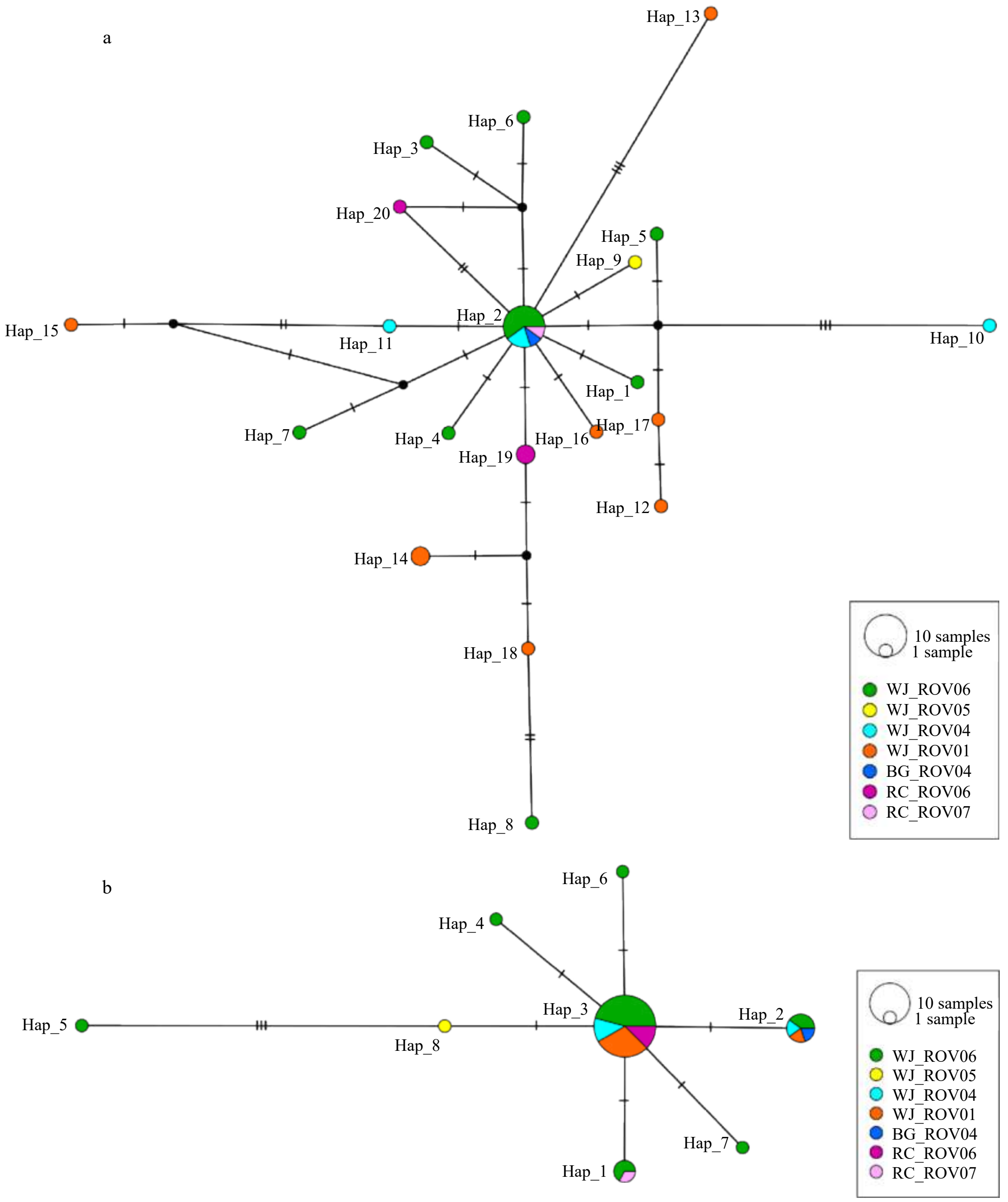

Fig. 5. *COI* (a) and *16S* (b) TCS haplotype networks. Each circle represents a haplotype and its diameter is proportional to the frequency of the haplotype. Median vectors (black circles, which represent missing intermediates haplotypes) are added to the network.

plicata and spherical or capitate, 0.3 mm in diameter in *O. pulchra*, and distributed not only centrally and marginally but also on disc plates surrounding the radial shields interradially in both the two species. In addition, *O. defensor* potentially differs from these two species in having a narrow genital split, shorter arm spines, and oral shields connecting the first lateral arm plate. Therefore, this study provide diagnose characters of *O. defensor* as follows: (1) spherical or conical disc spines, smooth or with few tiny thorns at the apex for adults, cylindrical disc spines with flaring thorny apex for juveniles; (2) large triangular radial shields covering a large proportion of the disc, at least half contiguous distally in adults, and triangular and about half contiguous distally in juveniles. Due to the high variation in morphological characters, further studies are required to clarify the relationships among species in the genus.

4.2 *Lack of a population structure between seamounts and within the Weijia Guyot*

Many studies have inferred extensive gene flow from regional to oceanic scales for invertebrates in deep-sea environments (Castelin et al., 2012; Etter et al., 2011; O'Hara et al., 2014; Taylor and Roterman, 2017). In the present study, the low and non-significant F_{st} and G_{st} values indicate a potentially high level of connectivity between the Weijia Guyot and the RC seamount *O. defensor* populations, or for populations within the Weijia Guyot, which is further supported by star-shaped network for both *COI*

Table 3. Pairwise population differences of *Ophioplinthaca defensor* among sampling sites with more than three individuals collected and between the Weijia Guyot and the RC seamount based on *COI* and *16S*

Gene	Population	WJ-ROV01	WJ-ROV04	WJ-ROV06	RC-ROV06	WJ-all	RC-all
COI	WJ-ROV01	–	0.064 5	0.083 4	0.117 0	–	–
	WJ-ROV04	0.056 0	–	0	0.129 0	–	–
	WJ-ROV06	0.060 9	0.000 2	–	0.088 0	–	–
	RC-ROV06	0.096 3	0.140 7	0.124 8	–	–	–
	WJ-all	–	–	–	–	–	0.050 9
	RC-all	–	–	–	–	0.049 3	–
16S	WJ-ROV01	–	0	0	0	–	–
	WJ-ROV04	0	–	0	0	–	–
	WJ-ROV06	0.023 6	0.012 3	–	0.020 2	–	–
	RC-ROV06	0.023 1	0.003 0	0.080 0	–	–	–
	WJ-all	–	–	–	–	–	0
	RC-all	–	–	–	–	0.027 9	–

Note: Above the diagonal are the F_{st} values, below the diagonal are the G_{st} values. WJ-all indicates the integrated population of the Weijia Guyot including all sequences (26 for *COI*, 32 for *16S*) from the four sampling sites; RC-all indicates the integrated population of the RC seamount including all sequences (4 for both *COI* and *16S*) from the two sampling sites; – represents no data.

Table 4. Demographic test results for *Ophioplinthaca defensor* populations from the Weijia Guyot and the RC seamount based on *COI* and *16S*

Site	*COI*			*16S*		
	D	Fu's Fs	*r*	*D*	Fu's Fs	*r*
WJ-ROV01	−2.009 40*	−1.182 1	0.075 3	−1.951 7*	−3.196 3	0.312 5
WJ-ROV04	−0.796 84	0.811 4	0.305 6	−0.612 4	0.171 9	0.250 0
WJ-ROV06	−0.663 17	−2.104 3	0.094 8	−1.054 8	−0.182 0	0.147 4
WJ-all	−2.082 10*	−12.592 0	0.020 2	−1.908 0*	−4.601 0	1.294 2
RC-all	0.167 70	−0.133 0	0.194 4	−0.612 4	0.172 0	0.250 0

Note: *D*, Tajima's D statistic neutrality tests; Fu's Fs, Fu's Fs statistic; *, significant values ($p<0.05$); *r*, the Harpending raggedness index.

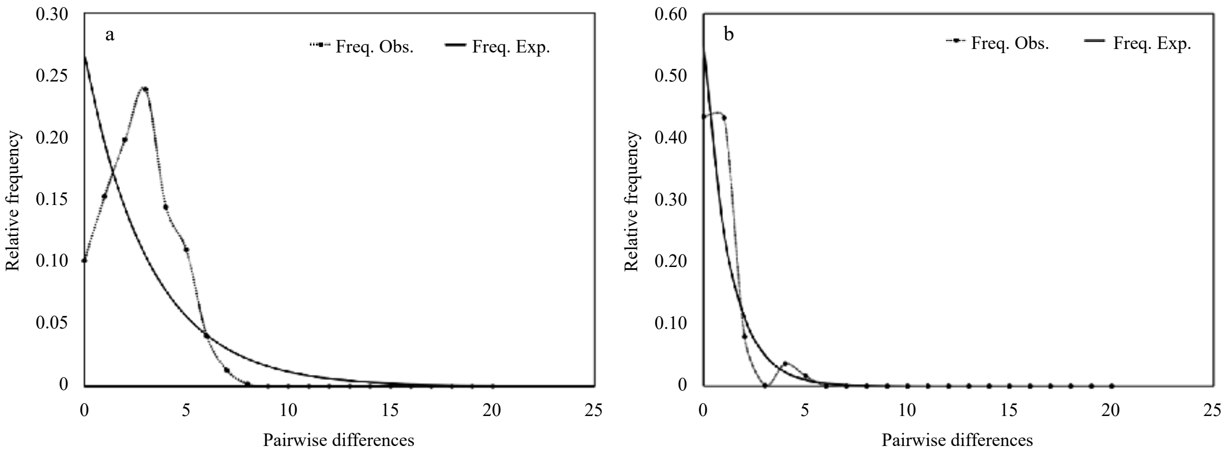

Fig. 6. Pairwise mismatch distribution for all *COI* (a) and *16S* (b) sequences of *Ophioplinthaca defensor* from the Weijia Guyot.

and *16S*.

Distance is potentially one of the most critical factors influencing the distribution of benthic organisms (O'Hara et al., 2010). The distance between the Weijia Guyot and the RC seamount used for population analyses was about 640 km, which seems beyond the maximum dispersal distance for most species from non-chemosynthetic ecosystems (Baco et al., 2016). However, it was summarized that populations at similar depths were generally well connected over 100 s–1 000 s km (Taylor and Roterman, 2017). Species from Ophiacanthidae have been demonstrated to have a capacity to disperse across abyssal plains more than a thousand kilometers wide (O'Hara et al., 2014). In this study, populations were sampled within narrow depth range, which may be a reason for the lack of genetic structure. In addition, the distances from the two targeted seamounts to their neighboring seamounts were much lower; mostly less than 100 km. A large number of seamounts distributed in the study area could provide discrete similar hard substrate habitats, which serve as "stepping-stones" (Shank, 2010) that facilitate the dispersal of deep-sea invertebrates over greater distances (Baco et al., 2016). Therefore, the distance between seamounts may not be a barrier to the dispersal and connectivity among *O. defensor* populations in the study area.

Depth is another key factor influencing population divergence in deep-sea ecosystems (Taylor and Roterman, 2017). According to this results, there was no significant vertical differenti-

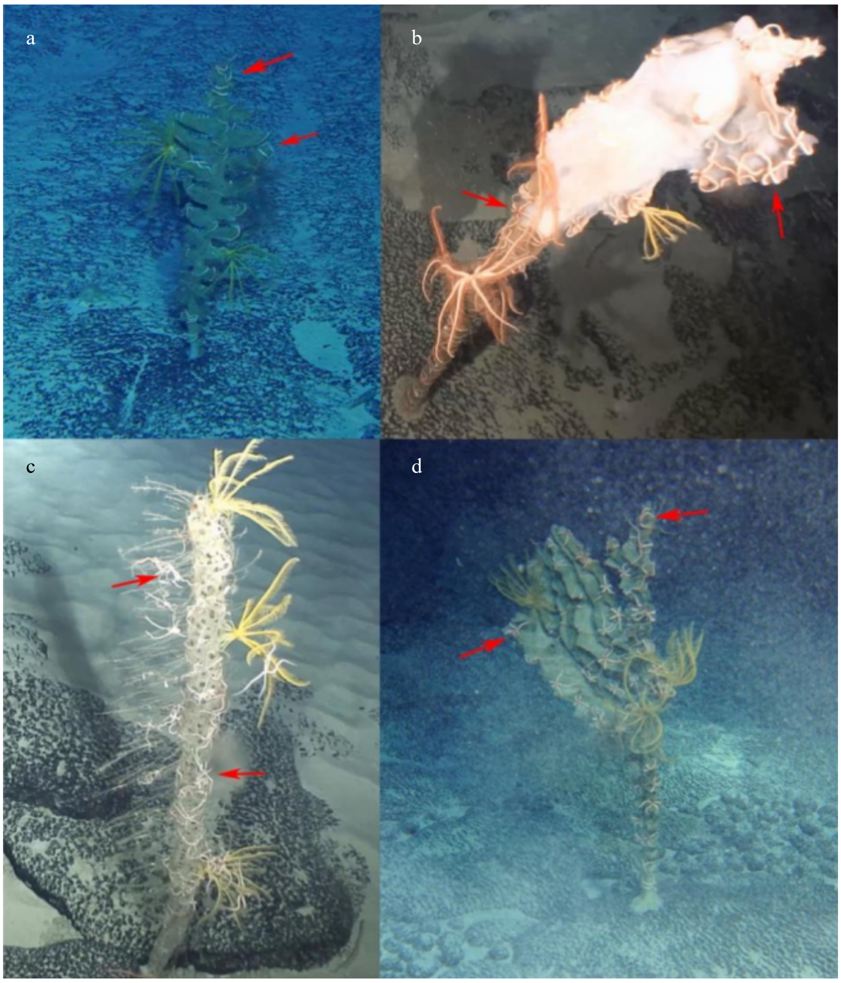

Fig. 7. Different sponge species with *Ophioplinthaca defensor* individuals attached in the Weijia Guyot. a. *Farreidae* sp. 1; b. *Farreidae/Euretidae* sp.; c. *Corbitellinae* sp.; d. *Farreidae* sp. 2. Red arrows indicate *Ophioplinthaca defensor*.

ation in the *O. defensor* population within the Weijia Guyot. Among four ophiuroid species from seamounts in the Atlantic Ocean, two other ophioplinthacids exhibited no genetic structure based on depth, conversely, two species from Euryalidae exhibited significant genetic differentiation based on depth (Cho and Shank, 2010). The dispersal of the latter two species could be limited hydrographically to specific depth ranges considering they are broadcast spawners; however, as brooders, it may be easier for ophioplinthacids to migrate vertically across hydrographical gradients when compared to spawners (Cho and Shank, 2010). This may also explain the lack of a genetic structure based on depth for *O. defensor* in the Weijia Guyot.

4.3 *Demographic expansion in the deep-sea seamount*

Deep-sea environments are expected to be stable when compared to shallow-water environments, but benthic fauna may be affected by periodical disturbance events (Thatje et al., 2008). In the present study, the neutrality statistics indicated the accumulation of a high number of low frequency gene mutations in the population. Demographic analysis suggested that the *O. defensor* population in the Weijia Guyot had possibly experienced a bottleneck event followed by population expansion or a recent selective sweep. It has been suggested that there was an anoxic event at the Cenomanian/Turonian boundary in the Northwest Pacific, coincided with the Cretaceous extinction event (Kaiho and Hasegawa, 1994). This could be a reason for the demographic change in *O. defensor* populations. However, it is impossible to differentiate between the effect of selective sweeps and other demographic and life history processes (Taylor and Roterman, 2017), thus, multi-locus studies are required to facilitate a better understanding of the demographic patterns of benthic invertebrates in this area.

4.4 *Implications for conservation and environmental management*

With the heightened interest in mineral resource extraction from deep-sea environments (Martino and Parson, 2012), the protection and conservation of marine environments are becoming more critical than ever. This is the first population structure study focusing on CRC seamounts in the Northwest Pacific. The small sample size and utilization of limited gene markers prevent us from providing conclusive or adequately robust recommendations, nonetheless, results in this study suggest that the degree of connectivity between the seamounts hundreds of kilometers apart is potentially high, which could offer key insights to facilitate REMP establishment activities (Taboada et al., 2018). In the Northwest Pacific, more sampling efforts from different localities are required, particularly from the west part of the “Triangle Area”, which would yield a more comprehensive perspective of population connectivity, and potentially reveal a greater spatial scale of the connectivity (Manel et al., 2019) in the area. Although the lack of a significant pairwise differences in populations in the present study is consistent with the findings of a previous study (Cho and Shank, 2010), the depth range (1 482–1 935 m) of the sampling sites in the present study may be inadequate to distinguish the variation with depth. *Ophioplinthaca* is a genus with a large depth range (O'Hara and Stöhr, 2006). Many studies have suggested unique characteristics in populations in deeper water below the upper bathyal zone (Havermans et al., 2013; Jennings et al., 2013); therefore, further sampling efforts at deeper slopes should be considered, which could offer additional insights on population structure within single seamounts. Ophiuroids represent benthic invertebrates with low-mobility, suggesting such a lack of population structure is applicable to swimmers (fish or amphipod) with higher dispersal capacity. Nevertheless, sessile taxa such as sponges and corals, which are more vulnerable to anthropogenic activities (Zeng et al., 2019), should be given greater attention in environmental impact assessment activities in the wake of anticipated CRC seamount exploration and mining expeditions.

Acknowledgements

We thank Zhenggang Li for helping produce the topographic map of the study area, Hongbin Liu for polishing the language. We also appreciate the *Jiaolong* team from the National Deep Sea Center, the *Haima* team from the Guangzhou Marine Geological Survey, the *Hailong III* team from the National Deep Sea Center, and Shanghai Jiaotong University for their wonderful job during the collecting samples.

References

Baco A R, Etter R J, Ribeiro P A, et al. 2016. A synthesis of genetic connectivity in deep-sea fauna and implications for marine reserve design. Molecular Ecology, 25(14): 3276–3298, doi: 10.1111/mec.13689

Balbar A C, Metaxas A. 2019. The current application of ecological connectivity in the design of marine protected areas. Global Ecology and Conservation, 17: e00569, doi: 10.1016/j.gecco.2019.e00569

Boissin E, Stöhr S, Chenuil A. 2011. Did vicariance and adaptation drive cryptic speciation and evolution of brooding in *Ophioderma longicauda* (Echinodermata: Ophiuroidea), a common Atlanto-Mediterranean ophiuroid?. Molecular Ecology, 20(22): 4734–4755

Boschen R E, Tyler P A, Copley J T. 2013. Distribution, population structure, reproduction and diet of *Ophiolimna antarctica* (Lyman, 1879) from Kemp Caldera in the Southern Ocean. Deep-Sea Research Part II: Topical Studies in Oceanography, 92: 27–35, doi: 10.1016/j.dsr2.2013.02.005

Castelin M, Lorion J, Brisset J, et al. 2012. Speciation patterns in gastropods with long-lived larvae from deep-sea seamounts. Molecular Ecology, 21(19): 4828–4853, doi: 10.1111/j.1365-294X.2012.05743.x

Cho W, Shank T M. 2010. Incongruent patterns of genetic connectivity among four ophiuroid species with differing coral host specificity on North Atlantic seamounts. Marine Ecology, 31(S1): 121–143

Clark M R, Bowden D A, Rowden A A, et al. 2019. Little evidence of benthic community resilience to bottom trawling on seamounts after 15 years. Frontiers in Marine Science, 6: 63, doi: 10.3389/fmars.2019.00063

Clark M R, Rowden R A, Schlacher T, et al. 2010. The ecology of seamounts: structure, function, and human impacts. Annual Review of Marine Science, 2: 253–278, doi: 10.1146/annurev-marine-120308-081109

Edgar R C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res, 32(5): 1792–1797, doi: 10.1093/nar/gkh340

Etter R J, Boyle E E, Glazier A, et al. 2011. Phylogeography of a pan-Atlantic abyssal protobranch bivalve: Implications for evolution in the Deep Atlantic. Molecular Ecology, 20(4): 829–843, doi: 10.1111/j.1365-294X.2010.04978.x

Excoffier L, Lischer H E L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10(3): 564–567, doi: 10.1111/j.1755-0998.2010.02847.x

Fu Yunxin. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics, 147(2): 915–925, doi: 10.1093/genetics/147.2.915

Galaska M P, Sands C J, Santos S R, et al. 2016. Geographic structure in the Southern Ocean circumpolar brittle star *Ophionotus victoriae* (Ophiuridae) revealed from mtDNA and single- nucleotide polymorphism data. Ecology and Evolution, 7(2): 475–485

Galaska M P, Sands C J, Santos S R, et al. 2017. Crossing the Divide: Admixture Across the Antarctic Polar Front Revealed by the Brittle Star *Astrotoma agassizii*. The Biological Bulletin, 232(3): 198–211, doi: 10.1086/693460

Harpending H C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human Biology, 66(4): 591–600

Havermans C, Sonet G, D'Acoz C D, et al. 2013. Genetic and morphological divergences in the cosmopolitan deep-sea amphipod *Eurythenes gryllus* reveal a diverse abyss and a bipolar species. PLoS ONE, 8(9): e74218, doi: 10.1371/journal.pone.0074218

Hein J R, Bohrson W A, Schulz M S, et al. 1992. Variations in the fine-scale composition of a central pacific ferromanganese crust: paleoceanographic implications. Paleoceanography and Paleoclimatology, 7(1): 63–77

Hilário A, Metaxas A, Gaudron S M, et al. 2015. Estimating dispersal distance in the deep sea: challenges and applications to marine reserves. Frontiers in Marine Science, 2: 6, doi: 10.3389/fmars.2015.00006

Hunter R L, Halanych K M. 2008. Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean. Journal of Heredity, 99(2): 137–148, doi: 10.1093/jhered/esm119

Hunter R L, Halanych K M. 2010. Phylogeography of the Antarctic planktotrophic brittle star *Ophionotus victoriae* reveals genetic structure inconsistent with early life history. Marine Biology, 157(8): 1693–1704, doi: 10.1007/s00227-010-1443-3

ISA. 2018. Towards the development of a regional environmental management plan for cobalt-rich ferromanganese crusts in the Northwest Pacific Ocean. ISA Technical Study No. 23. Kingston, Jamaica: International Seabed Authority

ISBA/19/LTC/8. 2013. Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the area. Jamaica: International Seabed Authority

Jennings R M, Etter R J, Lynn F, et al. 2013. Population differentiation and species formation in the deep sea: the potential role of environmental gradients and depth. PloS ONE, 8(10): e77594, doi: 10.1371/journal.pone.0077594

Kaiho K, Hasegawa T. 1994. End-Cenomanian benthic foraminiferal extinctions and oceanic dysoxic events in the northwestern Pacific Ocean. Palaeogeography, Palaeoclimatology, Palaeoecology, 111(1–2): 29–43, doi: 10.1016/0031-0182(94)90346-8

Koehler R. 1930. Ophiures recueillies par le Docteur Th. Mortensen dans les Mers d'Australie et dans I'Archipel Malais. Papers from Dr. Th. Mortensen's Pacific Expedition 1914-16. LIV. Videnskabelige Meddelelser fra Dansk naturhistorisk Forening, 89: 1–295

Leigh J W, Bryant D. 2015. Popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution, 6(9): 1110–1116, doi: 10.1111/2041-210X.12410

Manel S, Loiseau N, Andrello M, et al. 2019. Long-distance benefits of marine reserves: myth or reality?. Trends in Ecology & Evolution, 34(4): 342–354

Martino S, Parson L M. 2012. A comparison between manganese nodules and cobalt crust economics in a scenario of mutual exclusivity. Marine Policy, 36(3): 790–800, doi: 10.1016/j.marpol.2011.11.008

Miller K J, Gunasekera R M. 2017. A comparison of genetic connectivity in two deep sea corals to examine whether seamounts are isolated islands or stepping stones for dispersal. Scientific Reports, 7: 46103, doi: 10.1038/srep46103

Morgan N B, Cairns S, Reiswig H, et al. 2015. Benthic megafaunal community structure of cobalt-rich manganese crusts on Necker Ridge. Deep-Sea Research Part I: Oceanographic Research Papers, 104: 92–105, doi: 10.1016/j.dsr.2015.07.003

O'Driscoll R L, Clark M R. 2005. Quantifying the relative intensity of fishing on New Zealand seamounts. New Zealand Journal of Marine and Freshwater Research, 39(4): 839–850, doi: 10.1080/00288330.2005.9517356

O'Hara T D. 2007. Seamounts: centres of endemism or species-richness for ophiuroids?. Global Ecology and Biogeography, 16(6): 720–732, doi: 10.1111/j.1466-8238.2007.00329.x

O'Hara T D, Consalvey M, Lavrado H P, et al. 2010. Environmental predictors and turnover of biota along a seamount chain. Marine Ecology, 31(S1): 84–94

O'Hara T D, England P R, Gunasekera R M, et al. 2014. Limited phylogeographic structure for five bathyal ophiuroids at continental scales. Deep-Sea Research Part I: Oceanographic Research Papers, 84: 18–28, doi: 10.1016/j.dsr.2013.09.009

O'Hara T D, Smith P J, Mills V S, et al. 2013. Biogeographical and phylogeographical relationships of the bathyal ophiuroid fauna of the Macquarie Ridge, Southern Ocean. Polar Biology, 36(3): 321–333, doi: 10.1007/s00300-012-1261-9

O'Hara T D, Stöhr S. 2006. Deep water Ophiuroidea (Echinodermata) of New Caledonia: Ophiacanthidae and Hemieuryalidae. In: Richer de Forges B, Justine J L, eds. Tropical Deep Sea Benthos (Mémoires du Muséum national d'Histoire naturelle 193). Paris, France: Museum of Natural History of France, 24: 33–141

Palumbi S R. 1996. Nucleic acidS II: the polymerase chain reaction. In: Hilli D M, Moritz C, Mable B K, eds. Molecular Systematics. 2nd ed. Sunderland, MA, USA: Sinauer Associates, 205–247

Palumbi S R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecological Applications, 13(S1): 146–158, doi: 10.1890/1051-0761(2003)013[0146:PGDCAT]2.0.CO;2

Pérez-Portela R, Almada V, Turon X. 2012. Cryptic speciation and genetic structure of widely distributed brittle stars (Ophiuroidea) in Europe. Zoologica Scripta, 42(2): 151–169

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio J C, et al. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution, 34(12): 3299–3302, doi: 10.1093/molbev/msx248

Samadi S, Bottan L, Macpherson E, et al. 2006. Seamount endemism questioned by the geographic distribution and population genetic structure of marine invertebrates. Marine Biology, 149(6): 1463–1475, doi: 10.1007/s00227-006-0306-4

Sands C J, O'Hara T D, Barnes D K A, et al. 2015. Against the flow: evidence of multiple recent invasions of warmer continental shelf waters by a Southern Ocean brittle star. Frontiers in Ecology and Evolution, 3: 63, doi: 10.3389/fevo.2015.00063

Schlacher T A, Baco A R, Rowden A A, et al. 2014. Seamount benthos in a cobalt-rich crust region of the central Pacific: conservation challenges for future seabed mining. Diversity and Distributions, 20(5): 491–502, doi: 10.1111/ddi.12142

Shank T M. 2010. Seamounts: deep-ocean laboratories of faunal connectivity, evolution, and endemism. Oceanography, 23(1): 108–122, doi: 10.5670/oceanog.2010.65

Taboada S, Pérez-Portela R. 2016. Contrasted phylogeographic patterns on mitochondrial DNA of shallow and deep brittle stars across the Atlantic-Mediterranean area. Scientific Reports, 6: 32425, doi: 10.1038/srep32425

Taboada S, Riesgo A, Wiklund H, et al. 2018. Implications of population connectivity studies for the design of marine protected areas in the deep sea: An example of a demosponge from the Clarion-Clipperton Zone. Molecular Ecology, 27(23): 4657–4679, doi: 10.1111/mec.14888

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123(3): 585–595, doi: 10.1093/genetics/123.3.585

Taylor M L, Roterman C N. 2017. Invertebrate population genetics across Earth's largest habitat: The deep-sea floor. Molecular Ecology, 26(19): 4872–4896, doi: 10.1111/mec.14237

Thatje S, Hillenbrand C D, Mackensen A, et al. 2008. Life hung by a thread: endurance of Antarctic fauna in glacial periods. Ecology, 89(3): 682–692, doi: 10.1890/07-0498.1

Yesson C, Clark M R, Taylor M L, et al. 2011. The global distribution of seamounts based on 30 arc seconds bathymetry data. Deep-Sea Research Part I: Oceanographic Research Papers, 58(4): 442–453, doi: 10.1016/j.dsr.2011.02.004

Zeng Cong, Clark M R, Rowden A A, et al. 2019. The use of spatially explicit genetic variation data from four deep-sea sponges to inform the protection of Vulnerable Marine Ecosystems. Scientific Reports, 9: 5482, doi: 10.1038/241598-019-41877-9

Supplementary information:

Table S1. Specimen voucher of Ophioplinthaca defensor examined and their genbank accession

The supplementary information is available online at https://doi.org/10.1007/s13131-020-1666-1 and https://www.aosocean.com. The supplementary information is published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.