### Taxonomic and systematic remarks

This study complements the evolutionary study published by Christodoulou et al. (2019), thus providing detail information regarding the deep-sea brittle-star biodiversity in polymetallic nodule fields of the Pacific Ocean.

**Order Amphilepidida**

**Family Amphilepididae**

***Amphilepis* sp.** (Fig. 5A–B)

**Remarks.** One species of *Amphilepis* (species 5) was recorded from the abyss of both the CCZ and DEA (Peru Basin). The six specimens were small and do not possess characters that can be used to identify the species (Mills and O'Hara, 2010). However, they are likely to be *A. patens* Lyman, 1879, which was described from a specimen collected by the HMS Challenger from the abyssal plain of the southeastern Pacific (Lyman, 1879). Another species (*A. nuda* Tommasi, 1976) has been described from bathyal depths (2489–3541 m) in the Peru Trench (Tommasi, 1976).

**Family Amphiuridae**

***Amphioplus* cf. *daleus* sl** (Fig. 5C–E, 6A–B)

**Remarks.** The only amphiurids present were two clades (species 2 & 4) of *Amphioplus* cf. *daleus* (Fig. 3). The mean COI genetic distance between the two clades was 0.99 (p-distance) and 0.108 (K2P). Species 2 (Fig. 5C–E, 6A) was collected from Peru as well as the CCZ. Only two specimens of species 4 were collected (Fig. 6B). This is a complex of species (Clark, 1970) occurring at abyssal depths in all oceans, including the nominal species *A. daleus* (Lyman, 1879) (South Atlantic), *A. verrilli* (Lyman, 1879) (North Atlantic), *A. cernuus* (Lyman, 1879) (NW Pacific), *A. consors* (Koehler, 1908), *A. magnificus* (Koehler, 1908) and *A. patulus* (Lyman, 1879) (Antarctic). This complex is phylogenetically distinct from other *Amphioplus* species (O'Hara et al., 2017) and deserves recognition as a separate genus, for which the genus-level name *Silax* is available (type *A. verrilli*) (Paterson, 1985). The nominal species morphologically differ mainly in the number of arm segments that possess tentacle scales and the presence of swellings on the basal ventral arm plates, which can be variable characters. A worldwide phylogeography is required to delimit the species.

**Family Ophiernidae**

***Ophiernus* sp.** (Fig. 6C)

**Remarks.** COI sequences from two abyssal specimens of the CCZ and Peru were nested phylogenetically in the genus *Ophiernus* (species 31), within lineages identified (paraphyletically) as *O. adspersus* Lyman, 1883 (Christodoulou et al., 2019). This species is another widespread complex that requires revision (Madsen, 1977). The two specimens were too small for morphological identification. Previously, two names have been applied to abyssal *Ophiernus* from the eastern Pacific, *O. seminudus* Lütken & Mortensen, 1899 (2417–4600 m), which lacks the specialised bristle-like arm spines present on *O. adspersus*, and *O. barracloughi* Tommasi, 1976 (Peru-Chile Trench, 5746–5948 m) which was synonymised with *O. adspersus annectans* by Madsen (1983). A global phylogeography is required to determine the geographic and bathymetric range limits of these species.

**Family Ophiolepididae**

***Ophiotypa simplex* Koehler, 1987** (Fig. 6D–E, 7A)

**Remarks.** *Ophiotypa simplex* (species 30) is the sole abyssal species within this family, with a worldwide distribution at equatorial and temperate latitudes (60N to 50S) (Litvinova, 2010, 1975). No global phylogeography has been undertaken so far, however, the sequences from CCZ did form a single COI clade. This species is strongly paedomorphic, with only 6 plates being present on the adult aboral disc surface, however, this is a derived rather than primitive character (Stöhr and Martynov, 2016).

**Family Ophioleucidae** (Fig. 7B–E, 8A–F, 9A)

**Remarks.** Eight clades were identified within the Ophioleucidae, provisionally recognised as species (Fig. 3), however, only one can be confidently assigned taxonomically to a known species, the bathyal species *Ophioleuce gracilis* Belyaev & Litvinova, 1976 (Fig. 7B) formerly known only from the Peru-Chile Trench at 2900 m water depth. This species is distinguishable from other *Ophioleuce* species by contiguous ventral arm plates along the arm and two thin arm spines (Belaev and Litvinova, 1976; Madsen, 1983). The abyssal CCZ species 34 (Fig. 8C), 35 (Fig. 8D–F) and 37 (Fig. 9A) morphologically resembled to two monotypic genera, *Uriopha* and *Bathylepta* in having granules covering the disc (and onto the oral shields), genital slits, small oral shields, thin skeletal arms, reduced dorsal and ventral arm plates, and two sharp arm spines (Paterson, 1980). Nevertheless, they differed in having a pair of large swollen tube feet at the base of each arm and relatively long arm spines (longer than an arm segment in length), and therefore putatively represent a new genus. Species 33 (Fig. 8B) was represented by a unique abyssal CCZ sequence, sister to the clade encompassing species 34-35-37 (Fig. 3). It derives from a tiny juvenile specimen but did not appear to have the swollen basal tube feet. Species 22 (Fig. 7C–D) formed a monophyltic clade represented by four juveniles from the CCZ abyss (Fig. 3). Species 29 (Fig. 7E, 8A) was a relatively common species that occurs in both the CCZ and Peru regions. One specimen (SO239\_192\_5) was quite genetically divergent and possible represents another species (species 42). It was morphologically similar to the Atlantic *Uriopha ios* Paterson, 1980 (but had 3 sharp arm spines basally) and to *Bathylepta pacifica* Belyaev & Litvinova, 1972 (but had longer arm spines to half an arm segment in length and an oval tentacle scale). Comparison was impeded by the relatively large size of the types of these two described species (4 mm disc diameter) and the suboptimal condition of all specimens.

**Order Euryalida**

**Family Euryalidae**

***Asteroschema* sp.**(Fig. 9B)

**Remarks.** A single specimen (species 12) was collected from a seamount in the CCZ (1667 m), provisionally identified as *Asteroschema*. Recent genetic evidence suggests that the previous morphological distinction between species having granules (*Asteroschema*) or thick skin (*Ophiocreas*) on the disc does not always hold and these two genera require revision (Okanishi et al., 2011). The specimen is genetically very similar to specimens collected recently from seamounts in the Galapagos Marine Park (1226–1286 m) (Christodoulou et al., 2019).

**Order Ophiacanthida**

**Family Ophiacanthidae**

***Ophiocantha cosmica* Lyman, 1878** (Fig. 9C, 10A–B)

**Remarks.** Numerous specimens from both the CCZ and Peru of the large species *Ophiacantha cosmica* (species 27) form a shallow clade on the COI tree (Fig. 3). Nuclear DNA data suggests that this species occurs at lower bathyal and abyssal depths from Antarctica and southern Australia to the Kuril-Kamchatka Trench in the NW Pacific Ocean. Specimens have also been identified morphologically from the Indian and South Atlantic Oceans. Eastern Pacific specimens have been sometimes named as *O. pacifica* if they have a slightly wider distal oral papilla, but to date there is no genetic evidence to support this distinction.

**Family Ophiotomidae**

***Ophiotoma* sp.** (Fig. 10C)

**Remarks.** Two tiny specimens from abyss depths in both the CCZ and DEA phylogenetically cluster within *Ophiotoma*, a genus of both bathyal and abyssal species (Martynov, 2010). The subtending branch is relatively long (Fig. 3), suggesting a new species (species 21). The new specimens are small and do not allow identification of morphological characters.

**Order Ophioscolecida**

**Family Ophiohelidae** (Fig. 10D–E, 11A–G, 12A–C)

**Remarks.** Species 26 (Fig. 11D–F) is an *Ophiomyces* species. Several *Ophiomyces* species have been described from the abyssal Pacific Ocean (Litvinova, 2001), including *Ophiomyces nadiae* Litvinova, 2001 and *O. latissimus* Litvinova, 2001, however, we refrain from assigning the CCZ specimens to a known species until adult specimens with an intact disc have been found. Species 1 (Fig. 11G, 12A–B) and 28 (Fig. 12C) were assigned to *Ophiotholia*, both having characteristic umbrella-shaped spines distally on the lateral arm plates and widened oral papillae (Litvinova, 1992). They have been previously reported from the CCZ by Glover et al. (2016) as NHM\_303 and NHM\_076 respectively, with photos of NHM\_076 (Glover et al., 2016: Fig. 21) clearly showing the umbrella-shaped spines. Larger specimens and SEM images of the umbrellas are required to assign them confidently to a known species. Species 24 (Fig. 10E), 25 (not figured), 39 (Fig. 10D) and 40 (Fig. 11A–B) are provisionally assigned to the abyssal genus *Ophiocymbium* having large tube feet, a low convex disc covered in thin scales and inconspicuous radial shields (Martynov, 2010). Images show specimens lacking an elongated disc or upturned arms, no umbrellas, and no flattened oral papillae characteristic of *Ophiotholia* and *Ophiomyces*. No *Ophiocymbium* samples have been exon-captured to date (Christodoulou et al., 2019). Both species have two divergent clades indicative of cryptic speciation. Species 25 has been previously reported from the CCZ as species NHM\_072 (Glover et al. 2016). Species 32 (not figured) represented by one tiny juvenile with disc spines, sister to all sequenced Ophiohelidae to date.

**Family Ophioscolecidae** (Fig. 12D–H**)**

**Remarks.** Four clades (species 17–20), represented only by tiny juveniles without key identifying characters, were placed within the *Ophiophiura-Ophioplexa* clade on the super-matrix tree of Christodoulou et al. (2019). The Ophioscolecidae has been recovered as a paraphyletic family (with respect to the Ophiohelidae) and left unresolved in the phylogeny of O'Hara et al. (2017, 2018) due to incomplete sampling. All four species were found on the CCZ abyssal plain. Species 18 is composed of two COI clades, one of which was only collected from the Peru Basin.

**Order Ophiurida**

**Family Ophiopyrgidae** (Fig. 12I, 13A–E, 14A–D, 15A–D)

**Remarks.** *Amphiophiura bullata* (Fig. 12I, 13A–B) is a worldwide abyssal species complex that has been separated into four subspecies (*bullata*, *convexa*, *vitjazi* and *pacifica*) on the basis of plate fragmentation on the dorsal disc surface (Litvinova, 1971; Paterson, 1985). Preliminary results from nuclear DNA data indicate that these subspecies have little validity and the complex forms a shallow clade including specimens from abyssal depths from Antarctica to the Kuril-Kamchatka Trench, and from the south Atlantic to the eastern Pacific. Conversely, *Ophiuroglypha irrorata* recovers a large complex of lineages according to the phylogenetic trees of Christodoulou et al. (in press). Many of the listed synonyms of *O. irrorata* require recognition as full species. The subgenus *Ophiuroglypha* was raised to genus-rank by O'Hara et al. (2018) as the type species of *Ophiura* is in a separate family (Ophiuridae). Morphologically the *O.* *irrorata* complex has arm combs that do not meet across the dorsal base of the arm, characteristically widened adradial tentacle scales on basal pores that appear like supplementary ventral arm plates, and first and second ventral arm plates that are contiguous for at least half their width. Three lineages of the *irrorata* complex appeared to be present in the CCZ and/or DEA. Species 7 (Fig. 15B) and 14 (Fig. 14E) were each represented by one tiny juvenile. Species 6 (Fig. 14A–B) and 43 (Fig. 14C–D), occurring in both regions, clustered within the *O. figurata*/*polyacantha*/*lenticularis* clade of species that have been sampled from the Southern Ocean and western Pacific. There were large species with numerous arm spines, similar to other species in the latter clade. The two sister species (6 and 43) had 0.064 (p-distance) and 0.064 (K2P) mean genetic divergence (Table S2). Species 9 (Fig. 15C) from the abyssal CCZ looks superficially similar but lacks the diagnostic enlarged adradial tentacle scale and has very large flat arm comb papillae that almost meet across the base of the arm and very wide oral shields. Its sister lineage, Species 11 was a bathyal (2852 m) specimen collected from a seamount within the CCZ, and exhibits a round disc that looked superficially like an *Ophiocten* species but lacked their characteristic convex distal margin on the ventral arm plates. Species 6, 7, 9, 11, 14 and 43 were all designated to *Ophiuroglypha* pending a generic revision of the family. Species 10, also collected from a seamount in the CCZ, was a bathyal species (2852 m) that appeared to be morphologically similar to two *Anophiura* species described from the Indian Ocean (1046–1295 m) (Clark, 1939). On the phylogenetic tree of Christodoulou et al. (2019), species 10 was placed in a clade with *‘Ophiura’ tenera* (Caribbean), *O. falcifera* (Caribbean) and *O. costata* (South Africa). All these species had only three pairs of obvious tentacle pores (with scales) at the base of the arm and large disc plates separating the radial shields proximally and laterally. *Ophiura* is polyphyeletic (as mentioned above) and species 10 was provisionally designated to *Anophiura*. It differed from the two described species in having an oral shield that was as wide as long, relatively few irregular disc plates, and longer arm spines. Species 8 (Fig. 13E) was a tiny juvenile that didn’t show a genetic convergent with any sequenced Ophiopyrgidae lineage to date. Species 15 (Fig. 13D) has unusual flattened leaf-like basal arm spines and a fringe of marginal disc spines characteristic of the genus *Ophiophyllum* (see Vadon, 1991). This genus has not been previously sequenced and is here placed in the Ophiopyrgidae for the first time. The CCZ specimens appear to be a new species, the first in the genus reported from abyssal depths (4541–4822 m), differing from other species in having marginal disc spines which are spiniform and absent near the arm base.

**Family Ophiosphalmidae**

***Ophiosphalma glabrum* complex (Lütken & Mortensen, 1899)** (Fig. 15D, 16A–F)

**Remarks.** Two clades of *Ophiosphalma* were recovered in the COI tree present in the CCZ and DEA with mean intergenetic distance 0.05 (p-distance) and 0.052 (K2P) (Table S2). This divergence was confirmed by phylogenies based on nuclear DNA (O'Hara et al., 2017). One of these clades corresponding to Species 3 (Fig. 15D, 16A–C), referred herein as *Ophiosphalma glabrum*, was reported by Glover et al. (2016) under the name *Ophiomusium* cf. *glabrum* to be the most abundant megafaunal animals in the CCZ. Subsequently, Baker (2016) and O'Hara et al. (2017) transferred both (and other species) to *Ophiosphalma* based on morphological and genetic evidence. The other clade (Species 36; Fig. 16D–F), herein designated as *O.* cf. *glabrum*, is morphologically similar. Our attempts to separate the two clades morphologically had been unsuccessful. In particular, the number of visible tentacle pores at the base of the arm, which could vary between 3 and 5, did not appear to correlate with the genetic clades. However, there were substantial differences in morphology with size in both clades. The interradial and central disc plates, clearly visible on juveniles, were replaced by skin on large specimens. COI sequences on NCBI/Nucleotide (HM400322-3) identified as *O. glabrum* from off Vancouver Island (2076–2080 m) were phylogenetically distinct (K2P=0.170) and represented a separate species, for which the nominal name *O. multispinum* is available (type from offshore Washington, 1604 m). The distribution limits of these species requires clarification, as it is unclear whether *O. glabrum* and *O. multispinum* will be separated into distinct geographical (north vs tropical eastern Pacific) or bathymetric ranges (bathyal vs abyssal). The relationship to another species, *O. armatum*, described from juvenile bathyal specimens from the Philippines (2021 m), is also unclear. Unlike other abyssal ophiuroids, *Ophiosphalma glabrum* was not widespread. Exon-capture DNA evidence (Christodoulou et al. 2019) showed that *O. fimbriatum* from the western Pacific was distinct from *O. glabrum*, and therefore the synonymy proposed by Baker (2016) was rejected. All these species were replaced in the Atlantic and Indian Oceans by *O. armigerum* which differs morphologically in only having 4 arm spines compared to 8 or more in the other species discussed above.

**Ophiurida** incertae sedis

***Perlophiura profundissima* Belyaev & Litvinova, 1972** (Fig. 16G)

**Remarks.** The paedomorphic species *Perlophiura profundissima* was found only in the Peru Basin in this study, but other expeditions have previously collected it from the CCZ (Glover et al., 2016). It is another putatively widespread species, reported from all oceans except around Antarctica and the Arctic (Litvinova, 2010).

# References

Baker, A. N.: An illustrated catalogue of type specimens of the bathyal brittlestar genera *Ophiomusium* Lyman and *Ophiosphalma* H. L. Clark (Echinodermata: Ophiuroidea), Zootaxa, 4097, 1–40, doi: 10.11646/zootaxa.4097.1.1., 2016.

Belyaev, G. M., and Litvinova, N. M.: The new and rare ophiuroid species from the Pacific and Indian Oceans, Tr. Inst. Okeanol. im P. P. Shirshova, Akad. Nauk SSSR, 99, 126–139, 1976.

Clark, H. L.: Ophiuroidea., Scientific Reports. The John Murray Expedition, 6, 29–136, 1939.

Clark, A. M.: Notes on the family Amphiuridae (Ophiuroidea). Bull. Br. Mus. Nat. Hist. (Zool), 19, 1–81, 1970.

Litvinova, N. M.: The brittle-stars of the genus *Amphiophiura* of the Pacific and Indian Oceans collected by Soviet Expeditions on the R/V “Vityaz” and “Akademician Kurchatov”, Tr. Inst. Okeanol. im P. P. Shirshova, Akad. Nauk, SSSR, 92, 298–316, 1971.

Litvinova, N. M.: Ophiuroids of the Caribbean and Gulf of Mexico collected during the 14th cruise of the R/V “Akademik Kurchatov”, Tr. Inst. Okeanol. im P. P. Shirshova, Akad. Nauk, SSSR, 100, 196–204, 1975.

Litvinova, N. M.: Revision of the genus *Ophiotholia* (Echinodermata: Ophiuroidea), Zool. Zh., 71, 47–57, 1992.

Litvinova, N. M.: The ophiuroid genus *Ophiomyces* (Echinodermata, Ophiuroidea), in: Composition and structure of the marine bottom biota: collected proceedings, edited by: Kuznetsov, A. P., and Zezina, O. N., VNIRO Publishing House, Moscow, 2001.

Litvinova, N. M.: Catalogue of brittle stars (Echinodermata: Ophiuroidea) of the world ocean: from the collection of Ocean Benthic Fauna, P.P. Shirshov Institute of Oceanology, Russian Academy of Sciences URSS, Moscow, 2010.

Madsen, F. J.: The Ophioleucidae, Galathea Report, 14, 109–122, 1977.

Madsen, F. J.: A review of the Ophioleucinae stat. rev. (Echinodermata, Ophiuroidea) with the erection of a new genus *Ophiostriatus*, Steenstrupia, 9, 29–69, 1983.

Martynov, A. V.: Reassessment of the classification of the Ophiuroidea (Echinodermata), based on morphological characters. I. General character evaluation and delineation of the families Ophiomyxidae and Ophiacanthidae, Zootaxa, 2697, 1–154, doi: [10.11646/zootaxa.2697.1.1](http://dx.doi.org/10.11646/zootaxa.2697.1.1), 2010.

Mills, V. S., and O'Hara, T. D.: *Amphilepis neozelandica* sp. nov., the first record of the Amphilepididae in New Zealand waters (Echinodermata: Ophiuroidea), Zootaxa, 2514, 47–54, https://doi.org/10.11646/zootaxa.2514.1.4, 2010.

O'Hara, T. D., Stöhr, S., Hugall, A. F., Thuy, B., and Martynov, A. V.: Morphological diagnoses of higher taxa in Ophiuroidea (Echinodermata) in support of a new classification, Eur. J. Taxon*.*, 416, 1–35, doi: [10.5852/ejt.2018.416](https://doi.org/10.5852/ejt.2018.416), 2018.

Okanishi, M., O'Hara, T. D., and Fujita, T.: A new genus *Squamophis* of Asteroschematidae (Echinodermata, Ophiuroidea, Euryalida) from Australia, ZooKeys 129: 1–15, doi: [10.3897/zookeys.129.1202](https://doi.org/10.3897/zookeys.129.1202), 2011.

Paterson, G. L. J.: The deep-sea Ophiuroidea of the North Atlantic Ocean, Bull. Br. Mus. Nat. Hist. (Zool.), 49, 1–162, 1985.

Paterson, G. L. J.: A new abyssal genus of the family Ophiuridae (Echinodermata: Ophiuroidea), Bull. Br. Mus. Nat. Hist. (Zool.), 38, 211–218, 1980.

Stöhr, S., and Martynov, A. V.: Paedomorphosis as an Evolutionary Driving Force: Insights from Deep-Sea Brittle Stars, PLoS ONE, 11, e0164562, doi: [10.1371/journal.pone.0164562](https://doi.org/10.1371/journal.pone.0164562), 2016.

Tommasi, L. R.: Ophiuroidea collected in the Peru-Chile Trench by the USNS “Eltanin" during cruise III, Pap. Avulsos de Zool., Sao Paulo, 29, 281–318, 1976.

Vadon, C.: Echinodermata: Ophiuridea profunds de Novelle-Calédonie: Formes paedomorphes: Resultats des Campagnes MUSORSTOM vol. 8, Mém. Mus. Natl. Hist. Nat., Paris, 151, 335–356, 1991.