Zoological Studies **62:**23 (2023)
doi:10.6620/ZS.2023.62-23

Zoological Studies

Open Access

Discovery of a New Sea Spider Belonging to the Genus *Pycnogonum* (Pycnogonida: Pycnogonidae) in the Mesophotic Zone of Jejudo Island, Korea

Damin Lee[1], Jin-Ho Park[2], and Taeseo Park[3,*]

[1]*Division of Ecoscience, Ewha Womans University, Seodaemun-gu, Seoul 03760, Republic of Korea. E-mail: dlekals2@naver.com (Lee)*
[2]*Computational Biology Institute, The George Washington University, Washington DC 20052, USA. E-mail: jhpark1985@gmail.com (Park)*
[3]*National Institute of Biological Resources, Incheon 22689, Republic of Korea. *Correspondence: E-mail: polychaeta@gmail.com (Park)*

Received 5 January 2022 / Accepted 17 February 2023 / Published 22 May 2023
Communicated by Benny K.K. Chan

A new species of the genus *Pycnogonum* was discovered in the mesophotic zone of Munseom Islet, Jejudo Island, Korea. *Pycnogonum* (*Nulloviger*) *bifurcatum* sp. nov. is the first case of sea spiders in Korean fauna not only included in the subgenus *Nulloviger* but also collected in the mesophotic zone of Munseom Islet. The new species is morphologically close to *Pycnogonum* (*Pycnogonum*) *asiaticum* and *P.* (*N.*) *carinatum*, sharing the granular integument, the dorsomedian tubercles on the trunk, and the post-ocular tubercle. The new species can be easily distinguished from the congeners by combination of the following characteristics: the prominent dorsomedian tubercles on the trunk, the lateral processes 1–3 touching each other, and the small auxiliary claws. A key is provided to distinguish 12 species of the subgenus *Nulloviger* morphologically, and molecular data of the new species are provided for species identification and further studies.

Key words: Molecular data, Munseom Islet, *Nulloviger*, Sea spider, Subgenus.

BACKGROUND

Pycnogonum Brünnich, 1764 comprises 77 species, of which only seven have been newly recorded in the last decade (Staples 2019; Bamber et al. 2022). Diagnostic characteristics of this genus include having a stout and robust body and a well-developed proboscis, without chelifores and palps. Stock (1968) divided the genus *Pycnogonum* into three subgenera depending on the characteristics of ovigers as follows: (1) the subgenus *Pycnogonum* is characterized by 8–9 segmented ovigers with a terminal claw, (2) the subgenus *Retroviger* is categorized based on having 4–7 segmented ovigers with or without a terminal claw, and (3) the subgenus *Nulloviger* is characterized by the absence of ovigers in both sexes. Due to the absence of female ovigers in this genus, the subgenus designation depends entirely on the male specimens. The status of the ovigers and the position of the gonopores can determine the gender classification of this genus. The male gonopores are present ventrally on the inner surface of coxa 2 on each leg 4. Those of females are found at the dorsoposterior surface of coxa 2 on leg 4, which are larger and more noticeable. As the ovigers are not present in the subgenus *Nulloviger*, the position of the gonopores is the only reliable way to determine the sex of the specimen (Staples 2002).

Munseom Islet is located near the Seogwipo Harbor in Jejudo Island (Fig. 1). It is famous for its diverse and dense colonies of soft corals, and has been designated as Natural Monument No. 442 by the Cultural Heritage Administration, Korea. This islet has

Citation: Lee D, Park JH, Park T. 2023. Discovery of a new sea spider belonging to the genus *Pycnogonum* (Pycnogonida: Pycnogonidae) in the mesophotic zone of Jejudo Island, Korea. Zool Stud **62:**23. doi:10.6620/ZS.2023.62-23.

colorful soft coral communities and many subtropical fishes and invertebrates, making it a popular region for recreational SCUBA diving and one of the Korean islets where the biodiversity of marine flora and fauna is most actively investigated (Cho et al. 2014; Kwon et al. 2018). However, the investigations have been conducted mainly in shallow waters (euphotic zone). Research on the biota inhabiting areas below the shallow waters is barren because it is too shallow for submersible vehicle operations and too deep for recreational SCUBA diving (Menza et al. 2008; Reimer et al. 2019).

The mesophotic zone generally refers to a depth between 30–40 m to 150–200 m, depending on light penetration (Kahng et al. 2010; Reimer et al. 2019). Studies on this zone have mainly been conducted concerning coral ecology. As climate change and coral bleaching has proceeded, the zone's importance as a refuge for species from the euphotic zone emerged. Interestingly, species inhabiting the euphotic zone are also found in the mesophotic zone. Still, the dominant species found in each zone and their surrounding environments are very different. Especially in the hard substrata environment of the deep fore-reef (53–120 m), the dominant species found are coralline algae, azooxanthellate scleractinians and gorgonians, demosponges, and endolithic sponges, whereas in the euphotic zone, zooxanthellate corals and macroalgae are the primary periphytic organisms (Liddell and Ohlhorst 1988; Kahng et al. 2010). In contrast to the diverse investigation of species living in the euphotic zone, advanced techniques and safety precautions are required to discover the mesophotic zone. Consequently, there are few studies on the fauna of fish, molluscs, nematodes, and polychaetes, leaving a desperate need for research (Kahng et al. 2010; Bianchelli et al. 2013; Albano et al. 2020; Gravina et al. 2021).

The "Discovery of Invertebrate Species from Unexplored Habitats" project was initiated in 2018 under the leadership of the National Institute of Biological Resources, Incheon, Republic of Korea (NIBR). This project regarded fauna studies including the mesophotic zone, and the Munseom Islet located in the subtropical region was selected as a central research area. As we approached a water depth of about 50 m using trimix SCUBA diving, our visibility became very limited, making it difficult to discern our surroundings without artificial light. Sea whips, black corals, bryozoans, and sponges were mainly observed on the hard substrata, as opposed to dominant macroalgae and soft corals in the euphotic zone (Fig. 2). Researchers directly observed this ecological change, acquired various specimens, and conducted more extensive taxonomic studies on marine organisms (*i.e.*, finding unrecorded shrimps inhabiting the sponges and corals; see Park et al. 2020a b 2021).

Through underwater field surveys conducted at Munseom Islet, Jejudo Island, in 2018, a male specimen of the genus *Pycnogonum* was collected in the mesophotic zone (water depth of 58 m) by trimix SCUBA diving. Although typical features of the genus *Pycnogonum* were observed in this specimen, none of the species within the genus were morphologically

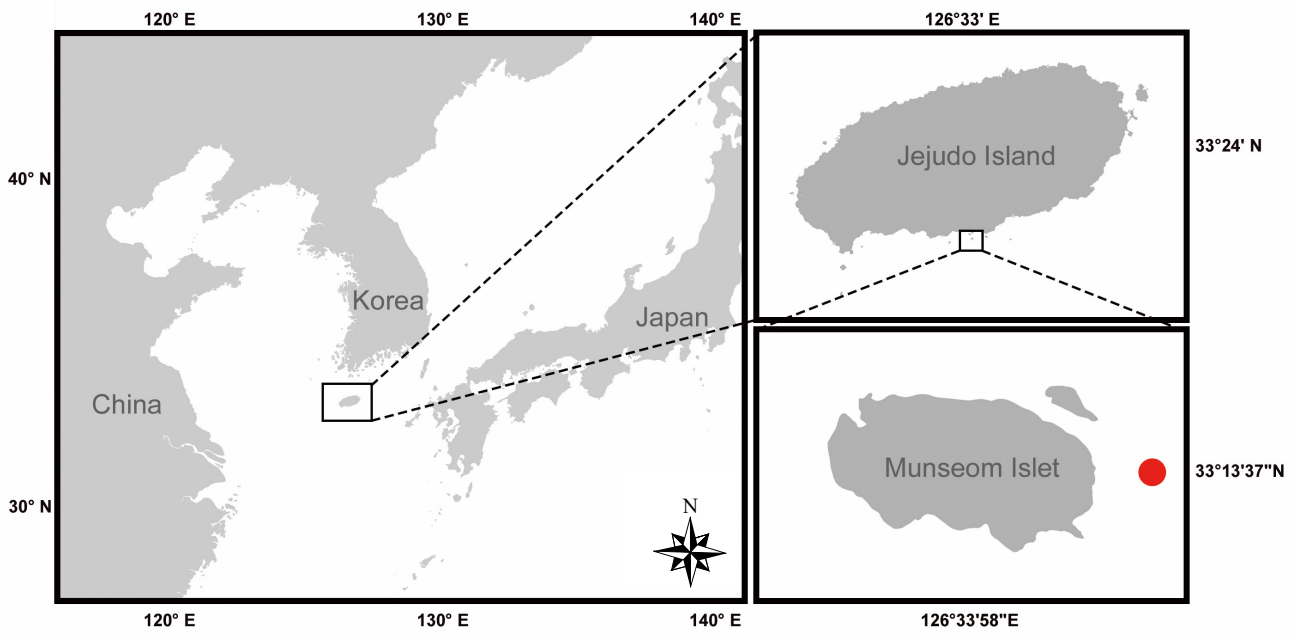

Fig. 1. Type locality of *Pycnogonum* (*Nulloviger*) *bifurcatum* sp. nov. in Korea. Red circle indicating the collection site near Munseom Islet.

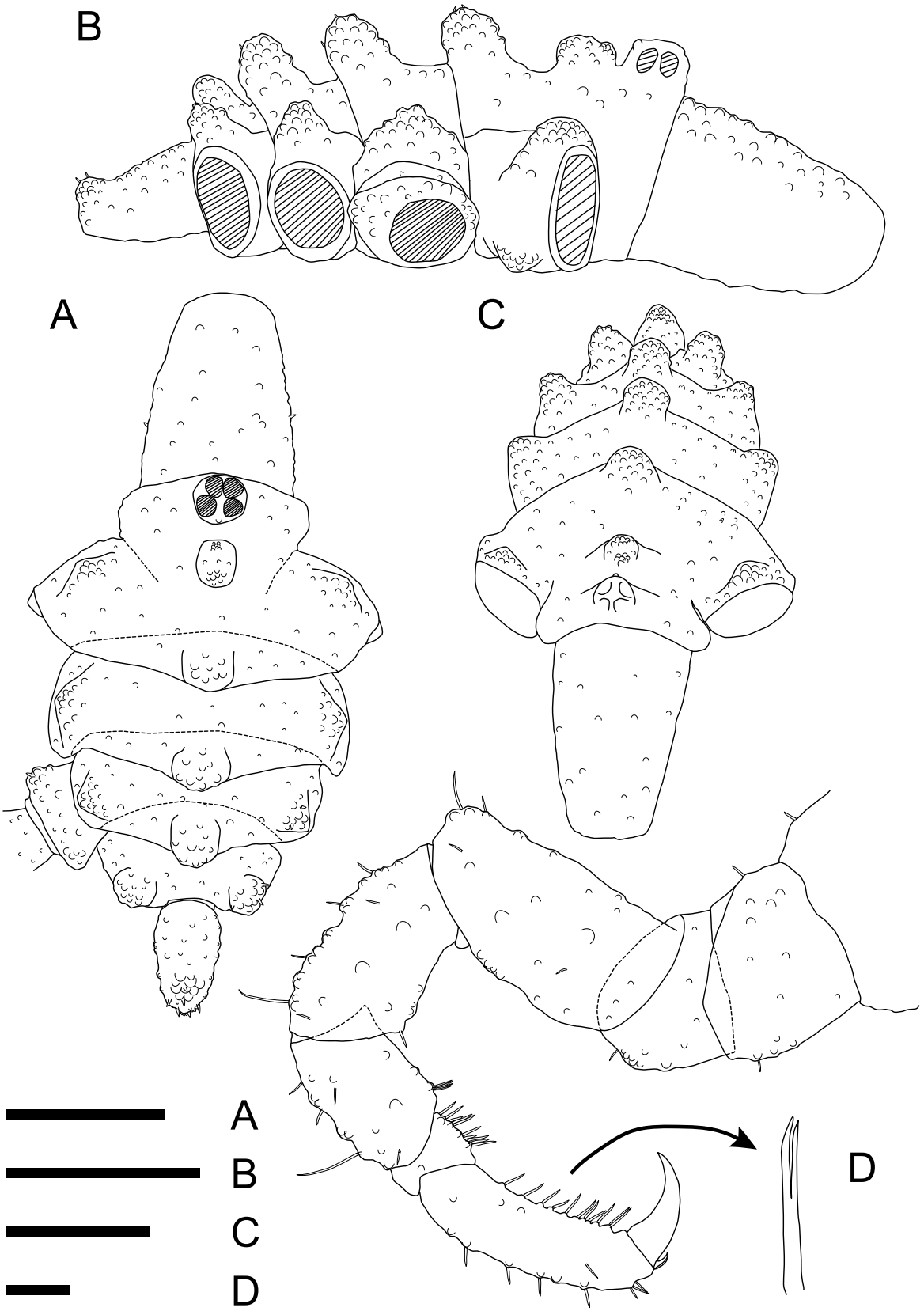

Fig. 2. Periphytic organisms on the hard substrata in the mesophotic zone of Munseom Islet. Images were recorded by GoPro between 50–60 m water depth.

consistent with the collected specimen. Additionally, the collected species had no ovigers, a characteristic of the subgenus *Nulloviger*. Here, we discuss the morphological descriptions of the new species in detail and provide molecular data on mitochondrial cox*1* and nuclear 18S rDNA genes for species identification and further studies.

MATERIALS AND METHODS

Specimen collection

A specimen was collected by trimix SCUBA diving at a depth of 58 m in the waters of the eastern Munseom Islet, Jejudo Island, Republic of Korea (Fig. 1). The specimen was hand-sorted from scraped debris of sessile organisms, including bryozoans and cnidarians, and fixed in 70% ethanol. The holotype was deposited at the National Institute of Biological Resources (NIBR). Comparative specimens of *Pycnogonum* (*Nulloviger*) *carinatum* Staples, 2002 were sourced from the Museums Victoria, Australia (NMV).

Morphological analysis

The trunk and legs of the specimen were examined under a stereomicroscope (Nikon SMZ1500, Japan) and a light microscope (Olympus BX51, Japan), and stained with methylene blue where necessary. Images were taken using a digital camera (Nikon D850, Japan) and stacked to improve the depth of the field using Helicon Focus software (Helicon Focus, Ukraine). Digital drawings followed Coleman's method (Coleman 2009). Measurements were performed as described by Fry and Hedgpeth (1969) and Staples (2002) using an ocular micrometer. Trunk length was measured from the anterior margin of the cephalic segment to the posterior margin of the lateral processes 4 in the dorsal view. Trunk width was measured between the midpoint of the distal sides of lateral processes 2 in the dorsal view. The proboscis was measured excluding the articular membrane in the lateral view. The abdomen was measured in the dorsal view. Legs were measured in the lateral view; segments were measured between the middle points of both ends; curved segments were measured using the chord length of the central arc.

Molecular analysis

Genomic DNA was extracted from leg 1 or 2 using a QIAamp DNA Micro Kit (QIAGEN, Germany) according to the manufacturer's instructions. The molecular data of mitochondrial *cox1* (mtCOI) and nuclear 18S rDNA genes were amplified by the polymerase chain reaction (PCR) using the universal primer set for mtCOI (Folmer et al. 1994) and three primer sets for the 18S rDNA gene (Arango and Wheeler 2007). The PCR mixture was a total of 50 µl: 37.25 µl of distilled water, 5 µl of 10 X Taq buffer including $MgCl_2$, 4 µl of dNTP mixture, 1 µl of each primer, 0.25 µl of Ex Taq polymerase (TaKaRa, Japan), and 1.5 µl of template DNA. PCR was conducted referring to Arango and Wheeler (2007) for the mtCOI gene as follows: initial denaturation at 94°C for 3 min, 40 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min 15 sec, and extension at 72°C for 1 min, ensuing final extension at 72°C for 5 min. PCR for the 18S rDNA gene was conducted following Sabroux et al. (2017). The amplified regions were sequenced with a 3730xl DNA Analyzer (Applied Biosystems, USA) and analyzed with Geneious Prime v.2022.2.2 (Biomatters, New Zealand).

RESULTS

TAXONOMY

Class Pycnogonida Latreille, 1810
Order Pantopoda Gerstaecker, 1863
Family Pycnogonidae Wilson 1878
Genus *Pycnogonum* Brünnich, 1764

***Pycnogonum* (*Nulloviger*) *bifurcatum* sp. nov.**
(Figs. 3, 4)
urn:lsid:zoobank.org:act:E60798B4-DDDA-4698-8A6F-62B55331CFF2

Material examined: Holotype, 1 ♂, NIBRIV0000837749 (fcn DM180109), East of Munseom Islet, Jeju-do, Republic of Korea (33°13'37"N 126°34'9.2"E), collected by trimix SCUBA diving at depth of 58 m, coll. D. Lee, 18 Jan. 2018.

Comparative material: *Pycnogonum* (*Nulloviger*) *carinatum*: Holotype, 1 ♂, NMV J48800, WV11, Beware Reef, near Cape Conran, Victoria, Australia (37°49'21.0"S 148°47'24.0"E), collected by SCUBA diving at depth of 5–6 m, coll. Dr. Tim D O'Hara, 15 Apr. 1998; 2 ♀♀, NMV J48806, WV5, Cheviot Beach, Point Nepean, Victoria, Australia (38°18'00.0"S 144°40'12.0"E), collected by SCUBA diving at depth of 3.5–5 m, coll. Dr. Tim D O'Hara, 31 Mar. 1998.

Description: Trunk tapering posteriorly, completely segmented, covered in intermittent spinules and tiny granules surrounded by spinules. Transverse ridge raised on posterior margin of segment 1–3, armed with dorsomedian tubercle; dorsomedian tubercle

granular, about 1/3 times of trunk height. Cephalic segment protruding anteriorly, having rectangular shape at anterior part (Figs. 3A, C, 4A).

Lateral processes very short, touching each other but separated distally between 3rd and 4th, with raised dorsodistal granular ridge and median tubercle; median tubercle larger and more distinct from lateral process 1 to 4. Lateral process 1 about 0.6 times as long as basal width, having posteroventral distal process. Lateral process 4 armed with distinct dorsal tubercle as high as

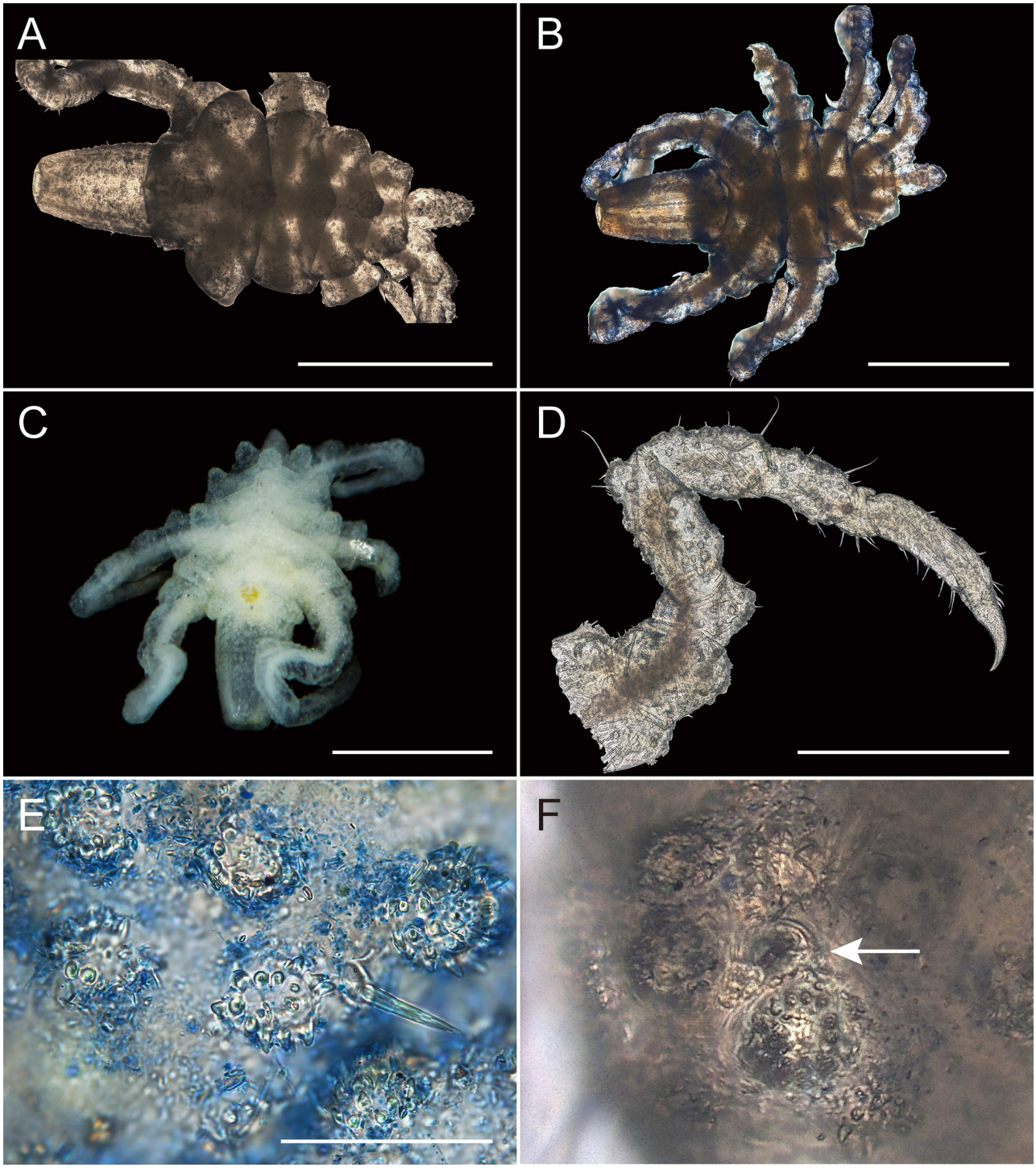

Fig. 3. *Pycnogonum* (*Nulloviger*) *bifurcatum* sp. nov., male (holotype). A, trunk, dorsal view; B, trunk, lateral view; C, trunk, anterodorsal view; D, leg 3, lateral view, arrow indicating bifurcated spine. Scale bars: A–C = 0.5 mm; D = 0.1 mm.

dorsomedian tubercle on trunk segment 1 (Fig. 3A, B).

Ocular tubercle 0.6 times of basal width, flat-topped with posterodorsal papilla; four eyes pigmented. Post-ocular tubercle half times as high as basal width, 0.7 times of ocular tubercle (Fig. 3A, B).

Proboscis truncated-cone shaped, covered in many granules; basal width about two times as wide as distal width (Figs. 3A, B, 4B). Oral glands present.

Abdomen articulated at base, reaching beyond posterior margin of coxa 2, directing horizontally, tapering and distally rounded in dorsal view, bearing nodulous dorsodistal elevation with several spines. Dorsal surface tapering toward end and ventral surface flat in lateral view (Figs. 3A, B, 4B).

Palps, chelifores, and ovigers not present.

Leg 3 stout, covered in intermittent spinules and tiny granules (Fig. 3D). Coxa 1 as long as basal width, as wide as lateral processes, with granular ridge on distal margin except ventral surface. Coxa 2 as long as basal width, half times as wide as distal width of coxa 1 in dorsal view, longest segment among coxae. Coxa 3 about 0.8 times as long as basal width, bearing granular low process on ventrodistal margin. Femur half times as long as basal width, longest among leg segments, with dorsodistal granular process bearing long and short spines. Tibia 1 half times as long as basal width, bearing long spine on dorsodistal margin and short spine on ventrodistal margin, having granular dorsal surface. Tibia 2 half times as long as basal width, as long as coxa 2, bearing long spine on dorsodistal margin and four spines on ventrodistal margin, having granular dorsal surface. Tarsus short, convex ventrally, 1/4 times as long as propodus, with several spines on ventral surface. Propodus almost straight, without heel spine, having 11 sole spines and seven dorsal spines. Bifurcated spines present on ventral surface of tibia, tarsus, and propodus. Main claw curved, 0.4 times as long as propodus. Auxiliary claws tiny, about 1/5 length of main claw.

Male gonopore present ventrally on the inner surface of coxa 2 of leg 4 (Fig. 4F). Coxal pellicula not observed.

Coloration: Specimen preserved without color notes or photo, no traces of color. Preserved specimen transparent with light yellow (Fig. 4C).

Habitat: The specimen was retrieved from scrapings off underwater wall face including bryozoans and cnidarians.

Etymology: Species epithet, bifurcatum, refers to the markedly bifurcated spines on the ventral surface of tibias, tarsi, and propodi in Latin. Korean name, DU-GAL-RAE-SONG-JANG-BA-DA-GEO-MI, also alludes to the shape of the spines.

Distribution: At present only type locality (Fig. 1).

Measurements (mm): Holotype, trunk length, 1.38; width, 0.94; proboscis, 0.61; abdomen, 0.36. Leg 3; coxa 1, 0.17; coxa 2, 0.23; coxa 3, 0.20; femur, 0.43; tibia 1, 0.28; tibia 2, 0.22; tarsus, 0.09; propodus, 0.36; main claw, 0.16; auxiliary claw, 0.03.

Molecular data: 329 bp of mtCOI gene and 1,776 bp of 18S rDNA gene (GenBank accession No.

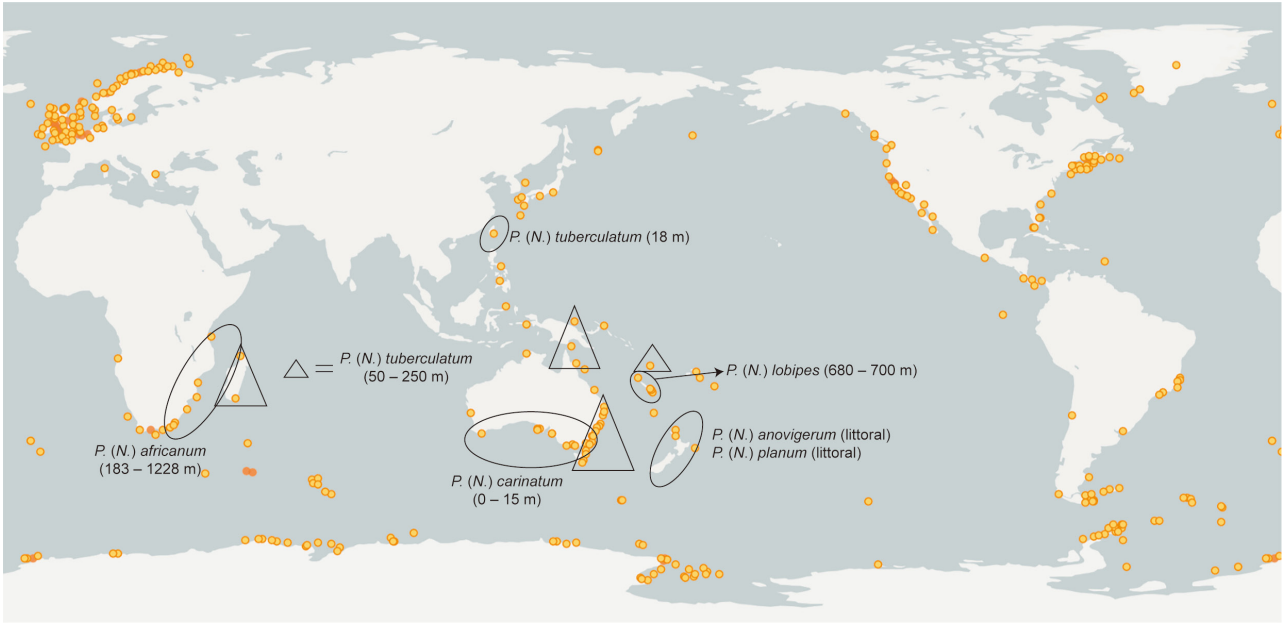

Fig. 4. *Pycnogonum* (*Nulloviger*) *bifurcatum* sp. nov., male (holotype). A, trunk, dorsal view; B, trunk, ventral view; C, trunk, anterodorsal view; D, leg 4, lateral view; E, granules; F, gonopore. Scale bars: A–D = 1 mm; E = 50 μm.

OQ127517; OQ134485).

DISCUSSION

The low granular process on the ventral surface of the coxa 3 is more protruded than that of leg 3 after comparing the leg 4 to leg3 of the present species. The dorsodistal granular process and ventroproximal swelling at the femur are more prominent (Figs. 3D, 4D). Additionally, the number of spines on the ventral surface of the tarsus and propodus differs from that of leg 3.

The integument of the present species is covered in tiny granules surrounded by spinules (Fig. 4E). This distinct decoration has been found in several *Pycnogonum* species: *Pycnogonum* (*Retroviger*) *pusillum* Dohrn, 1881, *P.* (*R.*) *pustulatum* Stock, 1994, *P.* (*Pycnogonum*) *asiaticum* Muller, 1992, *P. cranaobyrsa* Bamber, 2004, and *P. daguilarensis* Bamber, 1997. It has been described in many ways, such as granules crowned with minute spinules, rugose pimples, or papillose pores, but any evolutionary or ecological benefits are still unknown.

The present species is morphologically similar to *P.* (*P.*) *asiaticum* and *P.* (*N.*) *carinatum*, sharing granular integument, dorsomedian tubercles on the trunk, and a post-ocular tubercle. The new species has large dorsomedian tubercles on the trunk, lateral process 1−3 touching each other, ovigers, and small auxiliary claws, whereas *P.* (*P.*) *asiaticum* has small dorsomedian tubercles, separated lateral processes, no ovigers, and auxiliary claws about 2/3 length of the main claw. The ratio of the proboscis to the trunk is also noticeable (44.2% in *P.* (*N.*) *bifurcatum* sp. nov. vs. 31.4% in *P.* (*P.*) *asiaticum*). Still, this feature should be applied with caution, since the length of the proboscis may vary by up to 20% due to the articular membrane (Staples 2002). Compared with *P.* (*N.*) *carinatum* (Fig. 5; Staples 2002), the following differences are found in the new species: (1) dorsomedian tubercles on the trunk are more prominent (vs. less prominent in *P.* (*N.*) *carinatum*; see Figs. 4C, 5C). (2) Lateral processes are touching each other but distally separated between segment 3 and 4 (vs. all lateral processes separated in *P.* (*N.*) *carinatum*; see Figs. 4A, 5A). (3) Median tubercles on the lateral processes are larger and more distinct (vs. less distinct in *P.* (*N.*) *carinatum*; see Figs. 3B, 4C, 5C). (4) Lateral process 1 bears small posteroventral distal process (vs. large and obvious in *P.* (*N.*) *carinatum*; see Figs. 3B, 4B, 5D). (5) Auxiliary claws are present (vs. absent in *P.* (*N.*) *carinatum*; see Figs. 3D, 4D, 5B). (6) Deeper and more pronounced bifurcation is observed at the spines on the ventral surface of the tibia, tarsus, and propodus (vs. cleft at the tip in *P.* (*N.*) *carinatum*; see Fig. 3D and Staples 2002: Fig. 1C, 1H). Additionally, the ratio of the width of the proboscis to the trunk width is 52.2% in the present species but 36.9% in *P.* (*N.*) *carinatum.* Any ratio of the proboscis should be carefully examined, but it is a character that cannot be ignored.

Based on previous research, a total of nine species of the genus *Pycnogonum* are placed in the subgenus *Nulloviger*: type species *Pycnogonum* (*Nulloviger*) *africanum* Calman, 1938, *P.* (*N.*) *anovigerum* Clark, 1956, *P.* (*N.*) *bifurcatum* sp. nov., *P.* (*N.*) *carinatum*, *P.* (*N.*) *granulatum* Lee and Kim, 2020, *P.* (*N.*) *lobipes* Stock, 1991, *P.* (*N.*) *planum* Stock, 1954, *P.* (*N.*) *moolenbeeki* Stock, 1992, and *P.* (*N.*) *tuberculatum* Clark, 1963. The subgeneric status of three species, *P. cataphractum*, *P. portus*, and *P. spatium*, has not been mentioned. However, there are records that specimens carrying eggs or male gonopores were found without ovigers (Möbius 1902; Barnard 1946; Arnaud and Child 1988; Takahashi et al. 2007; Lee and Kim 2020; Miyazaki 2022). Therefore, these species need to be considered as belonging to the subgenus *Nulloviger*.

Contrary to these species, *P. elephas* Stock, 1966 and *P.* (*N.*) *moniliferum* Stock, 1991, have been described without male ovigers. However, we are doubtful about their sex determinations. Stock (1966) mentioned that the male gonopores of *P. elephas* were present on the dorsal surface of all legs, and the male was larger than the female. However, these "male gonopores" are considered to be coxal pellicular, and the male is usually smaller than the female, referring to the studies of *P. littorale* (Strøm 1762) and other sea spiders (Roberts 1981; Wilhelm et al. 1997; Arnaud and Bamber 1988; Bain and Govedich 2004; Barreto and Avise 2008). Stock (1991) observed that the male gonopores of *P.* (*N.*) *moniliferum* were present on the dorso-posterior surface of the coxa 2 of leg 4, which is now accepted as the position of the female gonopores. Additionally, it was noted that the female gonopores were present on the dorsal surface of the coxa 2 of all legs, in which position is the coxal pellicular. Therefore, it is necessary to re-examine the type specimens to determine their gender.

Most species in the subgenus *Nulloviger* have been found in the southern hemisphere, and only three species, *P.* (*N.*) *bifurcatum* sp. nov., *P.* (*N.*) *granulatum*, and *P.* (*N.*) *spatium*, have been found in the northern hemisphere (Fig. 6). The water depths at which the species are found also vary widely: seven species in the euphotic zone (*P.* (*N.*) *anovigerum*, *P.* (*N.*) *carinatum*, *P.* (*N.*) *cataphractum*, *P.* (*N.*) *granulatum*, *P.* (*N.*) *planum*, *P.* (*N.*) *moolenbeeki*, and *P.* (*N.*) *portus*), one species in the mesophotic zone (*P.* (*N.*) *bifurcatum* sp. nov.), three species in the aphotic zone (*P.* (*N.*)

africanum, *P.* (*N.*) *lobipes*, and *P.* (*N.*) *spatium*), and one species in the euphotic zone to aphotic zone (*P.* (*N.*) *tuberculatum*). Based on the collection records of previous research, the distribution of some species cast doubt on the accuracy of the species identification. Staples collected a specimen from the Timor Sea, Australia, and identified it as *P.* (*N.*) *moonlenbeeki*, even though it more likely represented an intermediate form between *P.* (*N.*) *moonlenbeeki* and *P.* (*N.*) *tuberuculatum*. This identification is based on four reasons, but seems incomplete. The ratio related to the proboscis should be applied carefully due to the articular membrane, and the other mentioned features are also observed in the later discovered species, *P.* (*N.*) *granulatum* and *P.* (*N.*) *spatium*, leaving no clear evidence to confirm species identification. Additionally, Staples (2002) mentions the necessity to re-examine the species through both comparison with type specimens and the collection of additional Australian specimens. In the case of geographical distribution, the collection site of Staples' specimen (Timor Sea, Australia), is far away from the type locality (Oman). There are no records of intermediates such as India, Taiwan, or Indonesia. Therefore, it is necessary to ascertain whether this species has a wide distribution or if the Australian record is misidentified. In the cases of *P.* (*N.*) *tuberculatum*, Clark (1963) and Staples (2002) mentioned that most specimens were collected in the aphotic zone and one specimen were collected in the mesophotic zone in southeast Australia. However, Stock (1994) expanded its range to the euphotic zone in Papua New Guinea. This record needs to be reconfirmed in that it is questionable whether this rare species truly lives in a wide range of water depths, and additional similar species such as *P.* (*N.*) *granulatum*, and *P.* (*N.*) *spatium*, have been discovered since 1994.

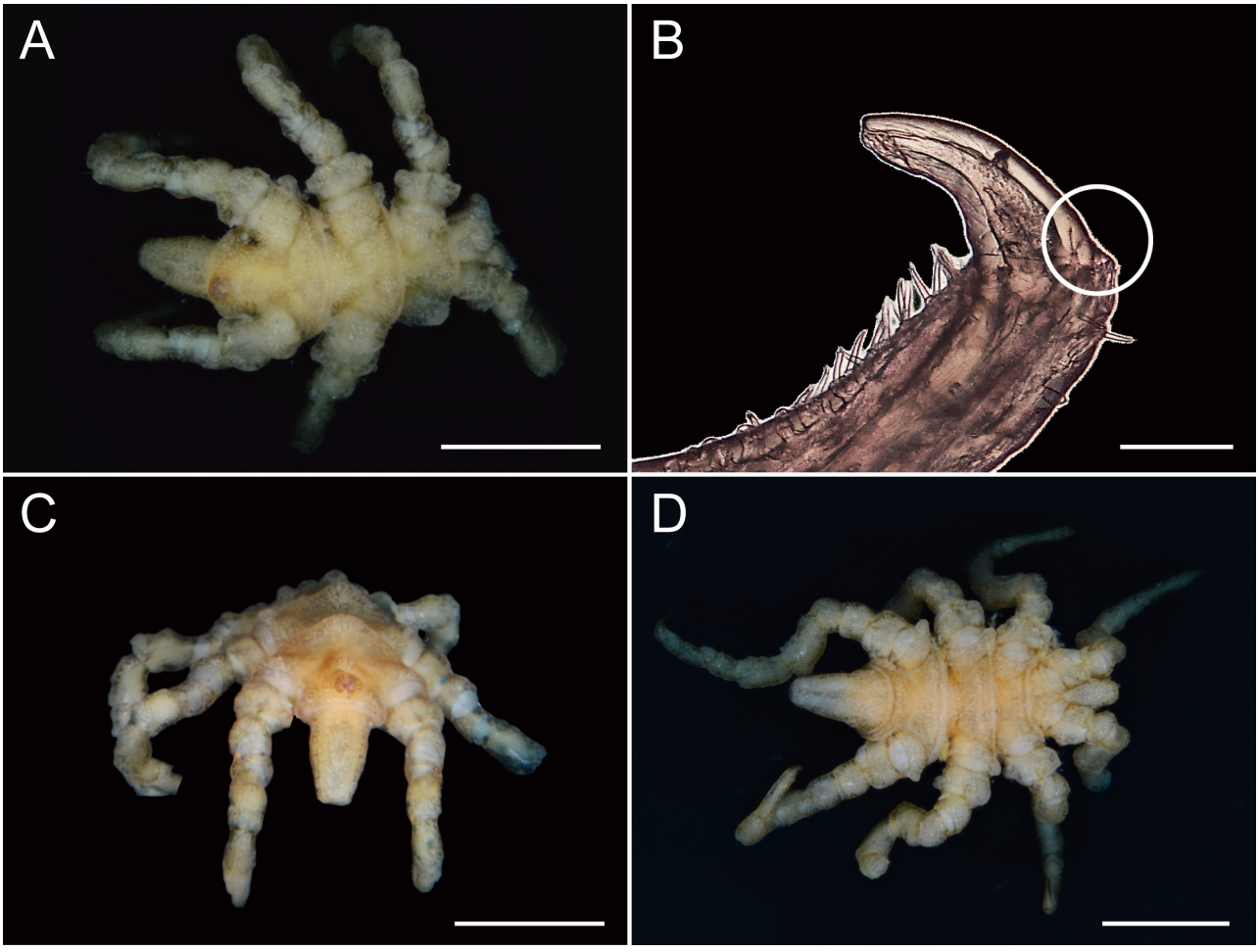

Fig. 5. *Pycnogonum* (*Nulloviger*) *carinatum*, male (holotype; NMV J48800; , A, B), female (NMV J48806; C, D). A, trunk, dorsal view; B, distal parts of leg 3, circle indicating absence of auxiliary claws; C, trunk, anterodistal view; D, trunk, ventral view. Scale bars: A, C, D = 1 mm; B = 0.1 mm.

Key to species in the subgenus *Nulloviger*

1. Post-ocular tubercle present 2
- Post-ocular tubercle absent 4
2. Proboscis conical-shaped. Dorsomedian tubercles on trunk armed with conical or digitiform processes ..... *P.* (*N.*) *portus*
- Proboscis tapering distally. Dorsomedian tubercles on trunk rounded 3
3. Dorsomedian tubercles on trunk more prominent. Lateral processes 1–3 touching each other. Auxiliary claws present ..... *P.* (*N.*) *bifurcatum* sp. nov.
- Dorsomedian tubercles on trunk less prominent. Lateral processes separated. Auxiliary claws absent ..... *P.* (*N.*) *carinatum*
4. Dorsal tubercle on proboscis present 5
- Dorsal tubercle on proboscis absent 8
5. Dorsal tubercle on proboscis small. Lateral processes slightly separated. Abdomen reaching half of coxa 3 ..... *P.* (*N.*) *tuberculatum*
- Dorsal tubercle on proboscis prominent. Lateral processes separated more than 1/4 of basal width. Abdomen reaching distal end of coxa 2 6
6. Transverse ridge less conspicuous. Proboscis tapering in lateral view. Femur armed with two lobes and three bosses on dorsal surface ..... *P.* (*N.*) *moolenbeeki*
- Transverse ridge prominent. Proboscis cylindrical in lateral view. Femur armed with two lobes and projection on dorsal surface .. 7
7. Integument granular. Trunk segment 4 without parallel part. Spines with bifurcated tips on ventral surface of tibia, tarsus, and propodus ..... *P.* (*N.*) *granulatum*
- Integument granular and pitted. Trunk segment 4 having parallel part. Spines with round tips on ventral surface of tibia, tarsus, and propodus ..... *P.* (*N.*) *spatium*
8. Integument reticulate 9
- Integument not reticulate 10
9. Lateral processes separated. Proboscis conical. Legs not conspicuously tuberculated ..... *P.* (*N.*) *africanum*
- Lateral processes touching each other. Proboscis tapering not conical. Legs conspicuously tuberculated .. *P.* (*N.*) *cataphractum*
10. Transverse ridge conspicuous. Lateral processes widely separated. Ocular tubercle with conical tip ..... *P.* (*N.*) *lobipes*
- Transverse ridge inconspicuous. Lateral processes slightly separated. Ocular tubercle with plat tip 11
11. Auxiliary claws absent ..... *P.* (*N.*) *anovigerum*
- Auxiliary claws present ..... *P.* (*N.*) *planum*

CONCLUSIONS

A specimen of *Pycnogonum* (*Nulloviger*) *bifurcatum* sp. nov. was collected by trimix SCUBA diving in the mesophotic zone of Munseom Islet, Jejudo Island. This is the first report of a species belonging to the subgenus *Nulloviger* in the Korean fauna, and it is a rare species found in the mesophotic zone. The

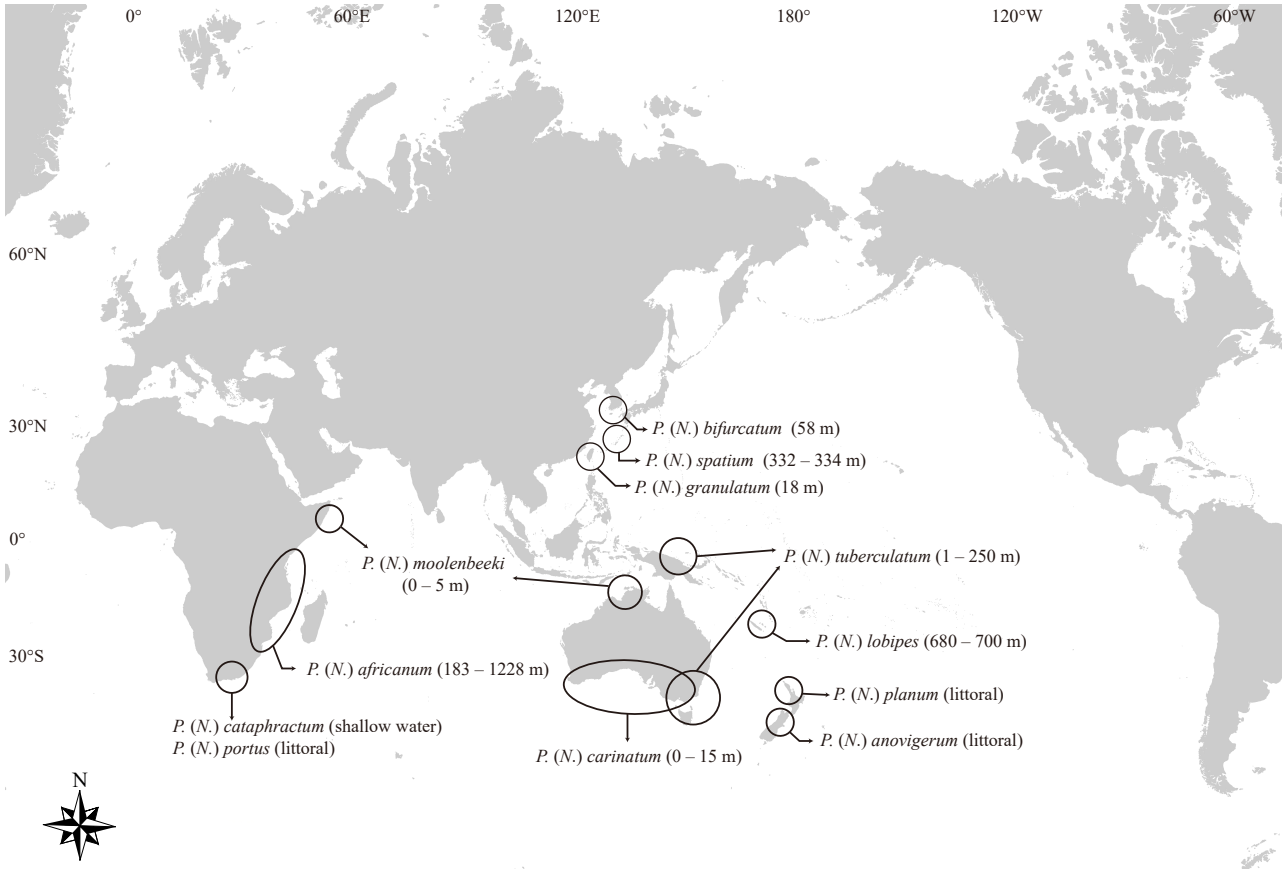

Fig. 6. Distribution of *Pycnogonum* species belonging to the subgenus *Nulloviger*. Parenthesis indicating collecting water depth.

holotype and non-type specimens of *P.* (*N.*) *carinatum*, the most similar congener, were loaned from the Museums Victoria, and morphological differences with the new species were examined. The new present species is characterized by its relatively small size, the integuments covered in the tiny granules surrounded by spinules, the post-ocular tubercle, and the auxiliary claws. Based on the absence of the ovigers in the male, this species shows a typical feature of the subgenus *Nulloviger*. Molecular data on mitochondrial cox*1* and nuclear 18S rDNA genes are provided for species identification and further studies.

List of abbreviations

NIBR, National Institute of Biological Resources, Republic of Korea.
NMV, Museums Victoria, Australia.
fcn, Field collection number.

Acknowledgments: This work and the new species name were registered with ZooBank under urn:lsid:zoobank.org:pub:45AB2ED7-67C7-48D9-A69C-D4BD1FA807D1. We are grateful to Capt. Jong-Moon Choi (Blue Research, Korea) for leading the field surveys. We thank Dr. Chris Rowley (Museums Victoria, Australia) for loaning the specimens deposited at the museum. Special thanks to Prof. Won Kim (Seoul National University, Korea) and Prof. Joong-Ki Park (Ewha Womans University, Korea) for supporting this research; Dr. Romain Sabroux (National Museum of Natural History, France) for sharing references; Mr. Kwang-Soo Kim (NIBR, Korea), Prof. Taekjun Lee (Sahmyook University, Korea), and Dr. Yucheol Lee (Korea National Park Service, Korea) for their assistance in the field survey and discussion. This research was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201801102) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2021R1A6A3A01086386).

Authors' contributions: Organized fieldwork and material collections: DL, JHP, and TP. Providing digital drawings and color photos: DL and JHP. Identification and article organization: DL, JHP, and TP.

Competing interests: The authors declare that they have no competing interests.

Availability of data and materials: The type specimen was deposited into the NIBR, Incheon, Republic of Korea.

Consent for publication: All authors agree to its publication in Zoological Studies.

Ethics approval consent to participate: Not applicable.

REFERENCES

Albano PG, Azzarone M, Amati B, Bogi C, Sabelli B, Rilov G. 2020. Low diversity or poorly explored? Mesophotic molluscs highlight undersampling in the Eastern Mediterranean. Biodivers Conserv **29(14):**4059–4072. doi:10.1007/s10531-020-02063-w.

Arango CP, Wheeler WC. 2007. Phylogeny of the sea spiders (Arthropoda, Pycnogonida) based on direct optimization of six loci and morphology. Cladistics **23:**255–293. doi:10.1111/j.1096-0031.2007.00143.x.

Arnaud F, Bamber RN. 1988. The biology of Pycnogonida. Adv Mar Biol **24:**1–96. doi:10.1016/S0065-2881(08)60073-5.

Arnaud F, Child CA. 1988. The South African Museum's Meiring Naude cruises. Part 17. Pycnogonida. Annals of the South African Museum **98(6):**121–187.

Bain BA, Govedich FR. 2004. Courtship and mating behavior in the Pycnogonida (Chelicerata: Class Pycnogonida): a summary. Invertebr Reprod Dev **46(1):**63–79. doi:10.1080/07924259.2004.9652607.

Bamber RN. 1997. Pycnogonids (Arthropoda: Pycnogonida) from the Cape d'Aguilar Marine Reserve, Hong Kong. The Marine Flora and Fauna of Hong Kong and southern China IV, pp. 143–157.

Bamber RN. 2004. Pycnogonids (Arthropoda: Pycnogonida) from Taiwan, with description of three new species. Zootaxa **458(1):**1–12. doi:10.11646/zootaxa.458.1.1.

Bamber RN, El Nagar A, Arango CP. 2022. Pycnobase: World Pycnogonida Database. *Pycnogonum* Brünnich, 1764. Available at: http://www.marinespecies.org/pycnobase. Accessed 6 Dec. 2022.

Barnard KH. 1946. V.—Diagnoses of new species and a new genus of Pycnogonida in the South African, Museum. J Nat Hist **13(97):**60–63.

Barreto FS, Avise JC. 2008. Polygynandry and sexual size dimorphism in the sea spider *Ammothea hilgendorfi* (Pycnogonida: Ammotheidae), a marine arthropod with brood-carrying males. Mol Ecol **17(18):**41644175. doi:10.1111/j.1365-294x.2008.03895.x.

Bianchelli S, Pusceddu A, Canese S, Greco S, Danovaro R. 2013. High meiofaunal and nematodes diversity around mesophotic coral oases in the Mediterranean Sea. PLoS ONE **8(6):**1–14. doi:10.1371/journal.pone.0066553.

Brünnich MT. 1764. Entomologia sistens Insectorium Tabulas Systematicas, cum Introductione et Incoibus etc. Copenhagen.

Calman WT. 1938. Pycnogonida. Scientific Reports John Murray Expedition 1933-34 **5(6):**147–166.

Cho IY, Kang DW, Kang J, Hwang H, Won JH, Paek WK, Seo SY. 2014. A study on the biodiversity of benthic invertebrates in the waters of Seogwipo, Jeju Island, Korea. Journal of Asia-Pacific Biodiversity **7(1):**e11–e18. doi:10.1016/j.japb.2014.03.003.

Clark WC. 1956. A New Species of *Pycnogonum* from Banks Peninsula, New Zealand. Records of the Canterbury Museum **7:**171–173.

Clark WC. 1963. Australian Pycnogonida. Records of the Australian Museum **26(1):**1–82.

Coleman CO. 2009. Drawing setae the digital way. Zoosyst Evol **85:**305–310. doi:10.1002/zoos.200900008.

Dohrn A. 1881. Die Pantopoden des Golfes von Neapel und der angrenzenden Meeres-Abschnitte. Monographie der Fauna und Flora des Golfes von Neapel **3:**1–252. doi:10.5962/bhl.title.16340.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome coxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech **3(5):**294–299.

Fry WG, Hedgpeth JW. 1969. The Fauna of the Ross Sea part 7 Pycnognida, 1 Colossendeidae, Pycnogonidae, Endeidae, Ammotheidae. New Zealand Oceanographic Institute Memoir **49:**1–139.

Gravina MF, Pierri C, Mercurio M, Marzano CN, Giangrande A. 2021. Polychaete Diversity Related to Different Mesophotic Bioconstructions along the Southeastern Italian Coast. Diversity **13(6):**1–13. doi:10.3390/d13060239.

Gerstaecker CEA. 1863. Arthropoden. *In*: Engelmann W. (ed) Handbuch der Zoologie, 2nd volume. Leipzig.

Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ. 2010. Community ecology of mesophotic coral reef ecosystems. Coral Reefs **29(2):**255–275. doi:10.1007/s00338-010-0593-6.

Kwon YJ, Kim HJ, Yoo SH. 2018. Assessment of the conservation value of Munseom area in Jeju Island, South Korea. International Journal of Sustainable Development & World Ecology **25(8):**739–746. doi:10.1080/13504509.2018.1457102.

Latreille PA. 1810. Considérations générales sur l'ordre naturel des animaux composant les classes des crustacés, des arachnides, et des insectes: avec un tableau méthodique de leurs genres, disposés en familles. Paris, France.

Lee D, Kim W. 2020. New species of *Pycnogonum* (Pycnogonida: Pycnogonidae) from Green Island, Taiwan, with an additional note on the holotype of *P. spatium*. Zootaxa **4750(1):**122–130. doi:10.11646/zootaxa.4750.1.6.

Liddell WD, Ohlhorst SL. 1988. Hard substrata community patterns, 1–120 M, North Jamaica. Palaios **3(4):**413–423. doi:10.2307/3514787.

Menza C, Kendall M, Hile S. 2008. The deeper we go the less we know. Revista de Biología Tropical **56:**11–24. doi:10.15517/RBT.V56I0.5575.

Miyazaki K. 2022. A taxonomic list of sea spiders (Arthropoda, Pycnogonida) from Japanese waters. Zool Sci **39(1):**1–25. doi:10.2108/zs210077.

Möbius KA. 1902. Die Pantopoden der deutschen Tiefsee-Expedition 1898–1899 (Vol. 3). G. Fischer, Jena, Germany.

Muller HG. 1992. Pycnogonida from Malaysian coral reefs, including descriptions of three new species. Bonner Zoologische Beiträge **43(1):**155–178.

Park JH, De Grave S, Park T. 2020a. On the genus *Mesopontonia* Bruce, 1967 (Crustacea: Decapoda: Palaemonidae) in Korea, with the description of a new species. PeerJ **8:**1–29. doi:10.7717/peerj.10190.

Park JH, Lee D, Lee SH, De Grave S. 2020b. First Record of the Sponge-Dwelling Palaemonid Shrimp, *Anchistioides compressus* (Crustacea: Decapoda: Palaemonidae) in Korea. Animal Systematics Evolution & Diversity **36(4):**319–329. doi:10.5635/ASED.2020.36.4.045.

Park JH, Lee D, Park T. 2021. First record of the symbiotic palaemonid shrimp *Hamodactylus boschmai* Holthuis, 1952 (Decapoda, Caridea) from Korea. Crustaceana **94(1):**115–129. doi:10.1163/15685403-bja10101.

Reimer JD, Kise H, Santos ME, Lindsay DJ, Pyle RL, Copus JM, Bowen BW, Nonaka M, Higashiji T, Benayahu Y. 2019. Exploring the biodiversity of understudied benthic taxa at mesophotic and deeper depths: examples from the order Zoantharia (Anthozoa: Hexacorallia). Front Mar Sci **6(305):**1–12. doi:10.3389/fmars.2019.00305.

Roberts D. 1981. Pycnogonids from Strangford Lough, Northern Ireland. The Irish Naturalists' Journal **20(5):**189–192.

Sabroux R, Corbari L, Krapp F, Bonillo C, Le Prieur S, Hassanin A. 2017. Biodiversity and phylogeny of Ammotheidae (Arthropoda: Pycnogonida). Eur J Taxo **286:**1–33. doi:10.5852/EJT.2017.286.

Staples DA. 2019. Pycnogonids (Arthropoda, Pycnogonida) from the Southwest Indian Ridge. Zootaxa **4567(3):**401–449. doi:10.11646/zootaxa.4567.3.1.

Staples DA. 2002. *Pycnogonum* (Pycnogonida: Pycnogonidae) from Australia with descriptions of two new species. Memoirs of the Museum of Victoria **59:**541–553. doi:10.24199/j.mmv.2002.59.12.

Stock JH. 1954. Pycnogonida from the Indo-West-Pacific, Australian and New Zealand waters. Papers from Dr Th. Mortensen's Pacific Expedition, 1914–1916. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjφbenhavn **116:**1–168.

Stock JH. 1966. Campagne de la Calypso au large des côtes atlantiques, de l'Amerique du sud (1961–62). 4. Pycnogonida. Résultats Scientifiques de la Campagne de la Calypso **7:**385–406.

Stock JH. 1968. Pycnogonida collected by the Galathea and Anton Bruun in the Indian and Pacific Oceans. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjφbenhavn **131:**7–65.

Stock JH. 1991. Deep-water Pycnogonida from the surroundings of New Caledonia. Résultats des Campagnes MUSORSTOM **8:**125–212.

Stock JH. 1992. Littoral pycnogonida from Oman. Bijdragen tot de Dierkunde **62(2):**81–98.

Stock JH. 1994. Indo-west pacific Pycnogonida collected by some major oceanographic expeditions. Beaufortia **44(3):**17–77.

Strøm H. 1762. Physisk og oeconomisk Beskrivelse over Fogderiet Sondmor, beligende i Bergens Stift i Norge. Forste part, Soroe, pp. 1–570.

Takahashi Y, Dick MH, Mawatari SF. 2007. Sea spiders (Arthropoda: Pycnogonida) from waters adjacent to the Nansei Islands of Japan. Journal of Natural History **41(1–4):**61–79. doi:10.1080/00222930601121783.

Wilhelm E, Bückmann D, Tomaschko KH. 1997. Life cycle and population dynamics of *Pycnogonum litorale* (Pycnogonida) in a natural habitat. Mar Biol **129(4):**601–606. doi:10.1007/S002270050202.

Wilson EB. 1878. Synopsis of the Pycnogonida of New England. Transaction Connecticut Academy of Arts and Sciences **5:**1–26.