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Description of a new abyssal copepod associated with the echinoid *Sperosoma grimaldii* Koehler, 1897

(Crustacea & Echinodermata)

Roland F. Anton, Angela Stevenson & Enrico Schwabe

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During an exploration of the Irish deep-sea in 2011, a second species of the genus *Pionodesmotes* Bonnier, 1898 was discovered in the Whittard Canyon system at the southern continental margin. It is described here based on scanning electron microscopic observations. The new species is distinguished from its sole congener, *Pionodesmotes phormosomae* Bonnier, 1898, by morphological characteristics, including a labrum that completely covers the mouth, the sharply pointed mandible, the unarmed triangular genital lobes, and by the fusion of the abdomen with the thorax in males. The new species *Pionodesmotes domhainfharraigeanus* spec. nov., is a gall-building copepod, which is only known from the North Atlantic deep-sea. Both members of *Pionodesmotes* infest echinothurids; the new species is only recorded from the sea urchin *Sperosoma grimaldii* Koehler, 1897 while the type species, *Pionodesmotes phormosomae*, infests *Hygrosoma petersii* (A. Agassiz, 1880).

Roland F. Anton (corresponding author), Bavarian State Collection of Zoology, Münchhausenstr. 21, 81247 München, Germany; e-mail: rolandanton1@googlemail.com

Angela Stevenson, Centre for the Environment, Trinity College Dublin, Dublin 2, Ireland; e-mail: stevenan@tcd.ie

Enrico Schwabe, Bavarian State Collection of Zoology, Münchhausenstr. 21, 81247 München, Germany; e-mail: Enrico.Schwabe@zsm.mwn.de

Introduction

Several groups of crustaceans contain associated and parasitic forms; of these the copepods are by far the most diverse, in terms of host utilisation and developmental plasticity (Röttger 1969). Fossil records of parasitic forms (e.g. Radwanska & Radwanski 2005, Radwanska & Poirot 2010) date back to the Middle Jurassic, and demonstrate that parasitism is an ancient life strategy. Within the copepods, the order Poecilostomatoida shows a broad range of hosts, from fish (e.g. Dojiri & Cressey 1987) to various marine invertebrates (e.g. Gotto 1979), including molluscs (e.g. Huys 2001, Anton & Schrödl 2013) and echinoderms (e.g. Boxshall & Ohtsuka 2001).

According to Boxshall & Ohtsuka (2001), there are 11 copepod families that are known to exclusively infest echinoderm hosts.

The different classes within the Echinodermata are infected with a diverse set of parasitic copepods. Almost all of the five microhabitat types defined by Marchenkov (2001) for copepods on invertebrate hosts are available in the Echinodermata, making it the second most important phylum (after the molluscs) in terms of providing a rich array of ecological niches for copepods. Among these copepods the gall building forms show a high degree of modification, including strong reduction of expressed body segmentation and loss of appendages (e.g. Hansen 1902, Stephenson 1918).

During a recent expedition to the Whittard Canyon system on the Irish southern shelf margin (Fig. 1), several echinothuriid sea urchins were collected by means of an ROV, including *Sperosoma grimaldii* Koehler, 1897 and *Hygrosoma petersii* (A. Agassiz, 1880). The latter species previously misidentified as *Phormosoma uranus* by Koehler (1898), is the original host species for the copepod *Pionodesmotes phormosomae* Bonnier, 1898 (Bonnier 1898a). This parasite builds galls in the sea urchin's test (Figs 2A,B) and is not visible externally. During dissections of the echinothuriids, we discovered an infection of *S. grimaldii* Koehler, 1897 by a gall building copepod that strongly resembled *P. phormosomae* (Figs 2C,D). Surprisingly, not a single individual of the co-occurring *H. petersii* showed evidence of infection, which led us to a more careful study of the observed copepod. A detailed study of the parasite by scanning electron microscopy revealed notable differences from *P. phormosomae*, justifying the establishment of a new species which is described here.

Material and methods

The hosts and their copepods were collected during survey CE11006 of RV Celtic Explorer. This cruise, entitled 'Survey Biodiscovery & Deep-Ocean Ecosystem', sampled the Whittard Canyon system at the edge of the northern continental margin to the Biscay abyssal plain (see Fig. 1), using the Irish deep-water ROV Holland I. The Quasar work class ROV Holland I is rated to 3000 m. It is equipped with several video camera systems including a Kongsberg OE14-502a high definition colour zoom, a Kongsberg OE14-208 digital stills camera and has two robotic arms and a slurp sampler. Slurp samples are stored in an enclosed system during the undersea work, while samples collected with the robotic arms are placed in extendable storage boxes. Once samples arrived on deck, they were handpicked from the ROV boxes. Prior to dissection, all taxa were carefully checked for evidence of parasitic infestation. Externally, there was some evidence of malnutrition and cell death obvious from the echinoids' discoloured test, but other parasite-free specimens were observed with the same discoloration. There were no other obvious indications of parasite infestation. The copepods described below were studied and identified at the Bavarian State collection of Zoology (ZSM) (by ES and RA, respectively), and only discovered as an unexpected consequence of dissections conducted for gut content and tissue sampling for stable isotope analyses by one of us (AS). Dissections were performed immediately after the samples arrived on deck; the sharp point of a pair of scissors was forced into the edge of the body wall and carefully cut along its equator. The aboral and adoral hemispheres were separated. Numerous large galls were discovered on the inside of the test of one specimen of *S. grimaldii* (Figs 2B,C).

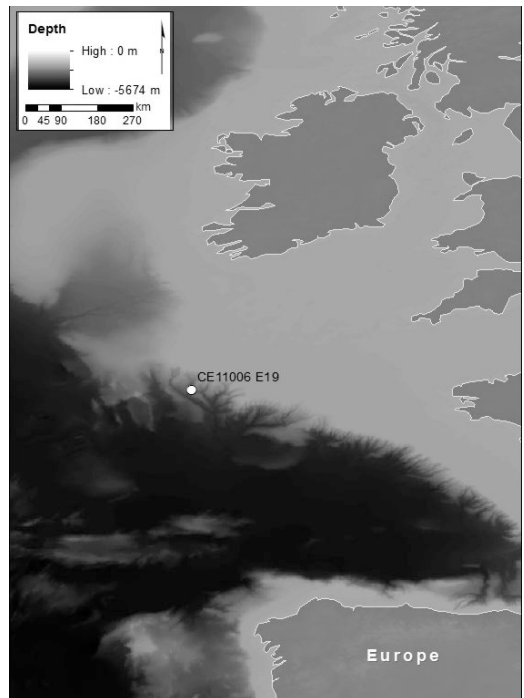


Fig. 1. The type locality of *Pionodesmotes domhainfharraigeanus* spec. nov.; Irish Sea, Whittard Canyon system, Event (= Station) 19: 48.491°N 10.692°W.

The echinoderms were examined in seawater, but once recognised, the parasites were immediately transferred to 99 % ethanol to permit subsequent genetic analysis. Accompanying environmental parameters were obtained from a 24-rosette conductivity-temperature-depth data logger from the nearest locality. Positional data for the ROV were determined using a Global Acoustic Positioning System, which incorporates inertial navigation systems and GPS using ultra-short baseline beacons.

Data from nearest CTD station (St. 18: 48.519°N 10.767°W): salinity 34.997, temperature 3.73 °C, pressure 2000 db.

Scanning electron microscopy (SEM). Most of the galls were opened during the internal examination of the host species (AS). The excavated copepods were immediately handpicked by ES, transferred to 99 % ethanol. Unfortunately, the initial treatment of the sample did not allow a careful check of the parasite's position within the galls.

Specimens for SEM were dehydrated through a graded acetone series, and then critical point dried in a BAL-TEC CPD 030 device and mounted on SEM-stubs. Stubs were sputter coated with gold for 120s (POLARON Equipment Ltd., Watford, United Kingdom) and examined on a LEO 1430VP SEM (Electron Microscopy Ltd., Cambridge, United Kingdom).

DNA extraction, amplification, and sequencing. DNA was extracted from the egg sacs of one female. We used a NucleoSpin Tissue Kit (Macherey-Nagel, Duren, Germany) and extraction procedures followed manufacturers' instructions. Universal primers LCO-1490 (forward) and HCO-2198 (reverse) (Folmer et al. 1994) were used to amplify an ~650 bp segment of the COI gene. For amplification Illustra PuRe Taq Ready-To-Go PCR beads (GE Healthcare) were used. A mix of 0.5 µl of each primer (conc. 10 pm, Metabion) plus 23 µl of molecular water was added to 1.0 µl of raw DNA. For PCR conditions we applied 94°C–300 s for the initial step, then 94°C–45 s, 45°C–50 s, 72°C–200 s for 36 cycles, with a final elongation of 72°C–600 s. For purification of the PCR-product a NucleoSpin Extract II kit (Macherey-Nagel, Duren, Germany) was used following the manufacturers' instructions. The complete sequencing process was carried out on an ABI 3730 48 capillary sequencer by the Sequencing Service Unit of the Ludwig-Maximilians-University Munich. All sequence amplicons were subjected to BLAST search to exclude contamination. The COI-sequences have been deposited in GenBank under the accession number KF652042.

Taxonomy

Subclass Copepoda Milne Edwards, 1840
 Order Poecilostomatoida Thorell, 1859
 Family Pionodesmotidae Bonnier, 1898
 Genus *Pionodesmotes* Bonnier, 1898

Type species: *Pionodesmotes phormosomae* Bonnier, 1898, by monotypy

Genus distribution: Northern Atlantic. Recent.

Pionodesmotes domhainfharraigeanus spec. nov.

Material. Holotype (SEM mounted ♀, ZSMA 20130001). – Paratypes: 1 SEM mounted ♂ ZSMA 20130002, 3 wet preserved ♀ ZSMA 20130003 (of one cephalic region removed, now SEM mounted ZSMA 20130006); 1 wet preserved ♂ ZSMA 20130004. – Additional material: 1 intact gall ZSMA 20130005.

Host. *Sperosoma grimaldii* Koehler, 1897

Microhabitat within the host. Within endocysts located in the ambulacral region of the adoral side of the host's test.

Type locality. Irish Sea, Whittard Canyon system, Event (= Station) 19: 48.491°N 10.692°W; on sediment in ca. 2000 m depth (Fig. 1).

Etymology. The species name derived from the Gaelic name for "deep-sea" – domhainfharraige.

Description

Female (holotype ZSMA 20130001). Body inflated, subspherical (Fig. 3A) measuring 3.4 × 3.2 mm, coloration yellowish-orange with white internal tissues visible through body wall (Fig. 2D); egg sacs white, about 2.5 mm in length. Body with no external segmentation expressed, anterior part (prosome) lacking distinct separation between head and thorax. Genito-abdomen very short, not segmented, distinctly separated from prosome, bearing paired genital lobes each carrying slit-shaped genital opening (Fig. 3D). Posterior margin of genito-abdomen with median anal slit and bearing paired caudal rami, each ramus armed with single seta at apex. Surface of prosome ornamented with papillae bearing sensilla.

Antennule tapering distally, 6-segmented (Figs 3B,C); setation as follows: first segment with 4 setae (distal seta originating on small swelling); second segment with 4 setae (all located on distinct swellings); third segment with 4 setae (1 anterior and 3 distal setae – 1 of latter located on swelling); fourth segment with 5 setae (1 ventral, 4 dorsal); fifth segment with 1 ventral seta; apical segment with two long distal setae.

Antenna uniramous (Figs 3E,F and 4F), 4-segmented: proximal segment largest, robust, armed with single seta; second segment about as long as wide, armed with small seta; third segment bearing 2 setae and produced into curved, inner distal claw; fourth segment offset, represented by small distal process bearing 2 long setae.

Labrum trilobate (Figs 4A,B) comprising long median lobe plus two shorter lateral lobes; median lobe covering mouth completely (Fig. 4B), but possibly moveable.

Labium small, formed by U-shaped raised region of ventral body surface closing off posterior of mouth area (Fig. 4B).

Mandibles small, sickle-shaped, covered by labrum, only distal part with hook visible (Fig. 4B). In a female paratype (ZSMA 20130006) the labrum was removed and the mandible excavated. It is comprised of two parts, the basal one pear-shaped (Figs 4C,F), and the distal part hook-shaped. The mandible is situated directly at the base of the largely extended maxillae.

Maxillule not detected.

Maxilla very broad and voluminous, comprising one segment only (Figs 4A,B,F).

Maxilliped well developed and carried on common pedestal (raised area of prosome surface), anteriorly directed, 3-segmented; first segment robust, unarmed, second segment more slender than first,

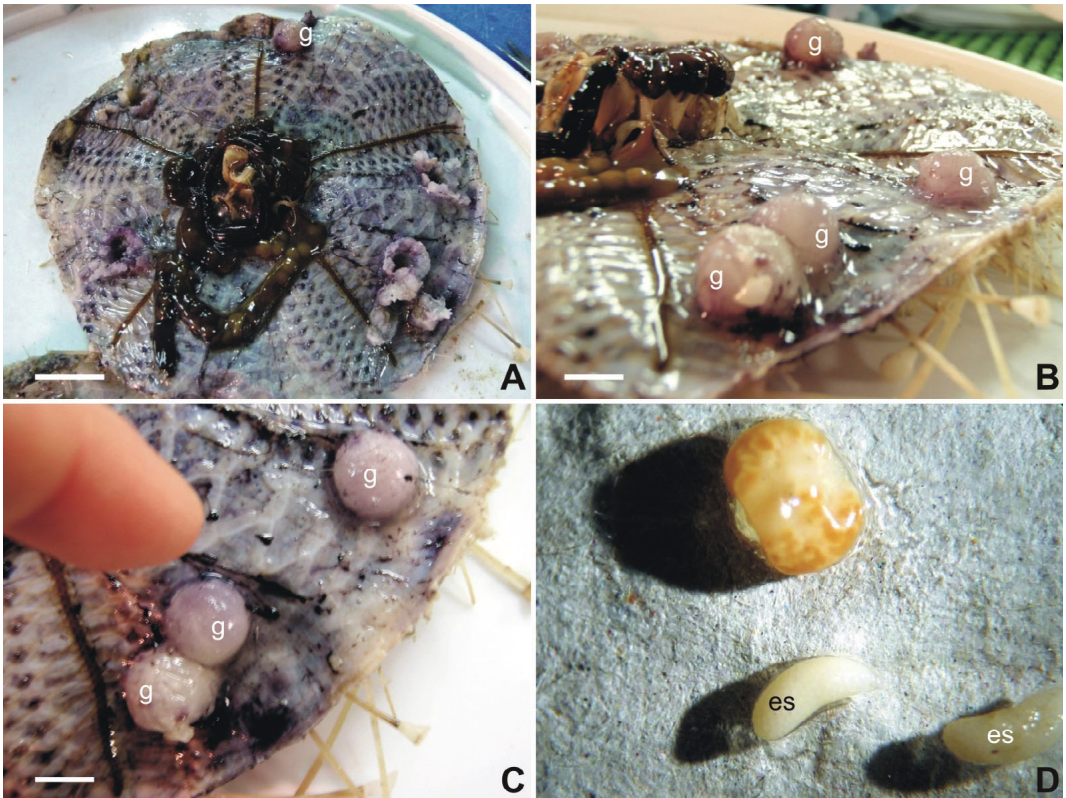


Fig. 2. A. Internal surface of the adoral side of a dissected *Sperosoma grimaldii* Koehler, 1897, showing opened and one intact endocysts of *Pionodesmotes domhainfharraigeanus* spec. nov. B. Lateral view of intact endocysts of *P. domhainfharraigeanus* spec. nov. C. Same as “B” from dorsal. D. Isolated female of *P. domhainfharraigeanus* spec. nov. with two egg sacs. g, gall; es, egg sac. Scale bars: A = 2 cm; B,C = 1 cm; D without scale.

unarmed; distal segment forming strongly recurved hook (Figs 4D,E,F).

Thoracopods absent.

Male (paratype ZSMA 20130002). Body inflated (Fig. 5A), about 1.8 mm in length; with slight traces of external segmentation visible as curving, transverse furrows on ventral surface of posterior part of prosome. Prosome showing no clear subdivision marking limits of original cephalothorax. Genito-abdomen (Fig. 5B) very short and rounded without distinct separation from prosome. Paired genital lobes triangular, defined by long, oblique, slit-like genital openings. Posterior margin of genito-abdomen with median anal slit and bearing paired caudal rami; each caudal ramus globular, armed with single seta at apex.

Antennule (Figs 5C,D,F) 5-segmented; proximal segment robust, distal segments narrower; segmental setal formula: 3, 8 (all on swellings), 5, 1, 1 (apical armature probably incomplete).

Antenna (Figs 5E,F, 6A) 4-segmented; proximal segment unarmed, second segment with 1 distal seta; third segment with one lateral seta and two strong distal setae; third segment drawn out into strong claw; fourth segment minute lobe bearing 2 setae.

Labrum with rounded lateral lobes (Figs 5E, 6B), median lobe covering mouth similar to female.

Labium not detected.

Mandible small, covered by labrum, only distal sickle-shaped part visible (Figs 5F, 6C).

Maxillule not observed.

Maxilla broad lobe, not clearly articulated at base with ventral body surface; with flattened apical part separated from lobate base by apparent articulation and curved swollen ridge (Figs 5F, 6B).

Maxilliped 3-segmented; first segment robust, unarmed, second segment more slender than first, unarmed; distal segment forming strongly recurved hook (Figs 5F, 6D).

Thoracopods absent.

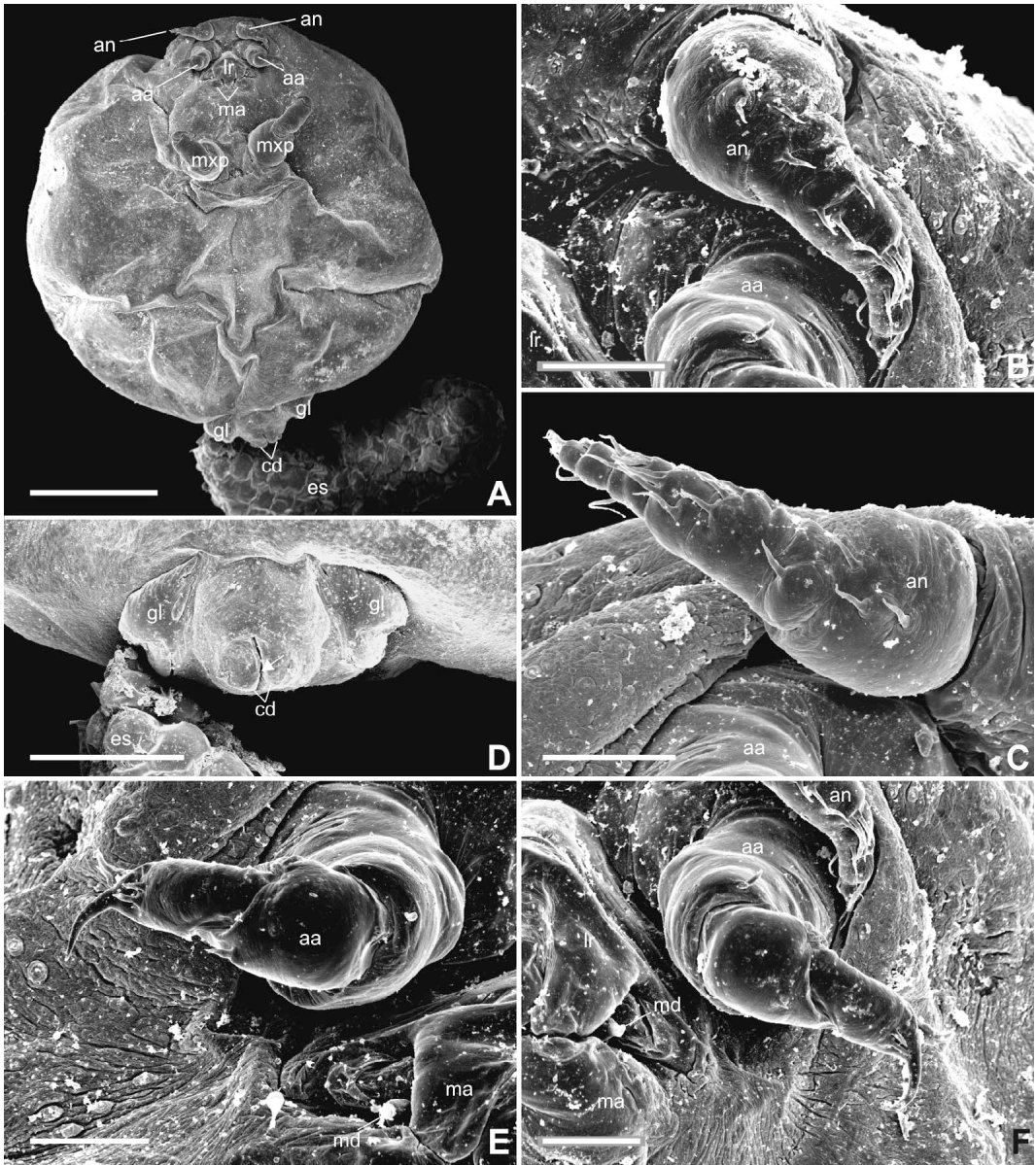


Fig. 3. SEM images of female *Pionodesmotus domhainfharraigeanus* spec. nov., holotype (ZSMA 20130001). **A.** Habitus, ventral; **B.** left antennule; **C.** right antennule; **D.** genito-abdomen with genital lobes and caudal rami, arrow indicating anus; **E.** right antenna; **F.** left antenna. **aa**, antenna; **an**, antennule; **cd**, caudal rami; **es**, egg sac; **gl**, genital lobes; **lr**, labrum; **ma**, maxilla; **md**, mandible; **mxp**, maxilliped. Scale bars: A = 1 mm; B, D-F = 100 μ m; C = 500 μ m.

Barcode gene COI. The extraction of DNA from the egg sacs of one female was successful using standard methods.

Remarks. Apart from the male's size and scarcely expressed segment borders there are few obvious

sexually dimorphic characters detectable. One difference is the possession of one segment less in the male's antennule compared to that of the female; another is the absence of a labium in males.

The only other species in the family Pionodesmotidae is the type species *Pionodesmotus phormo-*

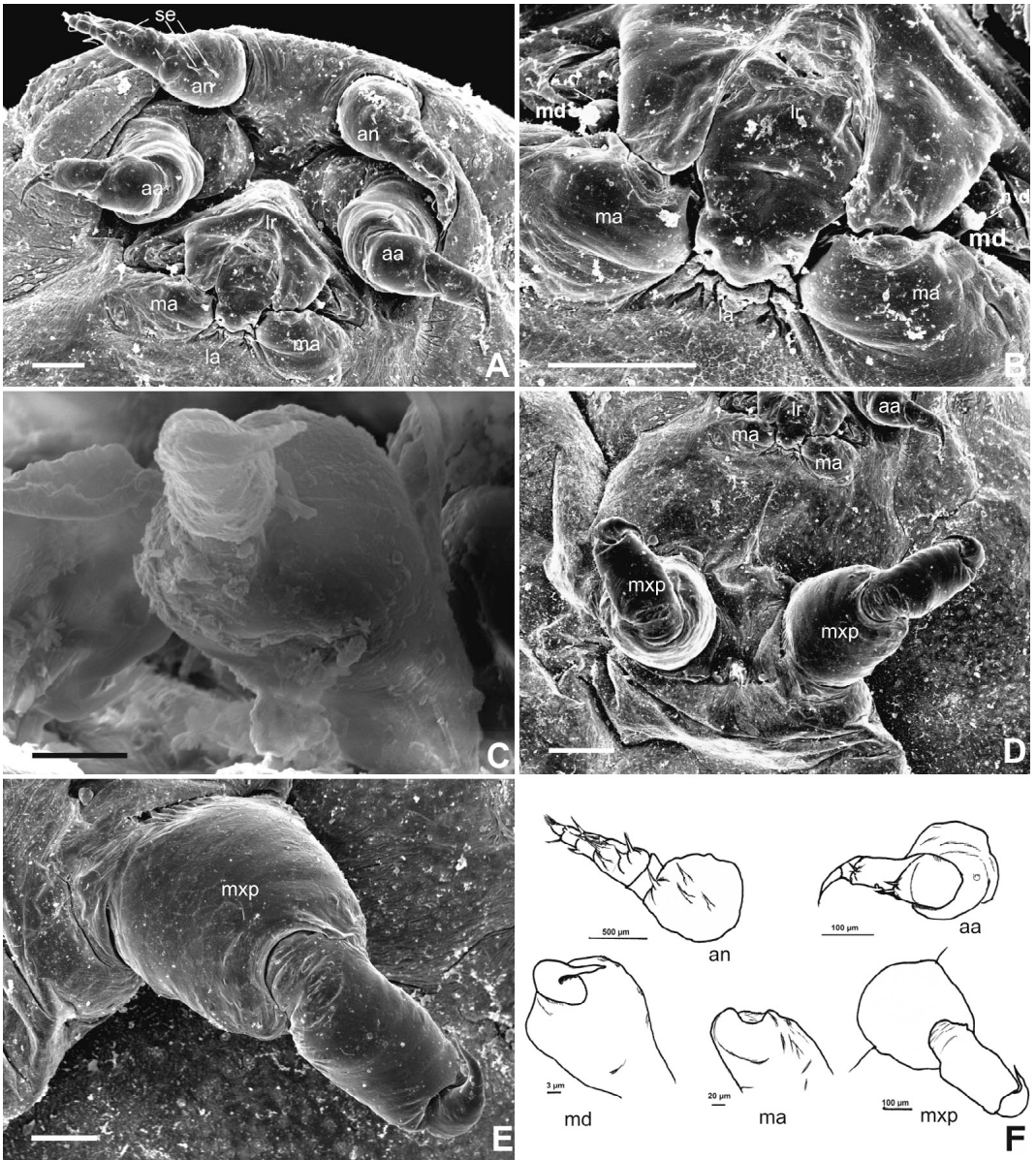


Fig. 4. A,B,D,E. SEM images of female *Pionodesmotes domhainfharraigeanus* spec. nov., holotype (ZSMA 20130001) and C. ventral view of female paratype (ZSMA 20130006). A. Cephalic region; B. mouth region; C. mandible of female paratype (ZSMA 20130006), after removing of the labrum; D. detail of prosome showing the maxillipeds; E. left maxilliped; F. line drawings of female mouthparts. aa, antenna; an, antennule; la, labium; lr, labrum; ma, maxilla; md, mandible; se, seta; mxp, maxilliped. Scale bars: A,B,D-E = 100 µm; C = 10 µm.

somae, which was found in galls in the sea urchin *Phormosoma uranus* Thomson, 1877 (Bonnier 1898a). In his extended description Bonnier (1898b) provided excellent illustrations of *P. phormosomae*. The host species however, was based on an incorrect

identification by Koehler (1898), which was subsequently re-identified by Mortensen (1935), as the echinothuriid *Hygrosoma petersii* (A. Agassiz, 1880) (see also Mortensen & Stephenson 1918).

Our attempt to obtain the syntypes of *P. phormo-*

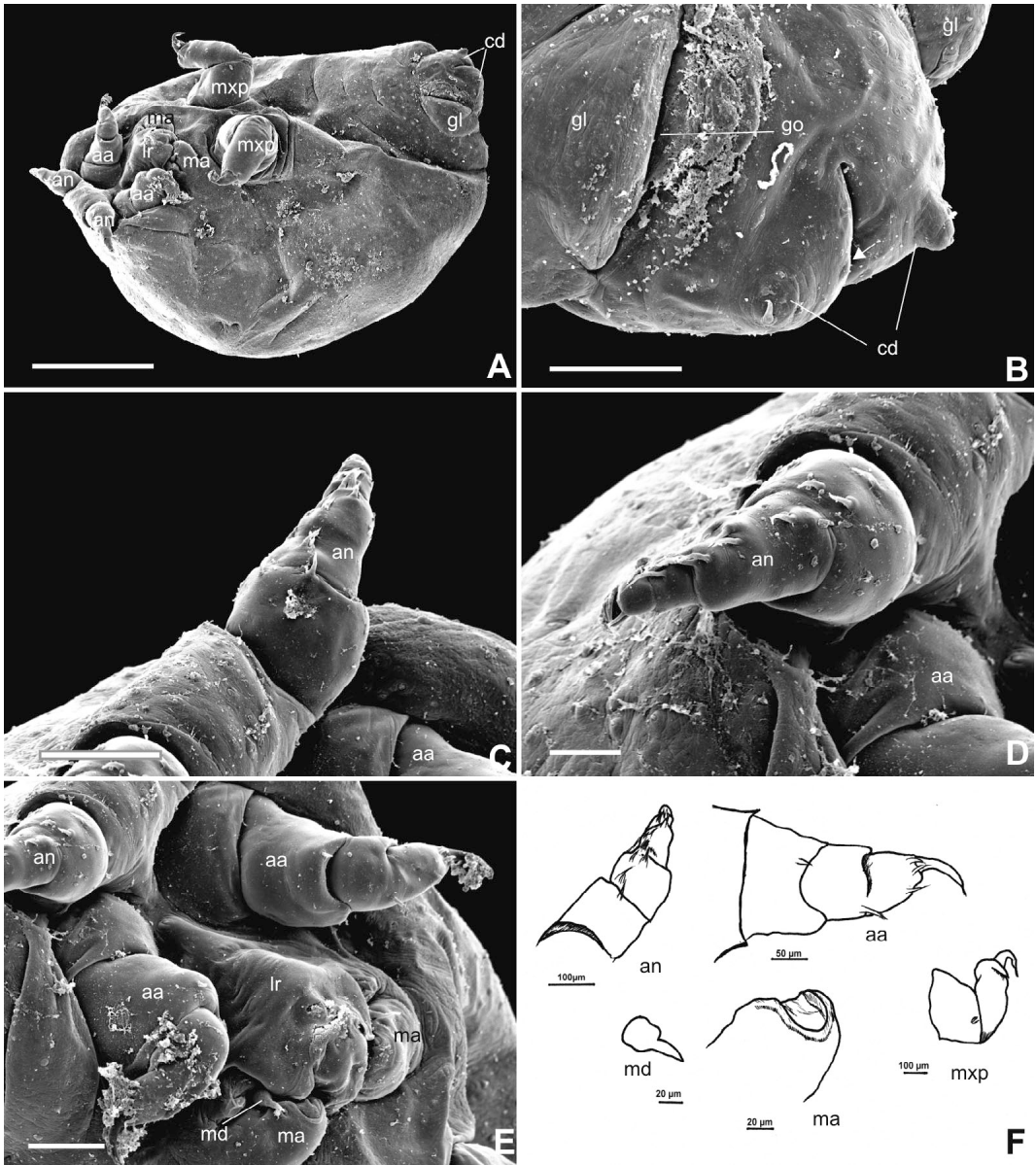


Fig. 5. SEM images of a male paratype (ZSMA 20130002) of *Pionodesmotes domhainfharraigeanus* spec. nov. **A.** Habitus, right lateral view; **B.** genito-abdomen, with anal slit arrowed; **C.** left antennule; **D.** right antennule; **E.** mouth region; **F.** line drawings of male mouthparts. aa, antenna; an, antennule; cd, caudal rami; go, genital opening; gl, genital lobes; lr, labrum; ma, maxilla; md, mandible; mxp, maxilliped. Scale bars: A = 500 µm; B,C,E = 100 µm; D = 50 µm.

somae for a comparative study failed as the material has been on loan for several years and has not yet been returned (pers. comm. Michèle Bruni, Oceanographic Museum, Monaco, e-mail: 07.05.2012). Hence our comparisons are based solely on the published

account of Bonnier (1898a,b) and the summary of Brian (1912).

The new species is sufficiently similar to *P. phormosomae* in tagmosis, and in the structure of the antennule, antenna and maxilliped to justify placing

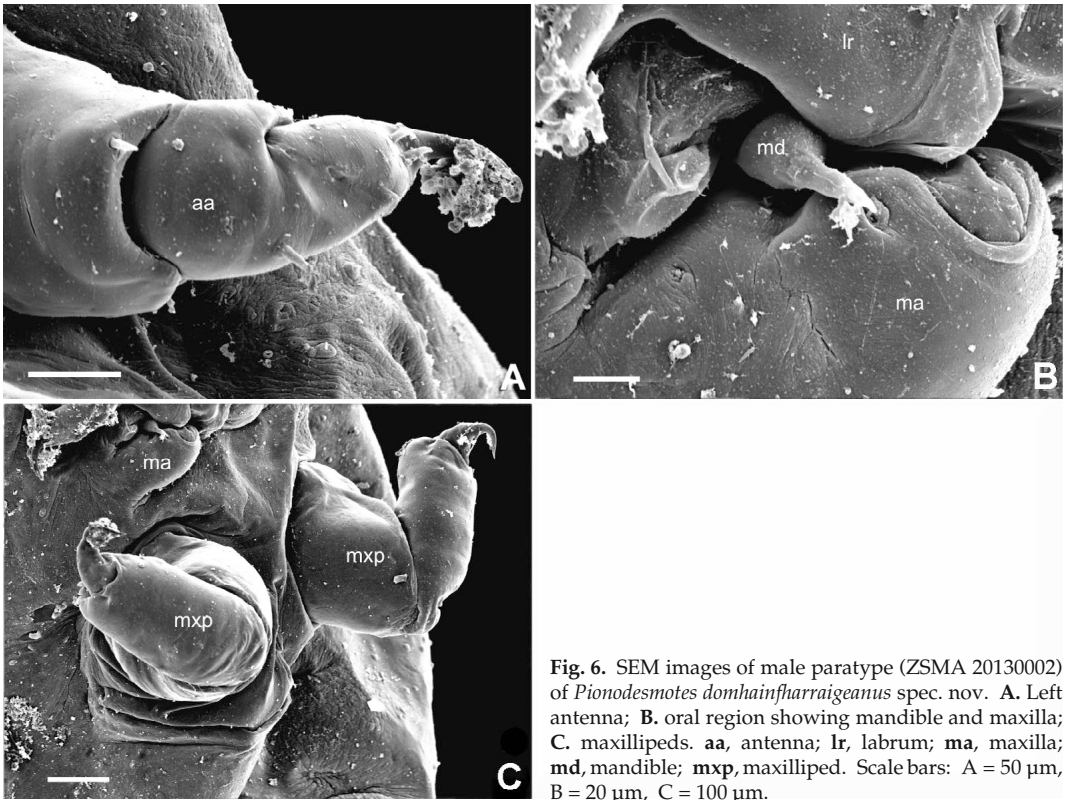


Fig. 6. SEM images of male paratype (ZSMA 20130002) of *Pionodesmotes domhainfharraigeanus* spec. nov. A. Left antenna; B. oral region showing mandible and maxilla; C. maxillipeds. aa, antenna; lr, labrum; ma, maxilla; md, mandible; mxp, maxilliped. Scale bars: A = 50 µm, B = 20 µm, C = 100 µm.

it in the same genus but there are several significant differences.

P. phormosomae differs from *P. domhainfharraigeanus* spec. nov. in the following characteristics: a) the abdomen is short with lateral extended genital lobes bearing four setae vs. short abdomen with triangular narrow unarmed genital lobes, b) the distal part of the mandible is cylindrical with a sharp pointed spine vs. pear-shaped with a sharp-pointed hook (Figs 4C,F), c) the maxilla has setae-bearing extensions vs. obtuse, without extensions (Figs 4B,F), d) the labrum is short and entirely horseshoe-shaped, partly covering the mouth vs. voluminous with two lobes covering the whole mouth, e) abdomen with small segment separated from thorax (♂) vs. merged with thorax (♂). In addition these copepods utilise different hosts, *Hygrosoma petersii* and *Sperosoma grimaldii*. *P. phormosomae* is currently only known from three stations around the Mid Atlantic Ridge north of the Azores (from the area: 39°22' N–40°05' N and 27°27' W–31°25' W; for details see Brian 1912) and from depths of 1384–1850 m, while *P. domhainfharraigeanus* spec. nov. is only known from its type locality.

Discussion

A single specimen of the echinoid *Sperosoma grimaldii* was heavily infested by an associated copepod, which, based on the obviously close similarity to the sole known species, was identified as a member of the genus *Pionodesmotes*. The type species *Pionodesmotes phormosomae* was described as parasite in the echinoid *Hygrosoma petersii* (= *Phormosoma uranus* sensu Koehler 1898). Both echinoderms are known to share a broad distributional range (Mortensen 1907) but infection of *S. grimaldii* by *P. phormosomae* is so far unknown. Therefore, since the parasite is only known from a small bathyal area north of the Azores at the Mid-Atlantic Ridge (Brian 1912) it is likely that *P. phormosomae* is not only host specific but might also have limited dispersal abilities. This is probably also dependent on the availability of the host species. Brian (1912) reported the occurrence of 10 specimens of *P. phormosomae* from three stations where *H. petersii* (as *Phormosoma uranus*) was found. According to Koehler (1898), who identified the echinoderms, the parasitic copepods were obtained from only six specimens of *H. petersii*. A single specimen

of *S. grimaldii* (Koehler 1898, p. 16; at station 184) was found together with three specimens of *H. petersii*, but the former was not infected.

Host usage by *P. domhainfharraigeanus* spec. nov., is the opposite. We detected at least six individuals (one gall was left intact) of the new species in one specimen of *S. grimaldii* at the type locality only. A total of seven *S. grimaldii* and five *H. petersii* were collected at this station. We interpret this as a strong indication of host specificity of the new species. Host specificity was also inferred for the second taxon within the family Pionodesmotidae: the ichnospecies *Castexia douvillei* Mercier, 1936. This species (ichnotaxon) is apparently exclusively found in the fossil collyritid echinoid *Collyrites dorsalis* d'Orbigny, 1851 (Radwanska & Radwanski 2005).

The successful extraction of DNA from the egg sacs reveals new possibilities since damaging of the often rare specimens is no longer necessary. Unfortunately a more detailed molecular analysis of the new species is currently impeded due to missing comparable data.

Due to sampling handling on board, we were unable to detect the exact position of the copepod within the gall. Positioning of the gall, namely in the ambulacral region and on the adoral side of the test, indicate that larval penetration to the interior of the test may have taken place through tubefeet pores (this is in accord with the ectoparasitic lifestyle noted by Marchenkov 2001 for his microhabitat class I; see also Radwanska & Radwanski 2005). This may mean that the associate is unable to penetrate through the calcium carbonate skeleton of the echinoid test, and/or that it may require a constant supply of water. We infer from the similar gross morphology of both *Pionodesmotes* species that *P. domhainfharraigeanus* spec. nov. may be positioned within cysts in a similar manner to that illustrated by Bonnier (1898b: pl. 10, fig. 1).

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