

Rapid Communication**Report of two Mediterranean species of *Stephos* T. Scott, 1892 (Copepoda: Calanoida) in the North-East Atlantic Ocean (Brittany, France), with a note on the “hyaline sheath” in the genus *Stephos***

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OPEN ACCESS**Abstract**

Stephos cryptospinosus Zagami, Campolmi and Costanzo, 2000 and *Stephos marsalensis* Costanzo, Campolmi and Zagami, 2000 were found at Concarneau (Brittany, France). These species were originally described at the same locality on the island of Sicily (Italy) in the western Mediterranean. This report is the first record of either species from outside the Mediterranean Basin. The description of *S. cryptospinosus* is supplemented with SEM observations, and compared with the original. In addition, the role of the hyaline sheath covering the genital double-somite of the female in some species of *Stephos* is discussed.

Key words: *Stephos cryptospinosus*, *Stephos marsalensis*, non-indigenous species (NIS), SEM, reproduction

Introduction

Stephos cryptospinosus Zagami, Campolmi and Costanzo, 2000 was originally described from the coastal waters of Sicily (Figure 1) in the western Mediterranean (37°52'N; 12°28'E). Prior to the present report it was known only from the type locality (Razouls et al. 2005–2018; Zagami, pers. com.). *Stephos marsalensis* Costanzo, Campolmi and Zagami, 2000 was originally described from the same locality but was subsequently reported from the Tunis lagoon, Tunisia (36°49'N; 10°13'E) (Annabi-Trabelsi et al. 2005).

Several *Stephos* species have been described from enclosed situations, such as submarine caves or aquarium systems (cf. Bradford-Grieve 1999) and many have only been recorded from their type locality. Although very rarely recorded, *Stephos scotti* Sars G.O., 1902 was known from the open sea of the eastern English Channel (Brylinski 2009). However, this species was recently observed as the dominant species (up to 90% of zooplankton, *personal observation*) in the sea water storage tank of the Marine Station of Wimereux (50°45'47"N; 1°36'19"E). We decided, therefore, to examine the plankton in other similar water tanks in search of species of *Stephos*.

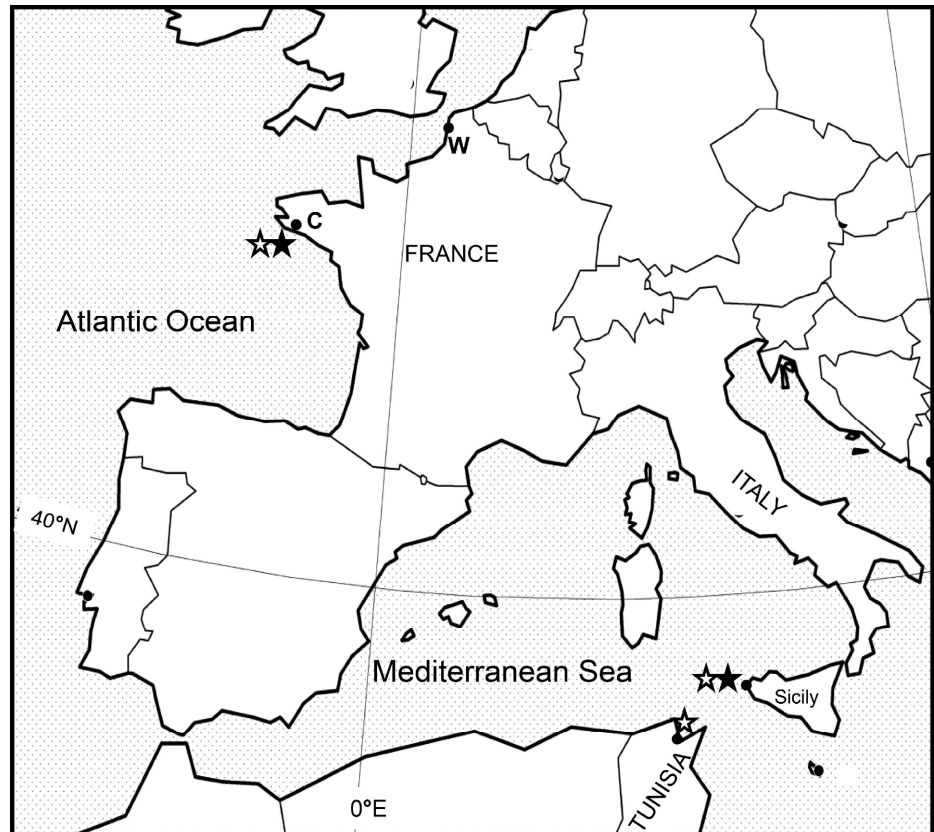


Figure 1. Global distribution of *Stephos cryptospinosus* (black star) and *S. marsalensis* (white star). C = Concarneau; W = Wimereux.

Materials and methods

Zooplankton was collected from the inner storage tank of the Marinarium at Concarneau (Brittany, France, 47°52'06"N; 03°55'00"W) through a trapdoor. Several vertical hauls were made down to 3 m depth using a conical net with a length of 40 cm and a mouth diameter of 40 cm. The mesh size was 200 µm. Quantitative data are not available. Four collections were made on November 15th, 2010; April 28th and September 9th, 2014; and July 8th, 2015. Temperature and salinity measurements were 14 °C – 36.0 PSU; 12.5° – 35.0; 18.5° – 35.5 and 19.5° – 35.0, respectively.

The plankton was fixed in 5% buffered formaldehyde. Copepods were sorted, cleared and dissected in lactic acid before optical observation.

Material was prepared for the scanning electron microscope (S.E.M.) by transferring to ultra-pure water (Millipore Synergy) and ultrasonic cleaning of the whole specimen for 2 minutes. Specimens were then dehydrated through graded alcohol series up to 100% ethanol (Merck PA) and subsequently immersed in HMDS (Hexamethyl disilazane, Molekula) for 0.5 h (twice). After the removal of excess HMDS, the specimens were placed in open vials and left overnight under a fume hood to enable evaporation. Finally, dried samples were mounted on aluminium stubs (Agar Scientific) with double-sided sticky carbon tabs (Agar Scientific) and

sputter coated under Argon flow with Au/Pd Polaron SC 7620 for 90 s. Coated specimens were examined on a SEM LEO 438 VP microscope.

Body length was measured from SEM observations, from the frontal margin of the prosome to the posterior tip of the caudal rami, excluding the caudal setae.

The general characteristics of the genus *Stephos* T. Scott, 1892 were outlined in Bradford-Grieve (1999). Species determination is mainly made using characteristics of the 5th legs.

Results

Relative abundance

The numbers per unit volume of zooplankton sampled were not quantified but all the copepods were counted. *Stephos cryptospinosus* as a percentage of total copepod counts was present as follows:

- 15 November 2010: *S. cryptosinosus* = 12.6% of copepods, after Harpacticoida (54.0 %) and *Oithona* sp. (17.2 %).
- 28 April 2014: Present (no quantitative data).
- 9 September 2014: *S. cryptosinosus* = 14 % of copepods, after *Eurytemora pacifica* (42%) and *Acartia clausi* (32%).
- 8 July 2015: *S. cryptosinosus* = 6.7 % of copepods, after Harpacticoida (50.0%) and *E. pacifica* (17.6%).

Stephos marsalensis was found on April 28th, 2014 and on July 8th, 2015, only one female was found on each date.

Morphology of S. cryptospinosus Zagami, Campolmi and Costanzo, 2000

Female

Body length: 0.90 mm (Figure 2).

Posterior prosomal margins slightly asymmetrical. Minute spinule present dorso-laterally on each side, demonstrated using SEM observations. Urosome 4-segmented, genital double-somite symmetrical with central genital aperture framed by two asymmetrical and very fine lappets, only observable with SEM. Tubular spermatophore implanted directly in opening, attached by long tubule. When carrying a spermatophore, the genital double-somite is partially covered by a hyaline sheath which tapers to a point dorsally (Figure 2A, B). This structure appeared fixed to the right side of the genital double-somite and extends forward as far as the basal articulation of the right fourth leg (Figure 2C). Fifth legs uniramous, 2-segmented (Figure 2D): proximal segment naked, distal segment armed with one short central spine on middle of anterior surface. Patch of very small spinules present proximally to the spine. Apical part of fifth legs slightly asymmetrical with row of 8–9 or 12–15 spinules on outer margin, and 6–7 or 4–5 spinules on inner margin, of right and left legs, respectively.

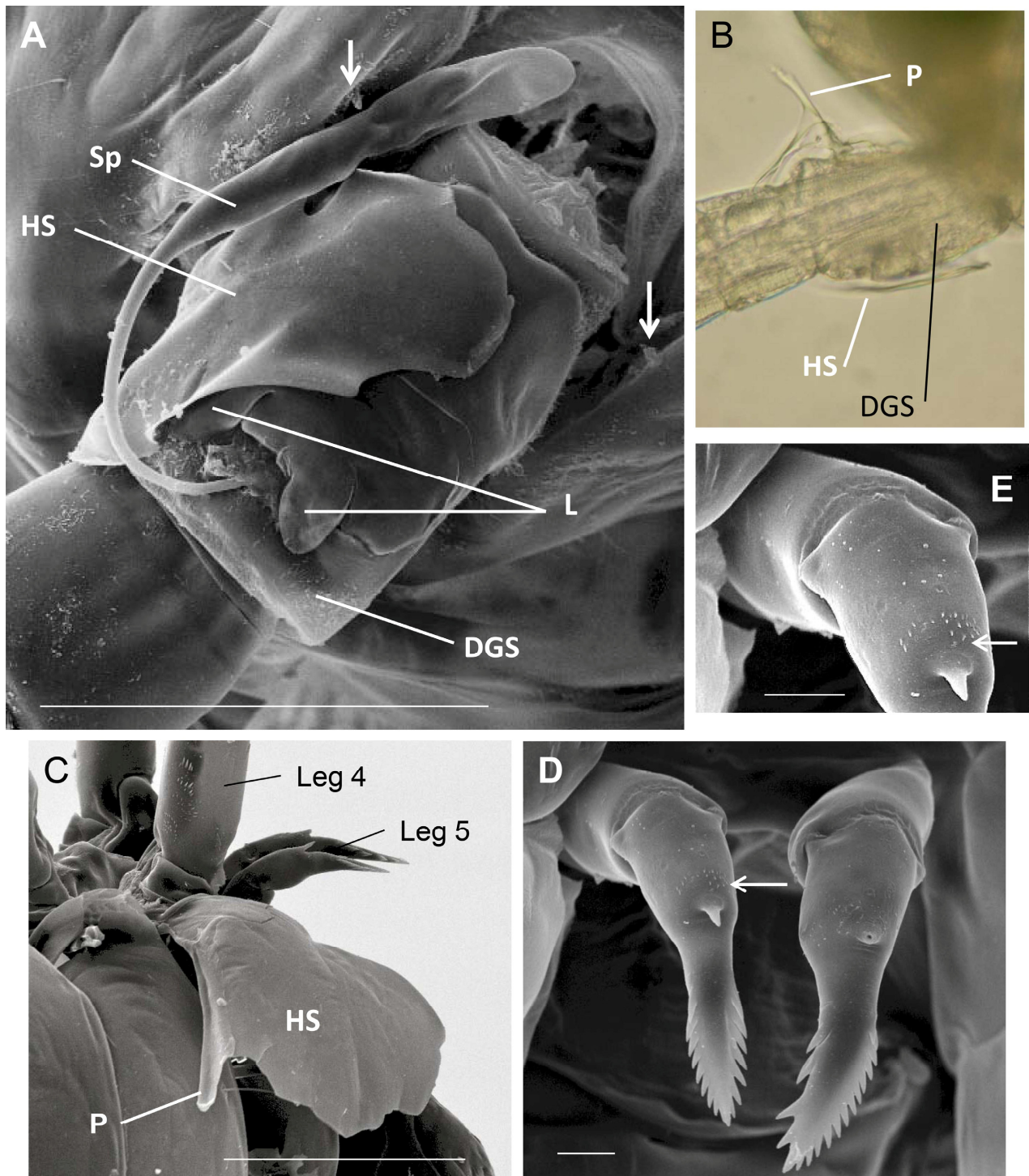


Figure 2. *Stephos cryptospinosus*, female. A: urosome, ventral view, B, C: lateral view; Scale bar: 100 μ m. D, E: leg 5; Scale bar: 10 μ m. DGS = genital double-somite, vertical arrow = “cryptospine” on last prosomite; L = lappets of genital operculum; Sp = spermatophore, HS = hyaline sheath, P = point of the hyaline sheath. Horizontal arrows: patch of micro-spinules. Photomicrographs by the authors.

Male

Body length: 0.75, 0.85 mm (Figure 3A).

Posterior prosomal margin symmetrical with minute spinule as in female. Urosome 5-segmented; second urosomite produced postero-ventrally into short process, directed towards left side (Figure 3B). Fifth legs

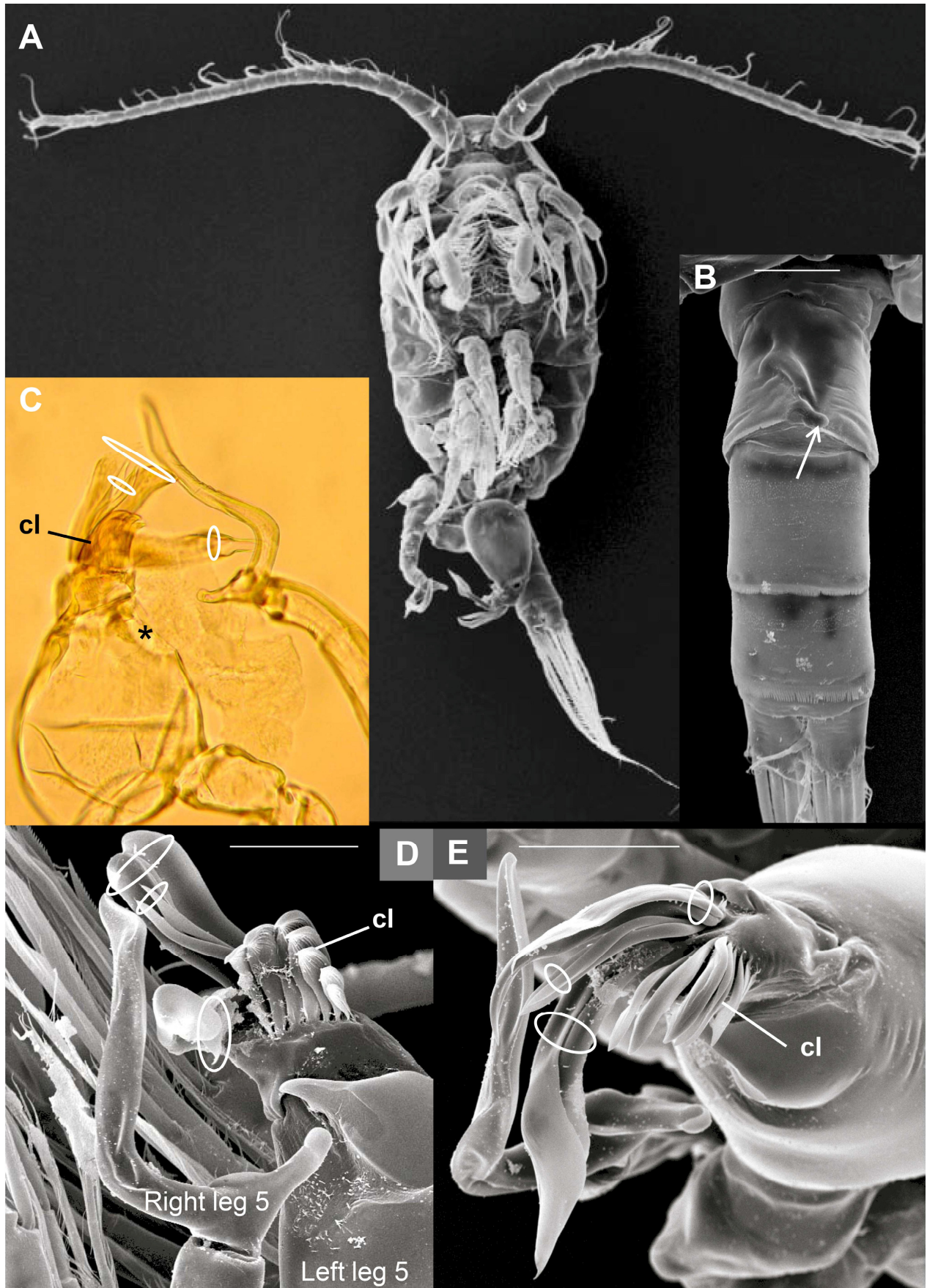


Figure 3. *Stephos cryptospinosus*, male. A: habitus, ventral view. B: urosome, ventral view. Scale bar: 40 μ m. C: last segments of fifth leg, anterior view. D: same, posterior view. E: same, apical view. cl = amber-coloured claws. Circles = pairs of lamellae. Asterisk = shrinkage of tissues. Photomicrographs by the authors.

elongate and markedly asymmetrical; both legs uniramous. Right leg slender, 4-segmented; first and second segments short, unarmed; third segment elongate, bearing acute outer process near base, slightly curving inwards; fourth segment comprising 2 processes of unequal length (Figure 3C, D). Left leg 5-segmented, first to third segments short, unarmed; fourth segment robust and swollen (Figure 3); fifth segment complex with 2 pairs of long, wide lamellar spines, 1 pair of long, narrow lamellar spines, and 1 row of 8 wide, amber-coloured claws directed posteriorly (Figure 3C, D, E).

Morphology of Stephos marsalensis Costanzo, Campolmi and Zagami, 2000

Female

Body length: 0.90 mm.

The morphological characters are in accordance with the original description (Costanzo et al. 2000) (Figure 4A). Posterolateral corners of prosome slightly asymmetrical, with left side more elongated than right side and compressed in distal part. Urosome 4-segmented. Genital double-somite with ventral genital aperture closed off by single unarmed and asymmetrical operculum. Proximal part of ventral and lateral surfaces on right side with incomplete transverse row of spinules. Left side and dorsal surface ornamented with patch of minute spinules.

Fifth legs uniramous, 2-segmented, twisted posteriorly and asymmetrical with right longer than left (Figure 4B, C). Terminal part of segment 2 fringed by marginal spinules and ornamented with patch of spinules in middle of anterior surface, thicker on right leg. One very small central spine present distally to patch of spinules in both legs (Figure 4B, C).

Discussion

Morphology

The first species was named *S. cryptospinosus* because it had “a small spinous process on the posterolateral margin of the last prosomite” (Zagami et al. 2000). We were unable to find this process using light microscopy because of its small size (4 µm), but it was visible using SEM (Figure 2A). The original description did not give precise dimensions for this spinous process but Zagami (*pers. com.*) confirmed that “the small spinous process is hardly visible”.

Some morphological differences were noted with regard to the original description of *S. cryptospinosus* and some supplementary observations are provided with SEM.

The genital aperture of the female was described as “closed off by single unarmed operculum” (Zagami et al. 2000: fig. 5) but in our material the operculum comprises two asymmetrical lappets. These lappets were not visible using light microscopy because they are very fine and transparent.

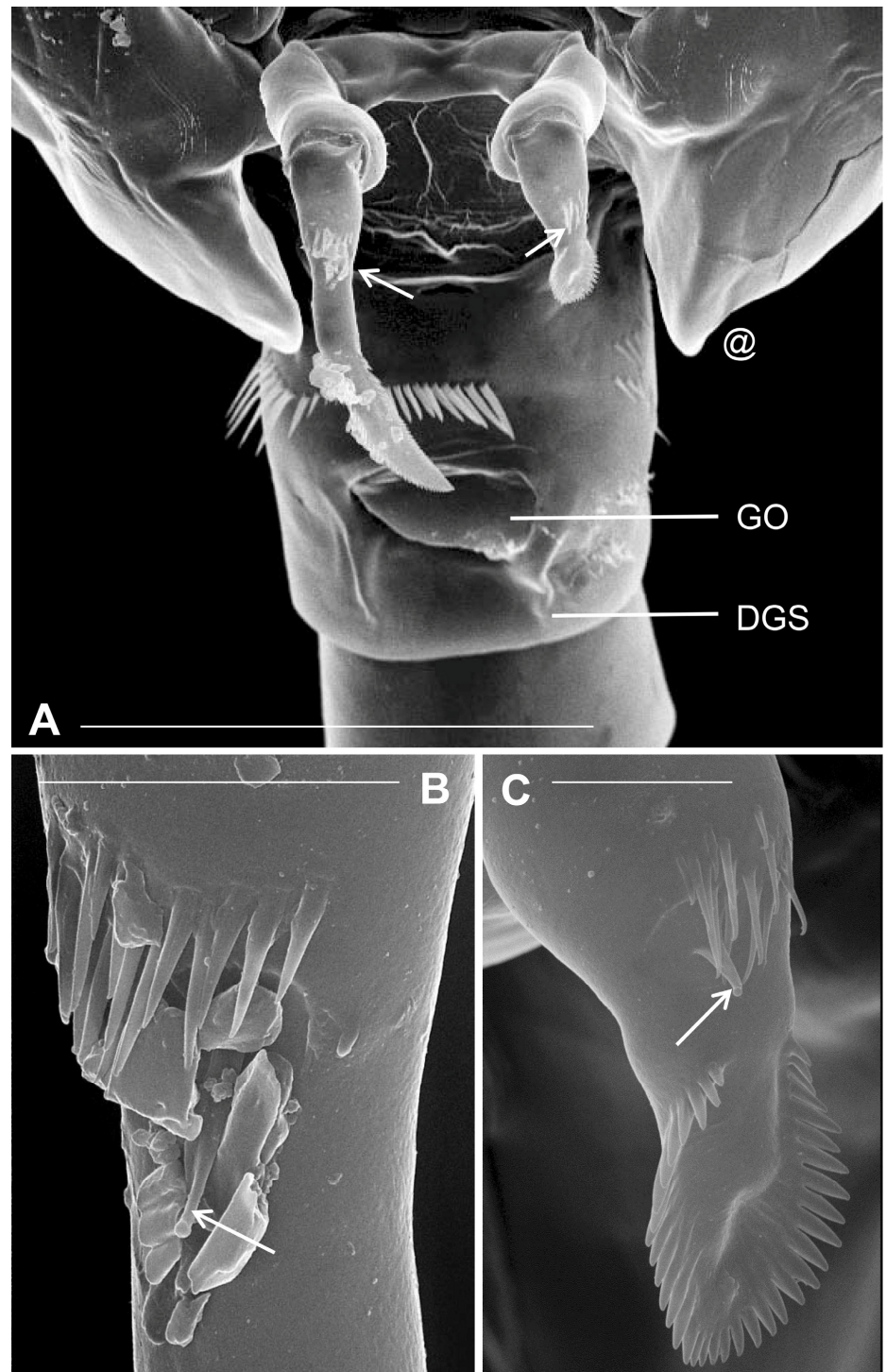


Figure 4. *Stephos marsalensis*, female. A: Scale bar: 100 μ m. Ventral view of genital double-somite (DGS) and anterior surface of fifth leg. @ = asymmetry of postero-lateral corner of prosome. GO = genital operculum. B and C: Scale bar 10 μ m. Right and left fifth leg; Diagonal arrows = central spines. Photomicrographs by the authors.

So, these structures were not able to be confirmed on paratypes (Boxshall, *pers. com.*). In the original paper, they were not clearly recognizable with SEM, because of fouling on the studied specimen (Zagami et al. 2000: fig. 5).

The original drawing of the fifth female legs (Zagami et al. 2000) was inverted because the central spine(s) on the distal segment is normally

located on the anterior surface in all *Stephos* sp. The inner margin in their material bears fewer spinules on the left than on the right leg. Both our data and Sicilian data showed variability in the number of these marginal spinules on the fifth legs. The right leg is slightly larger than left one and twisted towards the left.

Because many species of *Stephos* bear a patch of spinules and a central spine on the anterior face of the second segment of the female leg 5, we explored this association with SEM in both species. We demonstrated the presence of very small spinules, hitherto undescribed, in *S. cryptospinosus* (Figure 2D, E) and the presence of the previously undescribed central spine in *S. marsalensis* (Figures 4B, C). This spine is very short and grouped together with the proximal patch of spinules and is thus easy to overlook using optical observation in this species. Such defects in previous studies also exist for other species i.e. as *S. scotti*, and the use of this criterion (presence-absence of the central spine) to establish a biogeography of the species (Suarez-Morales et al. 2017) appears of limited reliability.

Our description of the male leg 5 agrees with the original description, particularly concerning the complex terminal structure, except for the fourth segment of the left leg. We consider the two rounded inner margin processes on this segment described by Zagami et al. (2000: fig. 4D) to be an artefact with the depression between these two processes probably resulting from shrinkage of the internal tissues within the cuticle (Figure 3C). Therefore, *S. cryptospinosus* belongs to type III, in which segment 4 of the left male P5 is swollen, according to the classification of Bradford-Grieve (1999).

Despite the apparent differences between our observations and the original descriptions, we consider that our two species are the two same species as found in Sicily. The presence of these two species in our four samples (2010 to 2015) indicates their permanent presence at the location.

The “hyaline sheath” and the reproduction in Stephos.

Bradford-Grieve (1999) noted that the female of *Stephos*, “when carrying a spermatophore, the genital double-somite is covered by a hyaline sheath of a complex structure”. Such a structure was observed in *S. cryptospinosus* (not in the original description but in paratypes, Boxshall, *pers. com.*, and in present study), *S. balearensis* (= *margalefi*) Carola and Razouls, 1996, *S. boettgerschnackae* Kršinić, 2012, *S. lucayensis* Fosshagen, 1970, *S. pacificus* Ohtsuka and Hiromi, 1987, *S. robustus* Ohtsuka and Hiromi, 1987, and finally in *S. projectus* Moon, Youn and Venmathi Maran, 2015. We suspect that it is not a general feature of the genus as we have never found this structure in *S. scotti*, despite examining numerous females both bearing spermatophore(s) and without.

The origin of this sheath is uncertain but Fosshagen (1970) suggested that “it seems to be secreted from two gland-like structures, one on either

side of the last prosomal segment next to the genital segment”. We did not find these glandular pores in either sex on any of the species we have studied using SEM. The hyaline sheath differs in shape in each species but the precise position can vary from specimen to specimen within a species (Fosshagen 1970), as also shown in our specimens. If the sheath were secreted by the female, it should be *a priori* always located in the same place. Our observations suggest it is produced by the male at the same time as the spermatophore. The variable positioning on the female might reflect variation in copulatory behaviour of males. This structure was suspected to be belonging to the spermatophore and to function as an attachment material by Kršinić (2012), but we were unable to demonstrate a direct connection with the spermatophore in the five specimens we studied by SEM (Figure 2). Most calanoid copepods produce simple tubular spermatophores as in the genus *Stephos*, but several produce more complex spermatophores. For example, some species of the subgenus *Tortanus* (*Atortus*) have highly complex coupling device on the flat spermatophore that almost entirely covers the female urosome (Barthélémy et al. 2003). In *Centropages* sp., the tubular spermatophore is connected via a bipartite coupling device (Lee 1972): the anterior hyaline coupler controls spermatophore fixation by closing around the genital double-somite of the female while the posterior coupler is a hyaline sheath cemented to the second urosomite. In calanoid copepods producing this complex spermatophore type, the female genital double-somite is usually ornamented with spines, processes and swellings which constitute a “lock-and-key” mechanism together with the coupler (Lee 1972). Some *Stephos* species’ descriptions lack sufficient detail, but the females of species known to have a hyaline sheath typically have an ornamented genital double somite which may help to secure the attachment of this structure. *S. cryptospinosus* is an exception which has a smooth surface on the genital double-somite and in which the hyaline sheath is sometimes “poorly positioned”. No other species of *Stephos* with a smooth surface to the genital double-somite has been reported in the literature as having a hyaline sheath.

We hypothesise that the hyaline sheath in *Stephos* is a relict structure derived from an ancestral complex spermatophore. It has been suggested that such structures can prevent subsequent mating by other males (Ohtsuka and Huys 2001). The efficiency of such a system is uncertain in *S. cryptospinosus* since it does not always cover the genital opening of the female and, furthermore, we found one specimen carrying two hyaline sheaths. However, species with a hyaline sheath, such as *S. lucayensis* Fosshagen, 1970, typically carry apparently only one spermatophore, whereas species without a hyaline sheath can carry two or more spermatophores as in *S. canariensis* (Boxshall et al. 1990) and *S. scotti* (up to 6; *personal observations*).

Further studies are necessary if we are to better understand the origin and clarify the role of this structure.

Zoogeography

The body size of *Stephos* species is small (up to or less than 1 mm) and the species exhibit a hyperbenthic mode of life (ref. in Razouls et al. 2005–2018). We consider that the current number of known species (32) is certainly an underestimate.

Some *Stephos* species have never been found outside of their type locality. Several species were described from specialised habitats. For example, *S. canariensis* Boxshall, Stock and Sanchez, 1990, *S. margalefi* Riera et al., 1991, *S. boettgerschnackae* Kršinić, 2012, *S. grievae* Kršinić, 2015 and *S. fernandoi* Suarez-Morales, Gutiérrez-Aguirre, Cervantes-Martinez and Iliffe, 2017 were all found in anchialine caves. *Stephos projectus* Moon et al., 2015 was collected in the stagnant water flooding the burrows excavated by ocypodid crabs. *Stephos gyrans* Giesbrecht, 1893 and *S. tsuyazakiensis* Tanaka, 1966 have only been recorded in aquarium tanks at Naples (Italy) and Kyushu (Japan), respectively. These restricted distributions may not necessarily indicate exclusive micro-distributions since these small copepods may be present in unexplored ecosystems, particularly outside of aquaria. The geographical gap between known sites of occurrence of *S. cryptospinosus* and *S. marsalensis* e.g. from Sicily in the Mediterranean to the French Brittany coast (North Atlantic Ocean) could be the result of anthropogenic exchanges between the two localities or maritime areas. The presence together of these two “Mediterranean” species could be interpreted as supporting this hypothesis. There are no recorded exchanges between the Marinarium located at Concarneau (built in 1859) and equivalent Mediterranean institutions. However, the outside storage tanks were under private ownership for a long time and were used for storage of edible shellfish. The details of this storage are not documented but it might have provided an opportunity for the introduction of the two *Stephos* species, as suspected for another copepod *Eurytemora pacifica* (Brylinski et al. 2016).

An alternative possibility is simply that the lack of scientific studies on this genus is factor of their hyperbenthic behaviour, since the hyperbenthic is under-sampled. Indeed, the description of these two species was relatively recent (2000) and the necessary data on their natural distributions are lacking.

In conclusion, there is no doubt that the number of *Stephos* species is underestimated. By way of example, Jacoby and Greenwood (1991) reported seven undetermined *Stephos* species at two stations off the coast of Queensland (Australia). According to Walter (1986), “further studies of demersal zooplankton should produce new records and species, allowing

better elucidation of phylogenetic relationships and zoogeographical distribution”. Because of the propensity of *Stephos* to live in the near-bottom zone and in dark localities, we encourage the exploration of such habitats in order to increase the number of known species. As this work suggests: if you search, you will find, whether endemic, non-indigenous, or new species.

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