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First occurrence of *Bonnierilla similis* Illg and Dudley, 1961 (Copepoda: notodelphyidae) in the central Mediterranean Sea

S. Lo Brutto [a,b], A. Badalucco[a,b], F. Mastrototaro[c] and J. P. Bernot [d]

[a]Department of Earth and Marine Sciences, University of Palermo, Palermo, Italy; [b]National Biodiversity Future Center (NBFC), Palermo, Italy; [c]Department of Biosciences, Biotechnologies and Environment, University of Bari, Bari and CoNISMa, Roma, Italy; [d]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA

ABSTRACT

Under a plan or monitoring the fouling community in Italian marinas, we report a new host and locality for the cyclopoid copepod *Bonnierilla similis*, found in the branchial sac of the ascidian *Styela plicata* along the Sicilian coast (southern Italy), in the central Mediterranean Sea. *Bonnierilla similis* was originally described from the ascidian *Pyura squamulosa* along the western Mediterranean coast of France, near Perpignan; until now, this copepod had not been documented in any other Mediterranean localities. In this study, 10 of 14 *S. plicata* specimens from the Palermo marina site were infected (71% prevalence); while in the Trapani marina site all the collected *S. plicata* specimens were uninfected. Given that *S. plicata* has been extensively sampled at the Sicilian site Palermo marina in the past and, until now, had never been found to host copepods, it is possible that the species has recently expanded its range into this region. To support future taxonomic identifications, the first COI sequence data was generated here for *B. similis*.

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Biofouling; Mediterranean Sea; parasite copepods; COI barcoding; tunicate-associated fauna

Introduction

Copepods occur in all aquatic habitats, including fresh water, estuaries, and all depths of the ocean, and are known to be benthic, demersal, and planktonic. Additionally, many copepods live in close association with other animals as commensals or parasites (Gotto 1979; Dudley & Illg 1991; Pastore 2001; Özak et al. 2012; Cruz-Rivera et al. 2022). Parasitic taxa often show drastic modifications of their external anatomy relative to free-living copepods, modifications that are typically related to host attachment and enhanced reproductive capacity (Holmes & Gotto 1987). In many cases, details regarding the exact nature of the association between copepods and their hosts are not known, and it is not clear whether the symbiotic interactions are mutualistic, commensalistic, or parasitic (Bernot et al. 2021; Cruz-Rivera et al. 2022). This is particularly true for the copepods associated with tunicates, which exist along a continuum from relatively unmodified cyclopoid copepods, such as species belonging to the family Archinotodelphyidae Lang, 1949, to highly derived taxa lacking evidence of external body segmentation and appendages, such as *Achelidelphys* Lafargue and Laubier, 1977, *Anoplodelphys* Lafargue and Laubier, 1978, *Brementia* Chatton and Harant, 1915, *Paranoplodelphys* Boxshall and Marchenkov, 2007, *Pholeterides* Illg, 1958, *Socotradelphys* Kim and Boxshall, 2020, and several other highly transformed monotypic genera (see Lang 1949; Illg 1955; Boxshall & Halsey 2004; Kim & Boxshall 2020).

Ascidian tunicates appear to be particularly good hosts for copepods in that they host a wide diversity of taxa at relatively high prevalence and intensities. Solitary and colonial tunicates collectively host over 600 species of copepods, most belonging to Cyclopoida, especially the families Notodelphyidae (383 species), Botryllophilidae (116 species), Enteropsidae (75 species), Archinotodelphyidae (20 species), Ascidicolidae (20 species), and Buproridae (3 species), which are all tunicate-specific symbionts, as well as several species of other families such as Asterocheridae and Lichomolgidae that parasitize a diversity of marine invertebrates (Boxshall & Halsey 2004; Kim & Boxshall 2021; Walter & Boxshall 2025). These copepods can reach high abundances in a single tunicate, and it is not uncommon for several copepod taxa to parasitize a single tunicate species (Pastore 2001; Kim & Boxshall 2020).

CONTACT S. Lo Brutto sabrina.lobrutto@unipa.it Department of Earth and Marine Sciences, University of Palermo, Via Archirafi 20, Palermo 90123, Italy

Currently, there are few records of copepods associated with ascidian tunicates along the central Mediterranean Sea. Existing records are for the families Ascidicolidae, Botryllophilidae, Enterognathidae, and Enteropsidae, reported in the Gulf of Naples (Illg & Dudley 1980), in the Gulf of Taranto (Pastore 2001), and Palermo's marina (Lo Brutto et al. 2024b). In this study, we report a new host and a new locality for *Bonnierilla similis* Illg and Dudley, 1961, found in the solitary ascidian *Styela plicata* (Lesueur, 1823) along the coast of Sicily Island (southern Italy).

Materials and methods

The sampling sites were within the recreational marinas of Palermo (La Cala site), near the huge commercial harbour of the city (38°07′14.04″N, 13°22′12.84″E), and Trapani (Molo Columbus site, 38°00′52.3″N, 12°30′02.9″E) (Figure 1). Both areas showed a diverse fouling community mostly represented by sessile suspension feeders attached to artificial substrates of the infrastructure. The fouling community was collected in 2023 and 2024 from nautical ropes at depths between 0 and 2 m. The sampling at the

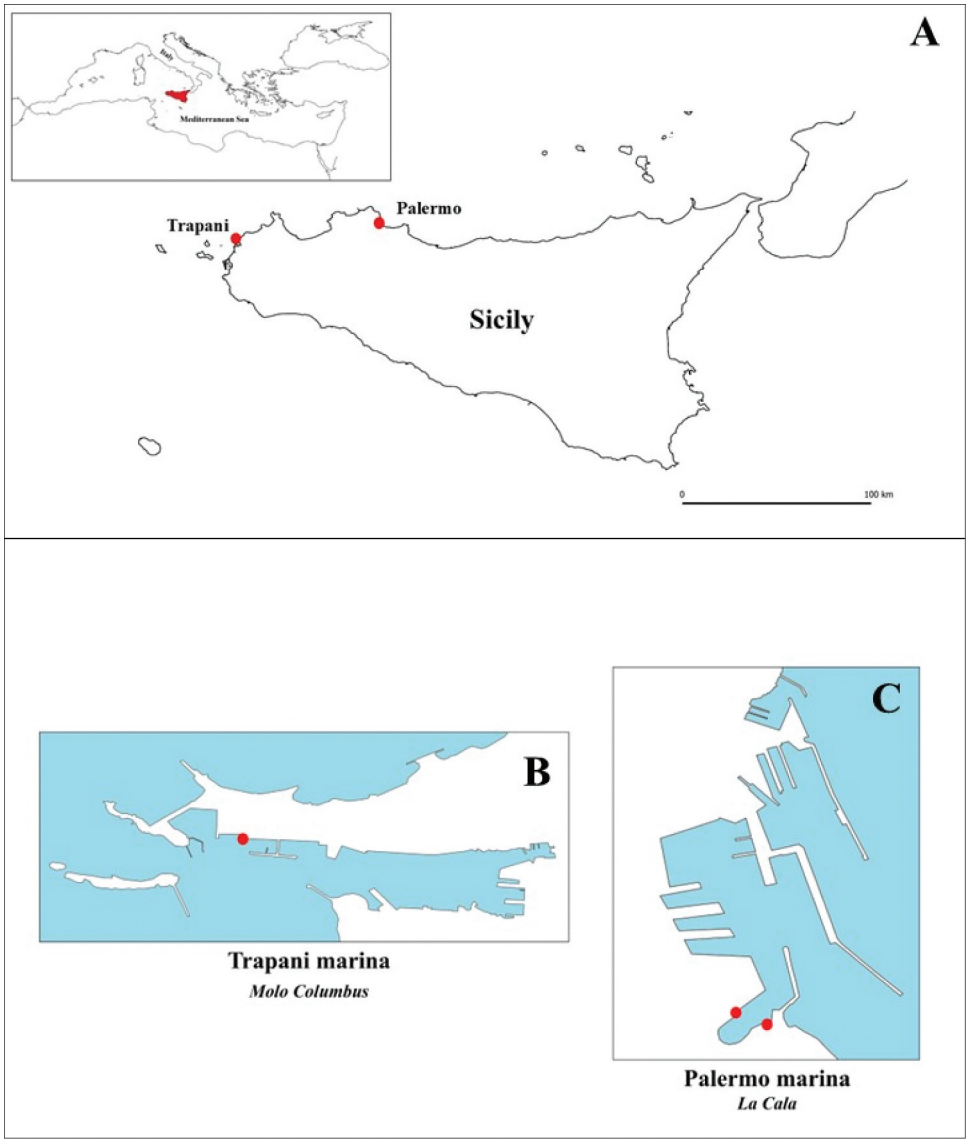

Figure 1. A, map showing the two sampling sites (Molo Columbus site within Trapani marina and La Cala site within Palermo marina) along the Sicilian coast. On the upper left side, the geographical position of Sicily (in red) is shown in the Mediterranean Sea. B, map of Trapani marina, within the harbour area of Trapani city; C, map of Palermo marina, within the harbour area of Palermo city. Red dots indicate the sampling sites.

Palermo marina was carried out on 5 December 2023, 20 and 28 March 2024, 18 April 2024 and 27 November 2024; while the Trapani marina was sampled on 24 March 2024. The sorting of the benthic assemblage was carried out under a stereomicroscope (Nikon SMZ25) to observe and collect small-sized organisms. The most common macrofauna species were identified to examine differences in the biofouling community between the two sites.

Copepods found inside ascidians were preserved in 95% ethanol at the time of collection. For detailed morphological observation and identification, copepod specimens were cleared and dissected in lactic acid, temporarily mounted on glass slides under a coverslip and examined with a Zeiss Axioscope 5 microscope. One female specimen was also observed with confocal laser scanning microscopy (CLSM) because CLSM can resolve minute morphological features and condense many focal planes into a single flattened image where all structures appear in sharp focus. For CLSM, the specimen was stained for 7 days in a 1:1 mixture of Congo Red and Acid Fuschin in which both stains were made from a solution of 0.25 g of stain and 50 mL of deionized water. After staining, the specimen was rinsed in approximately 15 changes of deionized water, letting it sit for several minutes between each water change, until no stain could be seen diffusing, and prepared as a temporary mount in a 20% solution of glycerine and deionized water on a glass slide under a coverslip. The specimen was imaged on a Nikon Ti2E AXR confocal system at the Advanced Light Microscopy Facility at the University of Connecticut using a 561 nm laser with the emitted fluorescence collected in two channels: a 571–630 nm artificially coloured green and 640–715 nm artificially coloured red, and a series of image stacks was collected; flat images were obtained by maximum projection of the overlaid channels using ImageJ v. 2.14. The copepod was identified to the genus *Bonnierilla* using the key to notodelphyid genera in Boxshall and Halsey (2004) and further identified to species by examining all descriptions of *Bonnierilla* species in the literature. Parasitology statistics with 95% confidence intervals were calculated using Quantitative Parasitology on the Web (QPweb) v. 1.0.15 (available at https://www2.univet.hu/qpweb/qp10/index.php) as follows: prevalence (Clopper-Pearson), intensity and mean abundance both with Bootstrap BCa with 2000 reps each (Reiczigel et al. 2019).

For molecular barcoding, total genomic DNA extraction was carried out for a female, and one target mitochondrial marker was amplified using universal primers (Folmer et al. 1994). A 658 base pair (bp) region of the cytochrome c oxidase subunit I gene (COI; accession number PV791688) was amplified following Baek et al. (2016).

Results

The notodelphyid copepod *B. similis* was collected from the examined tunicates (Figure 2(A–C)). It was identified based on the unique armature on the distal segment of the antenna, which consists of three parallel, curved spine-like elements that oppose the terminal claw (Figure 2(D)); the setal formulae of the legs were also congruent with the original description of *B. similis*. A total of 35 specimens were recorded in the branchial sac of 10 of the 14 specimens of *S. plicata* (Figure 2(B)) collected from the Palermo marina, for a prevalence of 71.4% (95% CI = 0.42–0.92), while no specimens of *B. similis* were found in the 29 *S. plicata* collected from the Trapani marina. The intensity of symbionts inside the ascidians ranged from 1 to 11 copepods per host, with an average intensity of 3.5 (95% CI = 1.8–6.1) and a mean abundance of 2.5 (95% CI = 1.2–4.8). Four tunicates were found to host a single individual of *B. similis*, 3 hosted 2 individuals, and 1 each hosted 6, 8, and 11 specimens of *B. similis*.

The Palermo marina is less wave-exposed and has a greater amount of nutrients than the Trapani marina, and the biofouling communities differed between the two sites. At Palermo marina, the fouling community was mainly composed of poriferans, anemones, polychaetes (such as serpulids, sillids), bivalves (e.g. *Brachidontes pharaonis* in massive abundance in March 2024), gastropods (e.g. patellids, *Haminoea* sp., and nudibranchs), pycnogonids (e.g. *Achelia sawayai*), crustaceans including copepods, balanids, isopods (*Paranthura japonica, Carpias stebbingi*), amphipods (*Elasmopus rapax*, *Caprella scaura*), tanaids and decapods, as well as bryozoans, ophiuroids, and botryllid ascidians.

At the Trapani marina, molluscs were dominant and showed a diversified composition (*Mytilaster minimus*, *Isognomon* sp., *Aplysia punctata*, *Euthria cornea*, trochids, *Limaria* sp., *Pinctada radiata*, *Placida* sp., *Janolus* cf. *cristatus*, *Tricolia pullus*, *Tritia incrassata*, *Columbella rustica*, *Diodora graeca*, *Fissurella nubecula*, ostreids), followed

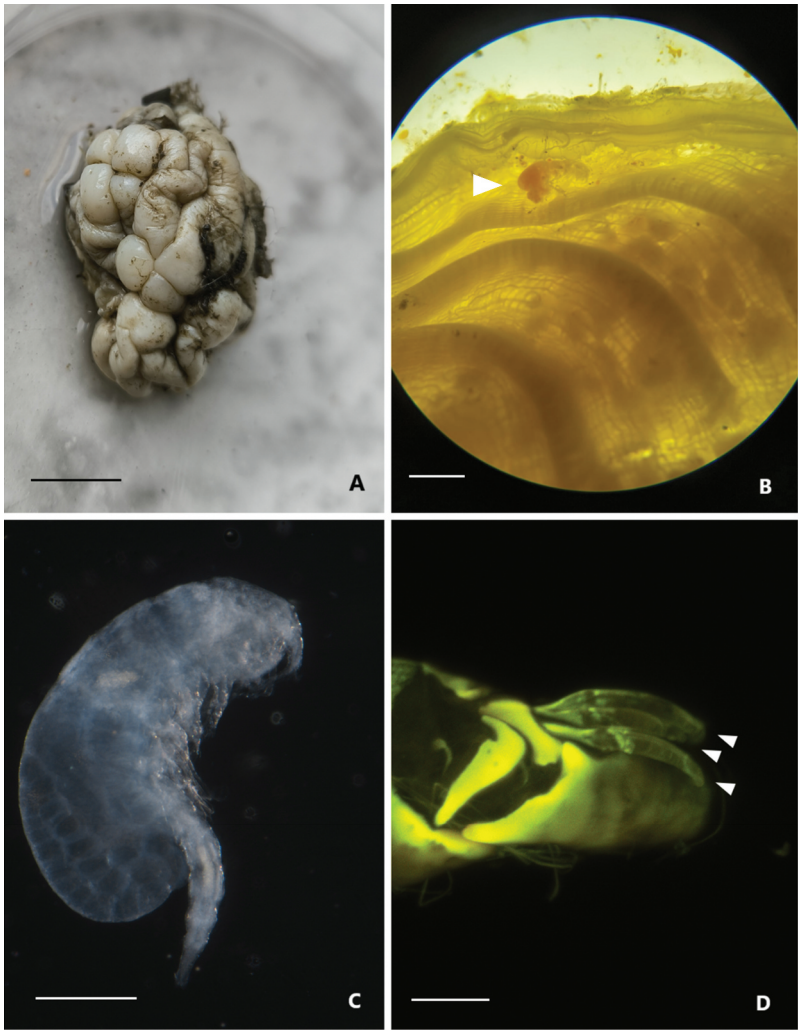

Figure 2. A, *Styela plicata*, scale bar: 1 cm; B, the notodelphyid copepod within the branchial sac, scale bar: 2 mm; C, *Bonnierilla similis*, scale bar: 500 µm; D, confocal laser scanning micrograph of the tip of the antenna, which shows three parallel claws diagnostic of *B. similis*, scale bar: 10 µm.

by peracarid crustaceans (including the amphipods *Lysianassa pilicornis*, *Elasmopus rapax*, *Caprella scaura*, *Phtisica marina*, and *Stenothoe* sp.; isopods *Carpias stebbingi*, *Paranthura japonica*, sphaeromatids; and tanaids); poriferans, the mite *Litarachna duboscqi*, ophiuroids, and tunicates (*Microcosmus squamiger*, *Ascidiella aspersa*).

Discussion

The island of Sicily is considered a hotspot for the early detection of exotic or rare species in the Mediterranean Sea due to its extensive port system that favours marine dispersal and puts the area at risk for colonization by non-native species (Servello et al. 2019; Katsanevakis et al. 2023). An increasing number of new records of overlooked or non-indigenous species (NIS) have been reported (Lo Brutto et al. 2018, 2024a, 2024b; Battiata et al. 2024).

A new record of the notodelphyid copepod *B. similis* is reported here. *Bonnierilla similis* was described by Illg and Dudley (1961) from the tunicate *Pyura squamulosa* from the southern Mediterranean coast of France, near Perpignan. Since then, it has been reported only once, from an unknown tunicate in Northern Ireland by Holmes and Gotto (1987).

Bonnierilla species inhabit the branchial cavity of the host ascidians. The genus has been reported in different solitary ascidians, where the effects on host health are not clear. According to Cruz-Rivera et al. (2022), their effect appears minimal and consistent with a commensalistic interaction; however, it would be

interesting to examine this more closely. The copepods might cause minor pathology if feeding on their host. Even if feeding only on particles trapped by the host, this could negatively impact host fitness by reducing nutrient intake.

Here we report *Bonnierilla similis* from the ascidian tunicate *Styela plicata* (Lesueur, 1823) collected in Palermo, a new host record and a new geographical area for the species. This species was readily identified here by the unique armature on the distal segment of the antenna, which consists of three parallel, curved, spinelike elements that oppose the terminal claw (Figure 2(D)). Holmes and Gotto (1987) also report this antennal armature from the specimens they collected. Given the disparate locality of their material (Northern Ireland vs. Mediterranean), we are left wondering about the native range of this species, its potential introduction into the Mediterranean and/or Ireland, or if the specimens reported by Holmes and Gotto (1987) represent a new species. We also note that the caption of Figure 1 in Holmes and Gotto (1987) incorrectly labels the three species in that figure. In particular, *B. similis*, is 1c, not 1a; the correct labels are given in their "Collecting details" section (p. 341), not in the figure caption.

It is possible that this copepod is currently undergoing a range expansion. The tunicate *Styela plicata* was collected from the same station (La Cala, Palermo) extensively in the past as the species was used for ecotoxicology analyses (e.g. Parrinello et al. 2017), physiological performance (e.g. Montalto et al. 2017) or immunotoxicology tests (e.g. Cammarata et al. 2007). However, *B. similis*, like other notodelphyids, was never found until now. So far, *B. similis* has been recorded only in the marina close to the large commercial and tourist harbour area of Palermo, which is the fourth largest in Italy in terms of cruise traffic. In contrast, despite the high number of *Styela plicata* examined from Trapani marina, none of the tunicates in that locality were infested. It is possible that *B. similis* has been introduced to Palermo by shipping traffic. Non-native ascidian parasites have been introduced to other regions, including the Gulf of California (Tovar-Hernández et al. 2010), but more extensive studies on Mediterranean tunicates and their symbionts are needed to better understand their range, dispersal, and potential anthropogenic introductions.

The different features of the localities could have influenced the different copepod prevalence (Palermo 71% and Trapani 0%), as supposed regarding previous observations of the tunicate *Phallusia nigra* collected along the Egyptian Red Sea coast (Cruz-Rivera et al. 2022). Cruz-Rivera et al. (2022) analysed different sites closer to each other (within 1 km) than the ones in the present paper (approximately 100 km), and supposed that local environmental factors might influence the nature of the copepod–host interactions and the density of copepods inside the ascidians.

As the scattered knowledge of the notodelphyid distribution could benefit from integrated taxonomy studies, the Sicilian sample was genetically analysed. According to previous studies on DNA barcoding within the order Cyclopoida (Baek et al. 2016), the COI marker provides a distinction within- and between-species and was selected to perform molecular characterization and facilitate future barcoding efforts. Additional sampling is planned to clarify the distribution of the species and the ecological role in the Mediterranean fouling communities; additional observations and analyses would be useful to establish the copepod–host interaction and identify factors that can affect the presence of such an understudied taxon.

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Disclosure statement

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ORCID

S. Lo Brutto http://orcid.org/0000-0002-9964-904X
J. P. Bernot http://orcid.org/0000-0002-1769-8631

References

Baek SY, Jang KH, Choi EH, Ryu SH, Kim SK, Lee JH, Lim YJ, Lee J, Jun J, Kwak M, Lee YS. 2016. DNA barcoding of metazoan zooplankton copepods from South Korea. PLOS ON E11(7):e0157307. DOI: 10.1371/journal.pone.0157307.

Battiata M, Curatolo T, Naser MD, Sarà G, Lo Brutto S. 2024. The state of the art of the global distribution of the invasive mytilid species *Brachidontes pharaonis* (P. Fischer, 1870). Diversity 16(7):381. DOI: 10.3390/d16070381.

Bernot JP, Boxshall GA, Crandall KA. 2021. A synthesis tree of the Copepoda: Integrating phylogenetic and taxonomic data reveals multiple origins of parasitism. PeerJ 9:e12034. DOI: 10.7717/peerj.12034.

Boxshall GA, Halsey SH. 2004. *An introduction to copepod diversity. Vol. I-II*. London: The Ray Society.

Cammarata M, Parisi MG, Benenati G, Arizza V, Cillari T, Piazzese D, Gianguzza A, Vazzana M, Vizzini A, Parrinello N. 2007. In vitro effects of methylmercury on ascidian (*Styela plicata*) immunocyte responses. Applied Organometallic Chemistry 21(12):1022–1028. DOI: 10.1002/aoc.1335.

Cruz-Rivera E, Sherif M-E-D, El-Sahhar S, Lombardi T. 2022. Spatial variability in a symbiont-diverse marine host and the use of observational data to assess ecological interactions. Diversity 14(3):197. DOI: 10.3390/d14030197.

Dudley PL, Illg PL. 1991. *Marine flora and fauna of the eastern United States: Copepoda, Cyclopoida: Archinotodelphyidae, notodelphyidae, and Ascidicolidae – associates of ascidians*. *Vol. 96*. NOAA Technical Report.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Biology and Biotechnology 3(5):294–299.

Gotto RV. 1979. The association of copepods with marine invertebrates. Advances in Marine Biology 16:1–109.

Holmes JMC, Gotto RV. 1987. Some ascidicolous copepods new to British and Irish waters. The Irish Naturalists Journal 22 (8):340–343.

Illg PL. 1955. A new species of *Pararchinotodelphys* (Copepoda: Cyclopoida) with remarks on its systematic position. Journal of the Washington Academy of Science 45(7):216–224.

Illg PL, Dudley PL. 1961. Notodelphyid copepods from Banyuls-sur-Mer. Vie et Milieu Supplement 12:1–126.

Illg PL, Dudley PL. 1980. The family Ascidicolidae and its subfamilies (Copepoda, Cyclopoida), with descriptions of new species. Mémoires du Muséum national d'Histoire naturelle. Série A, Zoologie 117:1–192.

Katsanevakis S, Olenin S, Puntila-Dodd R, Rilov G, Stæhr PAU, Teixeira H, Tsirintanis K, Birchenough SNR, Jakobsen HH, Knudsen SW, Lanzén A, Mazaris AD, Piraino S, Tidbury HJ. 2023. Marine invasive alien species in Europe: 9 years after the IAS regulation. Frontiers in Marine Science 10:1271755. DOI: 10.3389/fmars.2023.1271755.

Kim IH, Boxshall GA. 2020. Untold diversity: The astonishing species richness of the Notodelphyidae (Copepoda: Cyclopoida), a family of symbiotic copepods associated with ascidians (Tunicata). Megataxa. 4(1):001–660. DOI: 10.11646/megataxa.4.1.1.

Kim IH, Boxshall GA. 2021. Copepods (Cyclopoida) associated with ascidian hosts: Ascidicolidae, Buproridae, Botryllophilidae, and Enteropsidae, with descriptions of 84 new species. Zootaxa 4978(1):1286. DOI: 10.11646/zootaxa.4978.1.1.

Lang K. 1949. On a new copepod family related to Notodelphyidae and on two new copepod species from South-Georgia. Arkiv för Zoologi 42B(4):1–7.

Lo Brutto S, Badalucco A, Musco L. 2024a. First occurrence of the family Botryllophilidae Sars GO, 1921 (Copepoda, Cyclopoida) in Sicily, central Mediterranean Sea. Crustaceana 97(3–4):321–328. DOI: 10.1163/15685403-bja10374.

Lo Brutto S, Badalucco A, Smit H. 2024b. First occurrence of the mite *Litarachna duboscqi* Walter, 1925 (Acariformes: Pontarachnidae) in the central Mediterranean Sea. Ecologica Montenegrina 74:21–26. DOI: 10.37828/em.2024.74.3.

Lo Brutto S, Iaciofano D, Scipione MB. 2018. Citizen Science: un caso studio nel porto di Palermo. Biologia Marina Mediterranea 25(1):124–126.

Montalto V, Bagarella R, Rinaldi A, Sarà G, Mirto S. 2017. Thermal adaptation and physiological responses to environmental stress in tunicates. Aquatic Biology 26:179–184. DOI: 10.3354/ab00685.

Özak AA, Demirkale I, Yanar A. 2012. First record of two species of parasitic copepods on immigrant pufferfishes (Tetraodontiformes: Tetraodontidae) caught in the eastern Mediterranean Sea. Turkish Journal of Fisheries and Aquatic Sciences 12(3). DOI: 10.4194/1303-2712-v12_3_17.

Parrinello D, Bellante A, Parisi MG, Sanfratello MA, Indelicato S, Piazzese D, Cammarata M. 2017. The ascidian *Styela plicata* hemocytes as a potential biomarker of marine pollution: In vitro effects of seawater and organic mercury. Ecotoxicology & Environmental Safety 136:126–134. DOI: 10.1016/j.ecoenv.2016.11.001.

Pastore M. 2001. Copepods associated with *Phallusia mammillata* and *Ciona intestinalis* (Tunicata) in the area of Taranto (Ionian Sea, southern Italy). Journal of the Marine Biological Association of the United Kingdom 81(3):427–432. DOI: 10.1017/S0025315401004052.

Reiczigel J, Marozzi M, Fabian I, Rozsa L. 2019. Biostatistics for parasitologists – a primer to quantitative Parasitology. Trends in Parasitology 35(4):277–281. DOI: 10.1016/j.pt.2019.01.003.

Servello G, Andaloro F, Azzurro E, Castriota L, Catra M, Chiarore A, Crocetta F, D'Alessandro M, Denitto F, Froglia C, Gravili C, Langer M, Lo Brutto S, Mastrototaro F, Petrocelli A, Pipitone C, Piraino S, Relini G, Serio D, Xentidis N, Zenetos A. 2019. Marine alien species in Italy: A contribution to the implementation of descriptor D2 of the Marine strategy framework directive. Mediterranean Marine Science 20(1):1–48. DOI: 10.12681/mms.18711.

Tovar-Hernández MA, Suárez-Morales E, Yáñez-Rivera B. 2010. The parasitic copepod *Haplostomides hawaiiensis* (Cyclopoida) from the invasive ascidian *Polyclinum constellatum* in the southern Gulf of California. Bulletin of Marine Science 86(3):637–648.

Walter TC, Boxshall GA. 2025. World of copepods database. Available: https://www.marinespecies.org/copepoda. Accessed Feb 2025 7.