# **Original Article**

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A new species of *Herpyllobius* Steenstrup and Lütken, 1861 (Copepoda: Cyclopoida) parasitic on *Lamispina horsti* (Haswell, 1892) (Annelida, Flabelligeridae) from Western Australia

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# ABSTRACT

Herpyllobiid copepods are highly transformed mesoparasites that infect marine benthic polychaete annelids. The genus Herpyllobius Steenstrup and Lütken, 1861, the most diverse in the family, was known to infect exclusively polychaetes of the family Polynoidae, but it was recently reported also on another family. Species of Herpyllobius have been reported mainly from cold latitudes including Arctic and Antarctic areas, except for two subtropical species. During the taxonomic examination of flabelligerid polychaetes collected from a Western Australian coral reef system, a mesoparasitic copepod was found infecting a specimen of Lamispina horsti (Haswell, 1892). The copepod represents an undescribed species of *Herpyllobius*. The new species, Herpyllobius paulayi n. sp. belongs to a group of congeneric species (Group III) lacking intergenital processes or sclerotized dots. It diverges from the other species in this group by its possession of a unique combination of characters: 1) a globose ectosoma with pilose surface; 2) elongate, thick cylindrical egg sacs with +10 egg rows; 3) endosoma discoid, short, with two lateral, asymmetrical flattened processes and a medial lobe; 4) it attaches to the host prostomium; and 5) it infects a non-polynoid subtropical polychaete species.

# **KEYWORDS**

Benthic polychaetes, mesoparasitic copepods, reef systems, symbiosis, taxonomy

## INTRODUCTION

The cyclopoid copepod family Herpyllobiidae Hansen, 1892 comprises highly specialized mesoparasitic copepods infecting annelids (Lützen, 1968; Boxshall et al., 2019). They are part of the more than 120 copepod species known as external or internal parasites of polychaete annelids (Conradi et al., 2015; Björnberg and Radashevsky, 2011; Boxshall et al., 2019). The family Herpyllobiidae is known to contain five genera: Eurysilenium Sars M., 1870 (5 spp.), Gottoniella López-González, Bresciani and Conradi, 2006 (2 spp.), Herpyllobius Steenstrup and Lütken, 1861 (19 spp.), Phallusiella Leigh-Sharpe, 1926 (2 spp.), and Thylacoides Gravier, 1912 (5 spp.) (Walter and Boxshall, 2023). Some of these genera have been considered invalid or poorly defined (Lützen, 1964; Boxshall et al., 2019). Herpyllobius contains species that parasitize, almost exclusively, polynoid polychaetes (Lützen, 1964; Boxshall et al., 2019, Suárez-Morales and Salazar-Vallejo, 2022). Its type species, Herpyllobius arcticus Steenstrup and Lütken, 1861, was described as a parasite of a scale worm from Greenland (Bresciani and Lützen, 1961). Overall, herpyllobiids have been recognized as the most representative polychaete parasitic copepods known (Conradi et al., 2015). Their highly transformed body, lacking any trace of segmentation or appendages, comprises three main body parts: 1) an external reproductive ectosoma carrying the genital openings and paired egg sacs; 2) an internal endosoma embedded in the host body; and 3) a short intersomital stalk connecting the endosoma and the ectosoma (Lützen, 1964; Boxshall et al., 2019). Most species of Herpyllobius inhabit polar or cold temperate latitudes (Lützen 1964; López-González and Bresciani, 2001; López-González et al., 2000; Conradi et al., 2015), except for the subtropical H. nipponicus Lützen, 1964, from Japan. Members of Herpyllobius are known to parasitize only polynoid polychaetes, including some symbiotic on stylasterine corals, thus being constituent of the first reported case of hyperassociation among copepods (Stock, 1986).

During the taxonomic examination of flabelligerid polychaetes collected from a Western Australian coral reef system, a parasitic copepod was found infecting a specimen of *Lamispina horsti* (Haswell, 1892). The copepod was found to represent an undescribed species of *Herpyllobius*. The new species is here described and compared with its known congeneric species.

## **MATERIAL AND METHODS**

The infected specimen of the flabelligerid polychaete *L. horsti* is deposited in the University of Florida Natural History Museum (UF 1757). It was collected in Western Australia, Ningaloo Reef system, 27 m water depth, from rubble crevices, 30 May 2010, by C. Bagnato and A. Anker. The annelid host individual was observed with a stereoscope, temporarily stained with an oversaturated Methylgreen solution in 70% ethanol (Wisnes, 1985). Two left parapodia were removed for observing chaetal details. A series of digital photos were compressed with Helicon Focus<sup>®</sup>, and plates were prepared with PaintShopPro<sup>®</sup>.

We detected a single individual of the copepod parasite anchored close to the fused eyes and with its paired egg sacs exposed (Fig. 1A). By carefully separating the host tissues with a pair of sharpened needles, we obtained the complete copepod body (endosoma-stalk+ ectosoma) of this individual for further taxonomic examination. Morphological observations were made with an Olympus BX51 compound microscope equipped with Nomarski DIC. Line drawings of the main observable characters were prepared with the aid of a drawing tube. The holotype specimen was deposited in the collection of Zooplankton held at ECOSUR-Chetumal, Mexico (ECO-CH-Z).

## **SYSTEMATICS**

Class Copepoda Milne-Edwards, 1840

Order Cyclopoida Burmeister, 1834

Family Herpyllobiidae Hansen, 1892

Genus *Herpyllobius* Steenstrup and Lütken, 1861

Herpyllobius paulayi sp. nov. (Figs. 1–2) ZooBank: urn: lsid:zoobank.org:act:B497B75A-484D-4215-BDB5-770B679E8141



**Figure 1.** *Lamispina horsti* (Haswell, 1892), non-type specimen (UF 1757) infected by *H. paulayi* sp. nov. **A.** Complete specimen, right lateral view. **B.** Anterior end, right lateral view. **C.** Anterior end, frontal view, after Methyl-green staining (asterisks indicate parasite egg-sacs; Br, branchia, Pa, palp). **D.** Chaetiger 22, left parapodium, posterior view. **E.** Same, notochaetae. **F.** Same, neurochaetae. Scale bars: A = 1.9 mm; B = 0.6 mm; C = 0.4 mm; D = 0.3 mm;  $E = 125 \mu \text{m}$ ;  $F = 110 \mu \text{m}$ .

*Type material.* Ovigerous female, holotype (ECO-CHZ 11819), parasitizing *Lamispina horsti* (Haswell, 1902), Western Australia, Ningaloo Reef system, North Black Rock (27°42'11.7"S 113°36'19.02"E), specimen undissected, with two separate egg sacs, mounted in glycerin, sealed with acrylic varnish, slide. Collected 30 May 2010, 27 m water depth in rubble crevices, by C. Bagnato and A. Anker.

*Diagnosis* (female). Ectosoma almost spherical, integument pilose. Genital swellings moderately sclerotized, weakly developed. Intergenital surface smooth, lacking medial process or bulging protuberances. Sclerotized dots also absent (Fig. 2C). Anterior surface of ectosoma with rounded shield-like area ornamented with fan-like pattern. Intersomital stalk short and broad, originating from underside of ectosoma. Endosoma with 2 sections, one proximal discoid broadening into asymmetrical flap-like lateral lobes with irregular edges; medial lobe arises between lateral lobes. Sclerotized ring present at proximal half of endosoma, curved with borders toward distal part of endosoma. Egg sacs thick, cylindrical, almost twice as long as wide, multiseriate, with 10–12 rows of eggs. Male unknown.

Description of holotype. Ectosoma almost spherical, 1.29 times as long as wide, 240 µm long, 186 µm wide, ectosoma integument moderately pilose (Figs. 1A, C, 2A). Paired genital swellings weakly sclerotized, moderately prominent (gsw in Fig. 2B, C). Genital swellings carrying egg sacs; holotype carrying one complete (0.9 mm long, 0.47 mm wide, ca. 140 eggs) and one broken egg sac (0.66 mm, 48 eggs). Intergenital surface rounded, smooth, lacking medial processes, or protuberances (Fig. 2C); sclerotized dots absent. Intersomital stalk short, thick, ca. 140 µm in diameter, originating from the underside of the ectosoma close to mid-body (Fig. 2B). Endosoma 0.85 mm long, comprising a proximal lump structure, arranged horizontally, with 351  $\mu m$  in length and adjacent medial tongue-like lobe with irregular distal edges (Fig. 2A, B, D). Proximal endosomal section with oblong, narrow sclerotized ring (see Fig. 2B)



**Figure 2.** *Herpyllobius paulayi* sp. nov., holotype, adult female (ECO-CHZ-010673). **A**, complete body as extracted from host, including ectosoma and endosoma, anterior view. **B**, holotype in lateral view showing cement gland (cgl) and oblong sclerotized ring (dotted line band) and genital swelling (gsw). **C**, same as A, in slightly different (anterior) position showing ornamented shield area (osh). **D**, same, ventral view showing endosoma medial lobe (mlb) and adjacent lateral lobes (latl). Scale bars: A–D = 100 µm.

proximally (diameter =  $195 \mu m$ ,  $28 \mu m$  wide). Egg sacs thick, cylindrical, multiseriate, up to 0.85 mm long, 0.48 mm wide (Fig. 1A), eggs ca. 75  $\mu m$  in diameter.

Male. Unknown.

*Etymology.* The species is named after Dr. Gustav Paulay (Florida Museum of Natural History), who kindly allowed us to examine the worm host and the copepod parasite. *Distribution*. So far known only from the type locality.

Host. The host is an individual of the flabelligerid L. horsti; the species was redescribed elsewhere (Salazar-Vallejo, 2014), and was identified with a recently assembled taxonomic key (Jimi and Kajihara, 2018). The specimen is 18 mm long, 2 mm wide, with 48 chaetigers; the body is whitish, subcylindrical, tapering to a blunt posterior end (Fig. 1A). The cephalic cage is 2.5 times longer than body width, and the anterior end is fully exposed (Fig. 1B). In frontal view, the head has two corrugate thick palps, about 3-4 times wider than branchial filaments, but from the usual eight branchiae, only six are present (Fig. 1C). The head has four black, fused eyes, and above them there are egg-sacs of a single copepod parasite; one sac is complete, another one was broken (Fig. 1C). The parapodia include seven notochaetae and five lamispines per bundle (Fig. 1D); notochaetae are basally ankylosed, with long articles along most of chaetae (Fig. 1E), whereas lamispines are darker basally and paler distally, with tips falcate (Fig. 1F). The species has been recorded from southeastern and southern Australia (Haswell, 1892).

Attachment site. The studied individual was found attached to the head (prostomium), close to the eyes.

*Remarks.* The new species agrees with the main diagnostic characters of *Herpyllobius* according to Lützen (1964) and Boxshall et al. (2019). The known species of *Herpyllobius* were grouped by Lützen and Jones (1976) (Groups I–III) and then updated with additional characters by López-González et al. (2000) based on the position and size of the medial protuberances and the number and arrangement of cuticular processes (sclerotized dots) above or between the genital swellings, the endosoma shape and structure, and the attachment site on the host. Group I includes species with the genital swellings separated by a single, prominent, medio-terminal bulging process, with four adjacent sclerotised dots.

Group II comprises species with 2–4 sclerotized dots in the area above the genital swellings (Suárez-Morales and Salazar-Vallejo, 2022: tab. 1).

Group III includes species lacking both protruding intergenital processes and integumental sclerotized

dots. One of the members of this group is *H. nipponicus* Okada, 1932, confirmed by Lützen (1964) as lacking these characters; it should be placed in Group III (Suárez-Morales and Salazar-Vallejo, 2022).

The absence of intergenital processes and sclerotized dots in *H. paulayi* sp. nov. allows its inclusion in Group III, which, according to Suárez-Morales and Salazar-Vallejo (2022), includes several other species: *H. cluthensis* Boxshall, O'Reilly, Sikorski and Summerfield, 2019, *H. haddoni* Lützen, 1964, *H. luetzeni* López-González and Bresciani, 2001, *H. nipponicus* Lützen, 1964, *H. polarsterni* López-González, Bresciani and Conradi, 2000, and *H. stocki* López-González, Bresciani and Conradi, 2000.

Overall, *H. paulayi* n. sp. differs from the other members of *Herpyllobius* Group III (sensu Lützen and Jones, 1976; López-González et al., 2000) by possessing a unique combination of characters: 1) an spherical ectosoma with pilose surface; 2) elongate, thick cylindrical egg sacs with +10 egg rows; 3) endosoma short, with two lateral asymmetrical flattened processes and a medial lobe; 4) ectosoma with ornamented integumental shield; and 5) it infects a non-polynoid polychaete, the subtropical flabelligerid *L. horsti*.

*Herpyllobius paulayi* sp. nov. differs from *H. nipponicus* in having a roughly spherical ectosoma (versus asymmetrically globose in *H. nipponicus*) and cylindrical, straight, ovisacs, with 10–12 longitudinal egg rows (versus clearly thicker and shorter, curved, with 6–7 egg rows in *H. nipponicus*), as well as in having an endosoma with many blunt processes (Lützen, 1964, fig. 22) versus a tripartite one in *H. paulayi*. Also, *H. paulayi* was found living parasitically on the head, adjacent to the eyes, instead of on the ventral surface of the polynoid *Nonparahalosydna pleiolepis* (von Marenzeller, 1879) in *H. nipponicus*.

The new species differs from *H. cluthensis* in the shape of the ectosoma (roughly ovoid in *H. cluthensis* versus nearly spherical in the new species), the position of the genital swellings (posteroventral in *H. cluthensis* versus anterodorsal in *H. paulayi*) and in the endosomal morphology, it is remarkably elongate, slender in *H. cluthensis* (Boxshall et al., 2019: fig. 5A, B) (versus short, with three lobes in *H. paulayi* sp. nov.). In *H. haddoni*, the ectosoma is distinctly flat, arrowhead-like; the endosoma is lump-shaped,

lacking diverticulae, thus diverging from the spherical ectosoma and tripartite endosoma of the new species. In H. luetzeni, the ectosoma is triangular, with two divergent anterior humps, the genital swellings are prominent and heavily sclerotized, with a reduced intergenital space between them; the endosoma is bag-like, massive (López-González and Bresciani, 2001: fig. 2D, E), clearly differing from the ectosoma and endosoma of *H. paulayi* sp. nov. In *H. polarsterni*, the ectosoma is roughly pyriform, with strongly prominent genital swellings (López-González et al., 2000: fig. 4A-C) (versus poorly developed genital swellings in *H. paulayi* sp. nov.) and the endosoma is massive, lump-shaped, with a wrinkled surface (López-González et al., 2000: fig. 2D), versus poorly developed genital swellings and tripartite endosoma in the new species. Also, H. polarsterni is known to attach on the dorsal part of the host neuropodia (López-González et al., 2000: fig. 4A), whereas H. paulayi was observed on the head. The Antarctic H. stocki shares with H. polarsterni a pyriform ectosoma and strongly developed genital swellings (López-González et al., 2000: fig. 2F), but it has a medial intergenital protuberance and lacks sclerotized dots, thus diverging from the spherical ectosoma with poorly developed genital swellings and absent medial intergenital process observed in H. paulayi, which should be assigned to Herpyllobius species Group III (see Suárez-Morales and Salazar-Vallejo, 2022: table 1). We consider that these differences are sufficient to propose the new species, H. paulayi.

# DISCUSSION

The attachment site of species of *Herpyllobius* on its host is variable; it includes the neuropodium, between parapodia, the body wall, or between elytra (Lützen, 1964; López-González et al., 2000; López-González and Bresciani, 2001; Suárez-Morales and Salazar-Vallejo, 2022). According to Lützen (1964, 1967) the site of attachment may be an additional character to recognize species of this genus, as it relates to parasite biology; and the endosoma appears to show variable development according to the internal available space on the host at the attachment site, thus becoming valuable information in separating morphospecies of *Herpyllobius*. The species attached to the

prostomial area tend to have a short, flat endosoma, like that observed in H. paulayi, whereas the species with an interparapodial attachment like H. haddoni and H. australis have a relatively longer, lump-shaped endosoma lacking diverticulae (Lützen, 1964). In addition, the Antarctic H. vanhoeffeni is always found on the ventral surface of the parapodium, but H. polarsterni does not show any preference for particular sites along the host's main body axis (López-González et al., 2000; López-González and Bresciani, 2001). Specimens of H. paulayi sp. nov. were found attached to the prostomial area close to the eyes and, according to Lützen (1964) and López-González and Bresciani (2001), there are only two other species sharing this character: Herpyllobius antarcticus Vanhöffen, 1913, a member of Group I and Herpyllobius polynoes (Krøyer, 1863) (Group II) (see Suárez-Morales and Salazar-Vallejo, 2022: tab. 1). Herpyllobius paulayi is therefore the third species of the genus known to have a prostomial attachment site. More observations of this parasite will be necessary to confirm this preference in the new species.

Up to 11 of the 20 nominal species of the genus are known from the southern hemisphere (López-González and Bresciani, 2001), mostly in Antarctic areas. According to Lützen (1964), there are nine species of Herpyllobius reported from the northern hemisphere cold and Arctic waters. The author considered that the entire family was absent from tropical or subtropical latitudes, except for H. nipponicus from Southern Japan; but with the additions of H. piotrowskiae (Suárez-Morales and Salazar-Vallejo, 2022) from New Guinea and H. paulayi from an Australian subtropical reef system, the genus now has two subtropical species. The Ningaloo Marine Park in Western Australia encompasses the Ningaloo reef system, the type locality of H. paulayi. This fringing reef system straddles the Tropic of Capricorn and its hydrographic pattern produces a unique overlap of tropical and temperate biological organisms (van Keulen and Langdon, 2011).

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# ADDITIONAL INFORMATION AND DECLARATIONS

#### **Author Contributions**

Conceptualization and Design: ESM, SSV. Performed research: ESM, SSV. Acquisition of data: SSV. Analysis and intepretation of data: ESM. Preparation of figures/ tables/maps: ESM, SSV. Writing – original draft: ESM. Writing – critical review and editing: ESM, SSV.

## **Consent for publication**

Both authors declare that they have reviewed the content of the manuscript and gave their consent to submit the document.

## **Competing interests**

The authors declare no competing interest.

## Data availability

The holotype specimen is available for further examination by request to M.Sc. José Angel Cohuo Colli, collection of zooplankton at ECOSUR-Chetumal, Mexico (jose.cohuo@ ecosur.mx).

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#### Study association

All collection data are archived within the Florida Museum of Natural History, Gainesville

#### **Study permits**

The type host specimen of *Herpyllobius paulayi* belongs to the collection of Invertebrate Zoology of the University of Florida Natural History Museum and a catalog number is assigned to it (UF 1757). Permits data should be requested from the institution, if necessary.