

The mesoparasitic copepod *Trifur tortuosus* Wilson, 1917 (Pennellidae): Redescription with notes on its congeners

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Abstract

The genus *Trifur* Wilson, 1917 (Siphonostomatoida, Pennellidae) comprises relatively large and conspicuous parasitic copepods infecting the body surface and gills of several marine fishes. Variations in the gross morphology have been used to discriminate between the existing species of *Trifur*, however all previous descriptions of these species are brief, lacking many important characteristics, especially those of the head and trunk appendages, which usually were lost in the described specimens. In this paper, *T. tortuosus* is redescribed based on newly collected material from three species of fishes from Argentinean coasts. A detailed description of appendages morphology is given and the validity of *T. merluccii* Talice, 1936; *T. puntaniger* Thomé, 1963; *T. physiculi* Heegaard, 1962 and *T. lotellae* (Thomson, 1890) is herewith discussed.

Keywords

Copepoda, Pennellidae, Trifur tortuosus, fish, South America

Introduction

The genus Trifur Wilson, 1917 comprises relatively large and conspicuous parasitic copepods infecting the body surface and gills of several marine fishes. It was erected to accommodate T. tortuosus Wilson, 1917, a parasite of the body surface of Salilota australis (Günther, 1878) from the Smith Channel, Otter Bay Southern Chile (Wilson 1917). Later on, other species were described from the Southern Hemisphere: T. merluccii Talice, 1936, parasitizing "Merluccius gavi (Guichenot, 1848)", actually Merluccius hubbsi Marini, 1933 from Uruguay (Talice 1936); T. puntaniger Thomé, 1963 from an unknown host species caught in the coasts of Uruguay (Thomé 1963); T. chlorophthalmi Yamaguti, 1939 from Chlorophthalmus albatrossis Jordan et Starks, 1904 from Japan (Yamaguti 1963) and T. physiculi Heegaard, 1962 from the skin of "Physiculus barbatus (Günther, 1863)" = Pseudophysis barbata Günther, 1863 and "Physiculus sp." from Australia and Tasmania (Heegaard 1962).

Ringuelet (1947) synonymised *T. merluccii* with *T. tortu*osus, based on the fact that the only morphological characters used by Talice to create the new species were the relative similar size of the horns and the "absence of other appendages" in the head. This synonymy was later confirmed by Thomsen (1949). Later on, Yamaguti (1963) erected *Allotrifur* Yamaguti, 1963, characterized by having all pairs of legs uniramous and move *T. chlorophthalmi* to the new genus.

Finally, a species originally described as *Lernaea lotellae* by Thomson (1890) from the gills and abdominal wall of "*Lo-tella bachus* (Forster, 1801)" = *Pseudophysis bachus* (Forster, 1801) from the New Zealand waters, was cited as *Trifur lotellae* (Thomson, 1890) by Hewitt and Hine (1972). Therefore the genus is at present composed by five species.

After the generic description, numerous new host and locality records broadened the distribution of these species, most of them coming from the Atlantic coasts of South America. *Trifur tortuosus* was reported by Brian (1944) parasitizing hakes "*Merluccius* sp." and "*M. gayi*" from the coasts of Argentina (probably *M. hubbsi*, because *M. gayi* is distributed in the Pacific Ocean). Later Szidat (1955), Kovaleva and Gaevskaya (1975), Reimer and Jessen (1981), Bellisio *et al.* (1979), Rey and Cascudo (1986), Etchegoin and Sardella (1990), MacKenzie and Longshaw (1995), and Sardella and Timi (1996, 2004) reported this species from *M. hubbsi* in the south western Atlantic. *Trifur tortuosus* was also cited in other host species in this region, Boxshall and Montú (1997) reported this parasite from an unknown host from southern Brazil, whereas in Argentinean waters *T. tortuosus* was found parasitizing the body surface of *Merluccius australis* (Hutton, 1872) and the gills of *Percophis brasiliensis* Quoy et Gaimard, 1824 and *Pinguipes brasilianus* Cuvier, 1829 (cf. Mac-Kenzie and Longshaw 1995, Rohde *et al.* 1995, Braicovich and Timi 2008 and Timi *et al.* 2008). The presence of *T. tortuosus*, as well as of an unidentified species of *Trifur* was also reported in several host species in the Chilean coasts (Muñoz and Olmos 2007). On the other hand, *T. puntaniger* was also recorded in Chilean waters as a parasite of *M. australis* (cf. Fernández 1985, González and Carvajal 1994).

The gross morphology in representatives of the family Pennellidae has been used as the primary category of characters to differentiate among genera, with generic diagnoses based on postmetamorphosis females, which in turn are characterized by a high degree of variability and homoplasy (Boxshall 1986). Variations in the gross morphology (such as relative size, shape and orientation of cephalic holdfasts and morphology of cephalic appendages and legs) have been used to discriminate between the existing species of *Trifur*, however all previous descriptions of these species are brief, lacking many characteristics, especially those of the head and trunk appendages (Thomson 1890, Wilson 1917, Talice 1936, Heegaard 1962, Kovaleva and Gaevskaya 1975, Grabda 1991). A detailed description of appendages morphology and the analyses of variability of gross morphology can shed light on the species composition of the genus *Trifur*, especially taking into account that variations could be the result of the combined effect of different host species and microhabitats (body surface, bases of fins, gills) exploited by these copepods.

The presence of *Trifur* spp. in many commercially important fish species, their relevance in biogeographic studies of some of them (Szidat 1955, Kabata and Ho 1981) and their



Figs 1–9. *Trifur tortuosus* Wilson, 1917 from *Pseudopercis semifasciata*. Post-metamorphosis female. 1. Habitus, ventral view. 2. Habitus, dorsal view. 3. Anterior end, showing position of legs 1 to 4. 4. First antenna, ventral. 5. Second antenna, ventral. 6. Buccal stylets, ventral. 7. Mandible, ventral. 8. First maxilla, ventral. 9. Second maxilla, lateral. Scale bars = 5 mm (1 and 2), 0.5 mm (3), 0.05 (4 to 9)

potential value as biological tags for the discrimination of fish populations (Timi *et al.* 2008) makes also necessary to review the species composition of this imprecisely diagnosed genus.

During a parasitological survey carried out on the Argentinean sandperch, *Pseudopercis semifasciata* (Cuvier, 1829) from Argentinean waters, numerous specimens of *T. tortuosus* were found in the gills of fishes, providing new material for morphological analyses and allowing to describe in detail the morphology of cephalic and trunk appendages.

Materials and methods

A total of 100 specimens of the Argentinean sandperch, Pseudopercis semifasciata (Cuvier, 1829) from Argentinean waters were examined for parasitic copepods, the body surface and gills of each fish were examined. Additional specimens from previous studies on Argentine hake, M. hubbsi (cf. Sardella and Timi 2004), Brazilian sandperch, P. brasilianus (cf. Timi et al. 2008) were also studied. Parasites were recovered, fixed in 4% formalin, preserved in 70% ethanol; the appendages were dissected with lactic acid and studied under a light microscope. Drawings were made with a drawing tube. Some specimens were processed for scanning electron microscopy (SEM), specimens were dehydrated using a series of ethanol washes, dried by evaporation with hexamethyldisilazane, coated with gold palladium, and scanned in a JEOL JSM 6460-LV SEM (JEOL, Tokyo, Japan). Measurements are given in millimetres, with the mean followed by a range in parentheses. The studied material was deposited in the Carcinological Collection of the Museo de La Plata, La Plata, Argentina (Collection Nos. 26150-26152).

Issues of fish taxonomy were resolved based on Froese and Pauly (2008).

Results

Trifur tortuosus Wilson, 1917 (Figs 1-17)

Redescription (post-metamorphosis female): Body divided into holdfast-bearing head, long and cylindrical neck and sigmoid trunk (genital complex + abdomen). Total length (from tip to head to tip of trunk): 23.6 (20.79–27.7). Head nearly at right angle to neck, with a cylindrical anterior proboscis 1.71 (1.34–1.95) long, 1.4 (1.22–1.83) wide, and 3 cylindrical to conical posterior horns, one dorsal 3.69 (3.37-4.4) long and two lateral, right 3.6 (2.95-4.07) long, left 3.28 (2.67-4.07) long. Neck moderately thick, 7.87 (6.15-10.5) long, 0.97 (0.69–1.3) wide, longer than trunk, and smooth. Trunk swollen, bent into a sigmoid curve at right angles to neck; genital complex 7.64 (6.1–9.27) long, provided with pair of short processes over bases of egg strings on its convex ventral side. Abdomen club-shaped, unsegmented and devoid of processes, approximating half diameter of and shorter than genital complex, 5.09 (3.6-6.18) long; its long axis always at near



Figs 10–13. *Trifur tortuosus* Wilson, 1917 from *Pseudopercis semi-fasciata*. Post-metamorphosis female. **10.** First leg, ventral. **11.** Second leg, ventral. **12.** Third leg, ventral. **13.** Fourth leg, ventral. Scale bars = 0.1 mm

right angle to that of genital complex. Egg strings coiled into tight spirals, 6.81 (5.2–8.14) long. Eggs discoidal and uniseriate.

First antenna not distinctly segmented; divided into two segments by a shallow constriction. Basal segment cylindrical and carrying 22 naked setae of variable length, distributed mainly on its anterior margin. Terminal segment with rounded tip; apical armature consisting of one long, robust seta, 8 similarly long setae (two pairs of them fused at base); and 4 shorter setae. Second antenna three-segmented; basal segment stout, second segment with tooth-like process in inner distal corner; terminal segment as an unciform claw, with short seta at base. Tip of terminal segment fitting into cavity in dentiform process of second segment. Mouth tube at apex of proboscis, with three telescoping rings and fringed with a marginal membrane. Paired buccal stylets with blunt tips surmounted by two setiform processes. Mandible two-segmented; proximal segment cylindrical, shorter than distal; latter flat with 8 fairly uniform teeth. First maxilla short, with two setae on large lobe and one on small lobe. Second maxilla: lacertus robust with two large unciform processes; brachium with rows of spiniform setules; distal segment as a hook-like process (calamus) provided with parallel longitudinal rows of setules. Four pairs of swimming legs close together ventrally on the anterior region of neck, pairs 1-2 biramous, 3-4 uniramous. First to third with slender seta at base of exopod. Legs armature (all spines and setae naked) as follows:



Figs 14–17. *Trifur tortuosus* Wilson, 1917 from *Pseudopercis semifasciata*. Post-metamorphosis female. **14.** Tip of proboscis, ventral, showing buccal area. **15.** Buccal area, lateral. **16.** Mouth tube, ventro-lateral view. **17.** Tip of mandible, lateral. **Abbreviations:** Fm – first maxilla, Mb – mandible, Mt – mouth tube, R – rings of mouth tube, Sm – second maxilla. Scale bars = 0.1 mm (14), 0.02 mm (15), 0.05 (16), 0.002 mm (17)



Fig. 18. Variability in size and shape of the holdfast of *Trifur tortuosus* from different host species: a. *Merluccius hubbsi*; b-c. *Pinguipes brasilianus*; d-e. *Pseudopercis semifasciata*. Scale bars = 2 mm

	Exop	ood	Endopod		
	1	2	1	2	
Leg 1	0 - 1	I – 6	0 - 1	0 - 6	
Leg 2	0 - 1	I - 4, 2	0 - 1	1, 3, 2	
Leg 3	0 - 0	I – 5	_	_	
Leg 4	0 - 0	5, 1	_	_	

Measurements were variable between specimens from different host species, copepods from the body surface of hakes being larger (with longer neck, and trunk) than those from the gill arches of sandperches, which in turn were larger in *P. semifasciata* than in *P. brasilienis*. On the other hand, holdfasts were similar in size between specimens from *M. hubbsi* and *P. semifasciata*, but longer than in *P. brasiliensis* (Table I, Fig. 18).

Discussion

The original description of *T. tortuosus* by Wilson (1917) was based on only two ovigerous females, one of them lacking the head. For this reason, cephalic appendages and legs were not described in detail. Comparison of the presently described material with Wilson's description, revealed that the second antenna is three-segmented instead of two-segmented and leg 3 is uniramous rather than biramous.

In relation to the description of *T. tortuosus* provided by Brian (1944) the newly collected specimens differ in having the first antenna not distinctly segmented carrying 22 setae on its basal segment instead of three-segmented first antenna with 10 setae on its basal segment; apical armature of the first antenna consisting of one long, robust seta, and 12 additional setae rather than one robust seta and 6 additional setae (Brian 1944: Plate VI, fig. 52); and second antenna three-segmented instead of two-segmented. Brian (1944) and Thomsen (1949) also described leg 3 as being biramous. In fact, descriptions of *T. tortuosus* by both Brian (1944) and Thomsen (1949) were deeply influenced by Wilson's diagnosis and both authors based their descriptions on specimens lacking appendages as a consequence of the fragility of cephalic appendages and legs, which were surely damaged or lost by handling during the detachment or removal of host tissues surrounding the parasite's head.

Based on these previous descriptions of *T. tortuosus*, Thomé (1963) proposed a new species, *T. puntaniger*, from an unknown fish caught in the coasts of Uruguay. According to Thomé (1963), the morphological differences supporting the new species were: the second antenna three-segmented, legs 1 and 2 uniramous and legs 3 and 4 biramous. Examination of the presently described material revealed that these morphological characteristics are, in fact, shared with *T. tortuosus*. Locality of capture of the fish host also suggests that specimens studied by Thomé (1963) belong to *T. tortuosus*.

In 1962, Heegaard established T. physiculi to accommodate the copepods collected from the skin of "Physiculus sp." and "Physiculus barbatus" = Pseudophycis barbata. According to this author T. physiculi differs from T. tortuosus in having small differences in the shape of the horns and of the appendages (first antenna unsegmented, second antenna threesegmented and legs 1 trough 4 strongly reduced). Again, a new species was erected influenced by the incomplete description given by Wilson (1917). The variability in shape and size of horns and trunk observed by Heegaard (1962) was probably due to the space constrains imposed by the different microhabitats to which parasites attach (body surface, fins or branchial cavities). Indeed, according to Kabata (1979), in mesoparasitic copepods there is a broad range of morphological variability of holdfast due to the differential resistance exerted by host tissues, such as bones or hard connective tissue, which truncate or deviate the processes from their courses. Similar variations were observed in specimens from different host species and microhabitats in the present study, representing therefore intraspecific variations caused by the plasticity of holdfast in response to space constrains imposed by the host during the attachment process. With respect to the first and second antennae described by Heegaard (1962), the figures show that first antenna is divided into two segments by a shallow constriction and the second antenna is three-segmented (Heegaard 1962: figs 215 and 219) as was observed in T. tortuosus. Finally, the "strongly reduced legs" of T. physiculi were probably the result of a damage due to an inadequate handling of the parasites. Among the specimens collected from

Table 1. Comparative measurements (mean and range) of <i>Trifur tortuosus</i> witson, 1917 nom unce nost species in the Argenti	able L	I. (Comparative measurements ((mean and range) of 7	Frifur tortuosus Wils	son, 1917 fror	n three host s	pecies in the	Argentine	Se
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Host species	Pinguipes brasilianus	Pseudopercis semifasciata	Merluccius hubbsi
Length \pm SD of infected fish	35.9 ± 3.6 cm	71.6 ± 2.9 cm	$43.3 \pm 7.6 \text{ cm}$
Attachment site	gill arches	gill arches	body surface
Number of specimens measured	10	10	10
Total length	18.6 (12.2–24.1)	23.6 (20.8–27.7)	37.5 (31.6-46.3)
Proboscis length	1.4 (1.3–1.6)	1.7 (1.3–2.0)	3.9 (3.7–4.5)
Neck length	4.8 (4.9–10.7)	7.9 (6.3–10.5)	12.0 (7.5–15.9)
Genital complex length	4.4 (2.9–5.8)	7.6 (6.1–9.3)	13.8 (11.6–17.4)
Abdomen length	3.8 (2.9-4.9)	5.1 (3.6-6.2)	7.7 (6.4–9.3)
Dorsal horn length	2.2 (1.6–2.7)	3.7 (3.4–4.4)	3.7 (3.1-4.2)
Right horn length	2.3 (1.7-3.0)	3.6 (2.9-4.1)	2.9 (2.4–3.9)
Left horn length	2.1 (1.7–2.8)	3.3 (2.7–4.1)	3.0 (2.5–3.5)

M. hubbsi, *P. semifasciata*, and *P. brasilianus*, there were many copepods of apparently reduced legs; however, a detailed examination showed that the rami were actually lost during the dissection from the hosts tissues.

Finally, Thomson (1890) described *Lenaea lotellae* from specimens collected from *Lotella bachus*. The brief description of this species does not allow a comparison with the present description of *T. tortuosus*. Nevertheless, the original figures reflect morphological similarities with the genus *Trifur* (cf. Thomson 1980: Plate XXVIII, figs. 3 and 3a). Indeed, Hewitt and Hine (1972) refer to this species as *Trifur lotellae*. On the other hand, the hosts of both *T. physiculi (Pseudophycis barbata)* and *T. lotellae (Pseudophycis bachus)* are in fact congeneric and sympatric in Australian and New Zealand waters (Froese and Pauly 2008) and often confused each other (Cheung *et al.* 2005), supporting the idea that both host species harbour a single species of *Trifur*.

Based on these morphological comparisons, *T. puntani*ger, *T. physiculi* and *T. lotellae* should be considered as junior synonyms of *T. tortuosus*. The presence of *T. tortuosus* in a considerable number of phylogenetically unrelated fish species in the same geographic area demonstrates that parasite is nonspecific, constituting further evidence supporting the proposed synonymies. On the other hand, the presence of a single species of *Trifur* in distant geographic regions, such as South America, Australia and New Zealand can be explained by the effect of the circumpolar West Wind Drift (=Antarctic Circumpolar Current), which is known to be responsible for similar distributions of other species of parasitic copepods (Kabata and Ho 1981, Jones 1988).

With reference to Allotrifur, the diagnostic morphological characteristic put forward by its author (Yamaguti 1963) was the presence of four pairs of uniramous legs instead of two pairs of biramous legs and two pairs of uniramous legs as in Trifur. Nevertheless, the fourth pair was actually not observed by Yamaguti. In fact, Kabata (1979) characterized Allotrifur "apparently by the possession of only three pairs of legs", although the same author characterized the family Pennellidae as possessing four or five pairs of swimming legs. As stated earlier, legs are easily lost or damaged during handling of specimens; for this reason, examination of new material from type host and type locality is necessary to confirm the number of either pairs of legs and rami in each of them in order to assess the validity of this genus. Furthermore, the diameter of the abdomen relative to the genital complex and its presence in the Northern Hemisphere (Yamaguti 1963) precludes its identification as T. tortuosus.

As postulated by Kabata (1979) the observed variability in size of the holdfast and the trunk among specimens of *T. tor-tuosus* from the three host species should be a consequence of space restrictions imposed by the different microhabitats to which parasites attach. Larger specimens from *M. hubbsi* were attached to the body surface, with no space constrains conditioning the growth of the protruding trunk, while those parasites of sandperches, inhabiting the gill cavities, suffer such limitations. The differences in body size between the two pin-

guipedid species can account for the observed differences in the size of copepods. Therefore the place of attachment and the host identity can be considered as key factors determining the broad range of morphological and morphometric intraspecific variability in this species.

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