



Trifur tortuosus in whole Argentine hake

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ABSTRACT

This case report presents the basic parameters used in the identification of the species *Trifur tortuosus*, a parasitic copepod of the genus *Trifur*, family Pennellidae, and relevant literature data on this little-known parasite of marine fish. The parasite was identified in whole Argentine hake, with the fish delivered to our laboratory during the official import control of foodstuffs in 2024. *Trifur* is a relatively large ectoparasite and as such is easy to see, but this case report is the first time *Trifur* were detected in marine fish that were examined in our laboratory. Several species of marine fish in the Atlantic-Pacific region of South America are natural hosts of this parasite. As the import of fish and fish products is increasingly intensive, and *Trifur* spp. could become more frequent in many commercially important fish species, we believe that the presentation of the case can be useful in the practical work of laboratories that conduct examinations of animal origin foods in our region.

1. Introduction

The Food Safety Law in Serbia requires all food business operators, including importers, to ensure that the food they place on the market is safe for human consumption. Local regulations on animal health conditions, on the general and special conditions of hygiene of food of animal origin and on the hygiene of animal slaughter and trade (*Official Gazette RS*, 2011; *Official Gazette RS*, 2014 *Official Gazette RS*, 2023) concern fish and fish products. For these products, one of the requirements is the absence of visible parasites. Epidemiologically, the most important fish parasites are helminths from the group of Nematoda, including *Anisakis* spp. and *Eustrongylides excisus* (Ćirković *et al.*, 2013). In our

laboratory, the most commonly detected parasite in imported fish is the nematode *Anisakis*.

Over many years of conducting parasitological examinations of fish, parasites of the genus *Trifur*, or those with similar morphology, have not been detected in marine fish samples submitted to our laboratory. The damage caused by *Trifur* spp. is primarily aesthetic in nature, as the parasites leave unsightly scars deep within the flesh of affected fish. *Trifur tortuosus* parasitizes marine fish in the Atlantic-Pacific region of South America (Oliva & González, 2004; Timi *et al.*, 2008; Etchegoin *et al.*, 2009). The presence of this species in a considerable number of phylogenetically unrelated fish species in the same geographic area suggests that *Trifur tortuosus* is a nonspecific parasite (Etchegoin *et al.*, 2009).

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2. Materials and methods

Whole Argentine hake (*Merluccius hubbsi*), each weighing between 200 and 350 g, were delivered to the Food Safety Laboratory of the Novi Sad Scientific Veterinary Institute as part of the official inspection of imported fish during summer of 2024. According to the current Food Safety Law, as part of the control of animal origin food during import for safety, both sensory and parasitological testing must be performed on fish samples to ensure safety.

The fish were first identified, which included documenting the quantity, shape of the shipment, and label information. Following identification, parasitological examination was conducted through an external inspection, followed by an internal examination involving dissection of the body, including the abdominal region, digestive tract and other ventral organs.

The collected parasites were fixed in 70% ethanol and dissected using lactic acid for morphological observation.

3. Results

Ectoparasites were observed in the skin of dorsal part of Argentine hake (Figure 1A).

After being fixed in 70% ethanol and dissected with lactic acid, the following body segments were observed in the parasite: a holdfast-bearing head with three horns, a long, thick and cylindrical neck and a sigmoid trunk which includes the abdomen and genital complex. The ventral view is shown in the Figure 1B.

The measured dimensions of the parasite's body segments are presented in Table 1. Using the identification keys by Wilson (1917), Yamaguti (1963) and Etchegoin et al. (2009), the parasite was identified as the species *Trifur tortuosus*.



Figure 1. *Trifur tortuosus* isolated from Argentine hake (*Merluccius hubbsi*); A – Copepod in the skin of the dorsal part of Argentine hake; B – Ventral view of *Trifur tortuosus* showing body shape and segments

Table 1. Body measurements of *Trifur tortuosus* collected from Argentinian hake.

Part of the parasite body	Length (mm)
Total length	35.4
Proboscis length	3.7
Neck length	13
Genital complex length	10.4
Abdomen length	8.3
Dorsal horn length	3.6
Right horn length	2.8
Left horn length	2.7

4. Discussion

Parasitic copepods in the family *Lernaeidae* are known for their high morphological diversity and wide distribution among both marine and freshwater fish species. The classification within this group has evolved considerably over time as knowledge about their biology improved. In 1917, in the collection of the United States National Museum, Charles B. Wilson performed the thirteenth revision of the *Lernaeidae* family, pointing out the wrongly interpreted genera of that oldest family of parasitic copepods, due to incomplete understanding of their life cycles (Wilson, 1917). He noted that some of these copepods show highly unusual and distinctive forms. That author also recommended a method for examining their internal anatomy by thoroughly dehydrating specimens in alcohol and clearing them in clove oil, which provides clear visualization without the need for sectioning. Based on a careful study of external and internal morphological characteristics and their life history, Wilson proposed reorganizing the family *Lernaeidae* into 17 genera—introducing three new ones, including *Trifur* and described 80 species, of which fifteen were new to science. Among these new species was *Trifur tortuosus*, originally described from two females (one missing the head), found on the body of Patagonian cod (*Salilota australis*) from near South America's southern tip (Wilson, 1917). More recently, Etchegoin *et al.* (2009) revisited the species description and confirmed its taxonomic status based on modern morphological methods.

Regarding classification, different data can be found in the literature. World taxonomic databases, the World Register of Marine Species (WoRMS), the

Global Biodiversity Information Facility (GBIF), the Interim Register of Marine and Nonmarine Genera (IRMNG), and the Ocean Biodiversity Information System (OBIS), currently place *Trifur tortuosus* in the family *Pennellidae*. Classification according to WoRMS is presented in the Table 2.

The family *Pennellidae* contains copepod parasites commonly found in a wide range of marine fish species. Members of this family are typically distinguished based on the shape and proportions of the cephalothorax, neck, trunk and abdomen (Castro-romero *et al.*, 2016). However, due to their pronounced morphological plasticity and considerable intra-species variability, identifying species solely on the basis of morphology remains highly challenging and often controversial.

The genus *Trifur* Wilson, 1917 includes relatively large and conspicuous parasitic copepods that infect the body surface and gills of several marine fishes. Several species have been described so far, such as *T. tortuosus* (1878, 1917), *T. merlucii* (1936), *T. puntaniger* (1963), *T. chlorophthalmi* Yamaguti (1939), *T. physiculi* Heegaard (1962) and *T. lotellae* (1890). After several changes in systematization, the genus now includes five species: *T. tortuosus*, *T. merlucii*, *T. puntaniger*, *T. physiculi* and *T. lotellae* (Etchegoin *et al.*, 2009).

T. tortuosus has been reported the body surface of several marine fish species, including *Salilota australis*, *Merluccius gayi*, *M. hubbsi*, *M. australis*, *Percoptis brasiliensis*, *Pinguipes brasilianus* and several host species inhabiting the Chilean coasts. The main characteristics of *T. tortuosus* according to Wilson (1917) are: chitinized cephalothorax with small

Table 2. Classification of *Trifur tortuosus* according to the World Register of Marine Species (WoRMS, 2008)

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Crustacea
Superclass	Multicrustacea
Class	Copepoda
Infraclass	Neocoopoda
Superorder	Podoplea
Order	Siphonostomatoidea
Family	<i>Pennellidae</i>
Genus	<i>Trifur</i>
Species	<i>Trifur tortuosus</i>

lateral horns and a long, slightly curved posterior horn; curved neck, twice as long as the trunk, abdomen bent into a semicircle, color a brownish yellow, more of a brown on the head and neck, more of a yellow on the trunk; egg strings cinnamon-brown. The original description of *T. tortuosus* made by Wilson (1917) was based on only two ovigerous females—one of females lacking the head. For this reason, cephalic appendages and legs were not described in detail. By comparing the original description with the description given by Etchegoin et al. (2009), it was determined that the second antenna is three-segmented (not two-segmented) and leg 3 is uniramous (not biramous). Based on specimens collected from three fish species from the Argentine coast, the description of the species *Trifur tortuosus* was refined by Etchegoin et al. (2009). The refined description is presented in Table 2.

Variations in the shape and size of the horns and trunk in *T. tortuosus* are likely influenced by differences in host species and the specific microhabitats they inhabit, such as the fish body surface, fins or branchial cavities. The resistance provided by host tissue (bone or connective tissue) influences the variability of the parasite's shape, by truncating or

deviating the process of its development (Etchegoin et al., 2009). In most cases, only one parasite colonizes a single host. The resulting tissue damage is usually visible as deep scars on the skin, and in cases where the parasite dies *in situ*, abscesses measuring 2 cm or more long can develop (Garcia-Sepulveda, 2017).

In Germany, the sale of fish fillet batches with >5% parasite infestation is prohibited, and these fish are used for the production of animal feed and pet food (Garcia-Sepulveda, 2017). The fact that *T. tortuosus* has been found in a wide range of phylogenetically unrelated fish species suggests that it is not host-specific. Moreover, species from the genus *Trifur* have been recorded in geographically distant regions, including Australia and New Zealand (Etchegoin et al., 2009).

Due to its pronounced morphological variability, traditional identification methods based on morphology alone are insufficient. Therefore, modern approaches such as molecular techniques, including DNA sequencing (Muñoz et al., 2015) and DNA barcoding are increasingly used to achieve accurate species identification (Castro-Romero et al., 2016).

Tabel 2. Refined description of *Trifur tortuosus* (Etchegoin et al., 2009) removed from *Merluccius hubbsi* in the Argentine Sea

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)	37.5 (31.6 - 46.3) mm
Head	Nearly at right angle to neck, with a cylindrical anterior proboscis
Head length	1.71 (1.34 - 1.95) mm
Head width	1.4 (1.22 - 1.83) mm
Proboscis length	3.9 (3.7 - 4.5) mm
Horns	Three cylindrical to conical posterior horns
Dorsal horn length	3.7 (3.1 - 4.2) mm
Right horn length	2.9 (2.4 - 3.9) mm
Left horn length	3.0 (2.5 - 3.5) mm
Neck	Moderately thick, longer than trunk, and smooth.
Neck length	12.0 (7.5 - 15.9) mm
Neck width	0.97 (0.69 - 1.3) mm
Trunk	Swollen, bent into a sigmoid curve at right angles to neck
Genital complex length	13.8 (11.6 - 17.4) mm
Abdomen length	7.7 (6.4 - 9.3) mm

5. Conclusion

Considering the increased volume of imported fish that is a potential host of this type of parasite, this case report is significant as a useful source of information for the identification of the *Trifur* parasites. The presence of such ectoparasites caus-

es consumer disgust and affects sensory properties, although the parasites themselves do not pose a risk to the health of consumers. This case report summarizes important data for *Trifur* genus identification and the practical work of food safety laboratories in the region.

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