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### Title

First record for the Palearctic region of a rare rotifer from the *Ptygura elsteri* group (Rotifera: Monogononta: Flosculariaceae: Flosculariidae) with description and biogeography of a new species: *Ptygura innominata* n. sp.

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First record for the Palearctic region of a rare rotifer from the *Ptygura elsteri* group (Rotifera: Monogononta: Flosculariaceae: Flosculariidae) with description and biogeography of a new species:  
*Ptygura innominata* n. sp.

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## SUMMARY

Here, I describe a new rotifer species within the *Ptygura elsteri* group collected from Mallorca, Spain (Palearctic). Previously reported from the Nearctic, this form possesses anatomical and ecological characteristics that indicate it to be a separate species. While other morphotypes assigned to the *P. elsteri* group have cervical hooks, the hooks on this morphotype are clearly different. In this report, I discuss previously published observations of this form, describe its anatomical and ecological details, and discuss its taxonomic position within the genus *Ptygura*. The name for this new species is *Ptygura innominata* n. sp.

## INTRODUCTION

Sessile rotifers (superorder Gnesiotrocha) are frequently overlooked in the most common studies of rotifers because aquatic plants and other surfaces are not examined (Wallace et al. 2006). For this reason, Meksuwan et al. (2011) posited that their diversity and biogeography are underestimated. My finding of a stable population of a sessile rotifer, genus *Ptygura* in a pond on Mallorca (Spain), initially shared

through the iNaturalist<sup>1</sup> internet portal as *Ptygura furcillata* (Kellicott, 1889) and posteriorly identified as *Ptygura elsteri* Koste, 1972, allowed the description of the anatomical and ecological details of this rare form, clarification of its taxonomic position within the *P. elsteri* group, and its proposition as a new species.

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<sup>1</sup> <https://www.inaturalist.org/observations/37953610>, accessed November 16, 2021.

*Ptygura elsteri* belongs to a group of species all of which possess cervical hooks. This group also includes *Ptygura melicerta* Ehrenberg, 1832, *Ptygura mucicola* (Kellicott, 1888), *Ptygura furcillata*, *Ptygura kostei* José de Paggi, 1996, and *Ptygura ctenoida* Koste & Tobias, 1990. The structure of the cervical hooks in *P. elsteri*, *P. furcillata*, and *P. kostei* is very characteristic, being described as antler-like.

*Ptygura elsteri* was found in the Amazon River by Koste (1972) describing it as a new species with cervical hooks, separating it from *P. furcillata*. In this work he also compares the specimens observed by him with other previous reports of a certain “unnamed” *Ptygura* (Kellicott 1889, Jennings 1889, Wulfert 1966), also included with that name in the key of the genus *Ptygura* of Edmondson (1949).

Koste (1972) considers that the “unnamed” *Ptygura* morphotype is closely related to *P. elsteri* but shows significant differences in the morphology of the cervical hooks.

The morphotype that I found corresponds to the descriptions of that “unnamed” *Ptygura*.

The “unnamed” morphotype was first found by Stokes (1881), describing the cervical hooks as a “cervical process which is two parted, each division furcate and having a short supplemental spur projecting obliquely inward, upward and forward, the whole slightly curved toward the ventral aspect”.

Before its assignment to *P. elsteri* in 1972, it was reported at least four times, all in the Nearctic region (Stokes 1881, Kellicott 1889, Jennings 1894, Wulfert 1966).

Later, Shoemaker and Williams (1986) also found the “unnamed” morphotype in Texas, discussing the taxonomic situation of this rotifer and provided an interesting personal communication from W.T. Edmondson in which, after observing photographs of the

specimens, considered it likely to be a species other than *P. elsteri*. However, the authors proposed that until there was more evidence it should be kept assigned to *P. elsteri*.

Another possible observation was reported by Koste and Böttger (1989) from See San Pablo (Ecuador), a water body (~2700 m, msl) with abundant aquatic vegetation, pH 8.5, and temperature 20 °C. They identified the species as *P. elsteri*, but I infer from both their drawing and brief description that their specimen should be assigned to the “unnamed species”.

To my knowledge, no other reports of the “unnamed” *Ptygura* morphotype have been published.

Other observations initially assigned to *P. elsteri* in the Afrotropical (De Smet 1989, Segers et al. 1993) and Oriental (Koste 1975) regions were subsequently assigned to the new species *P. kostei* by José de Paggi (1996) from a specimen originating in subtropical Argentina. Later, *P. kostei* has also been registered in India (Arora and Mehra 2003) and Thailand (Meksuwan et al. 2011). Although this last observation was initially identified as *P. elsteri*, a personal communication with Phuripong Meksuwan, Phuket Rajabhat University, indicated that it was *P. kostei*.

Based on specimens collected from a small pond on Mallorca (Spain), I re-describe the “unnamed” *Ptygura* as *Ptygura innominata* n. sp.

## MATERIALS AND METHODS

The specimens were found in an old irrigation pond, currently out of use, located in an area known as *S'Estret* next to the *Torrent de Valldemossa*, on the island of Mallorca (Spain) (WGS84 reference system: 39° 41' 27.504" N, 2° 38' 10.320" E; 297 m asl) (Fig. 1).

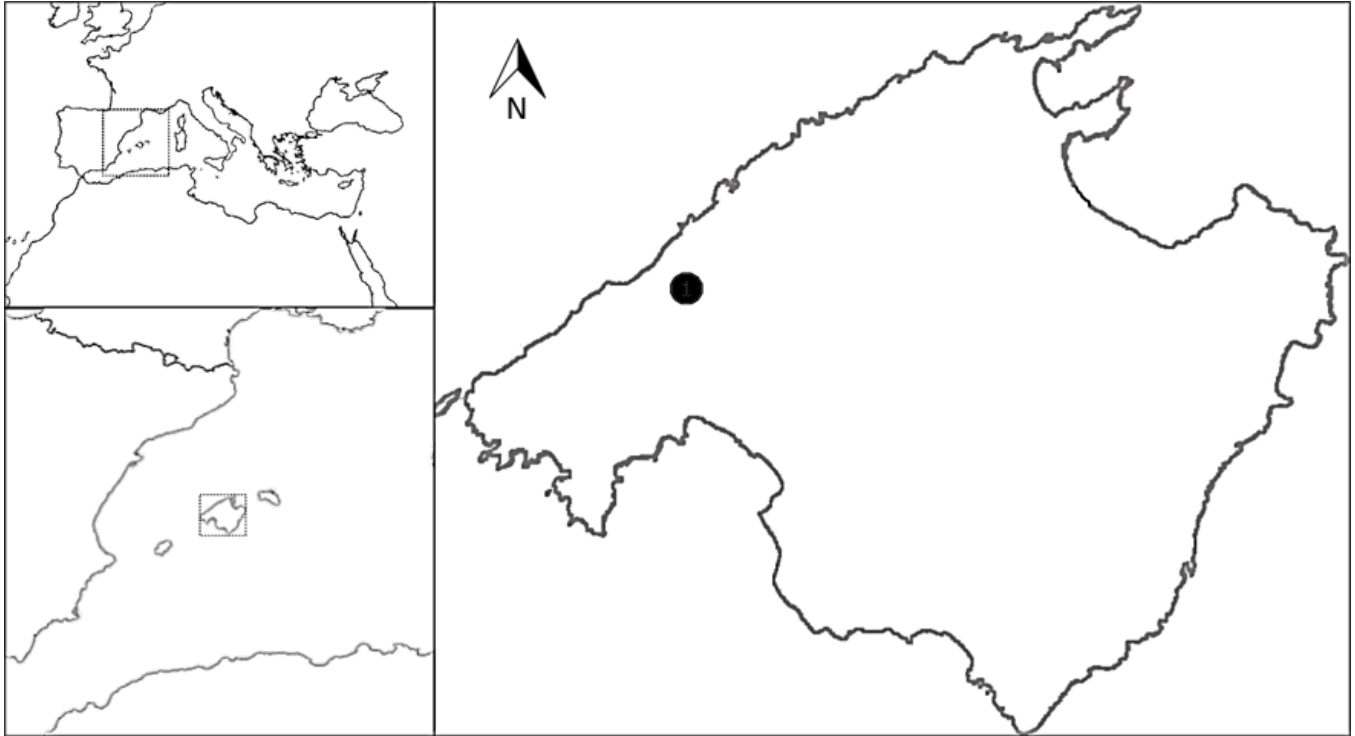


Figure 1. Location of the sampling site (black dot) on Mallorca Island (Spain).

I took two samples, the first in November 2019 when the pond had been permanently covered for several years by *Lemna minor* Linnaeus, a tiny vascular plant, commonly known as duckweed, on which sessile rotifers will attach (Wallace and Edmondson 1986). However, by the date of the second sample in October 2021, the duckweed layer had been removed by human action and two geese and some turtles and fish had been added, although it remains unused for irrigation (Fig. 2).

I kept the first sample of the pond's water with *L. minor* in a local laboratory in a glass crystallizer ca. 20 cm in diameter and 6 cm deep. This vessel was exposed to indirect sun light, and with *L. minor* covering the entire surface; evaporative loss was periodically replenished with tap water.

Two months after the first sampling, I made my initial observation of these rotifers. The temperature range on the observation dates was between a minimum of 10 and 22 °C. The

pH of the water was around 8 (Pampeha indicator pH paper; precision 0.5). Preparations were observed with an Olympus monocular microscope model (306654) equipped with a Hayear camcorder (1080P). Observations were video recorded, and images selected to extract the drawings using software built into Microsoft Office applications. In this sample, a total of 10 specimens were observed on four different occasions. On January 25, 2020, I observed two specimens sheltered between the chitinous trabeculae of a decaying insect head (Fig. 3a), probably the midge *Glyptotendipes barbipes* Staeger, 1839; on February 7, four specimens were observed all within the head of a larva; on February 9, three specimens were located within a flocculus mass; finally, on February 16, another specimen was seen in a flocculus mass. In these last two observations, I cannot rule out that among the organic material there were insect fragments. Observations ceased when the ostracods' population became very numerous.



Figure 2. Sampling pond covered with *Lemna minor* at the time of first sampling and devoid of it in the second sampling.

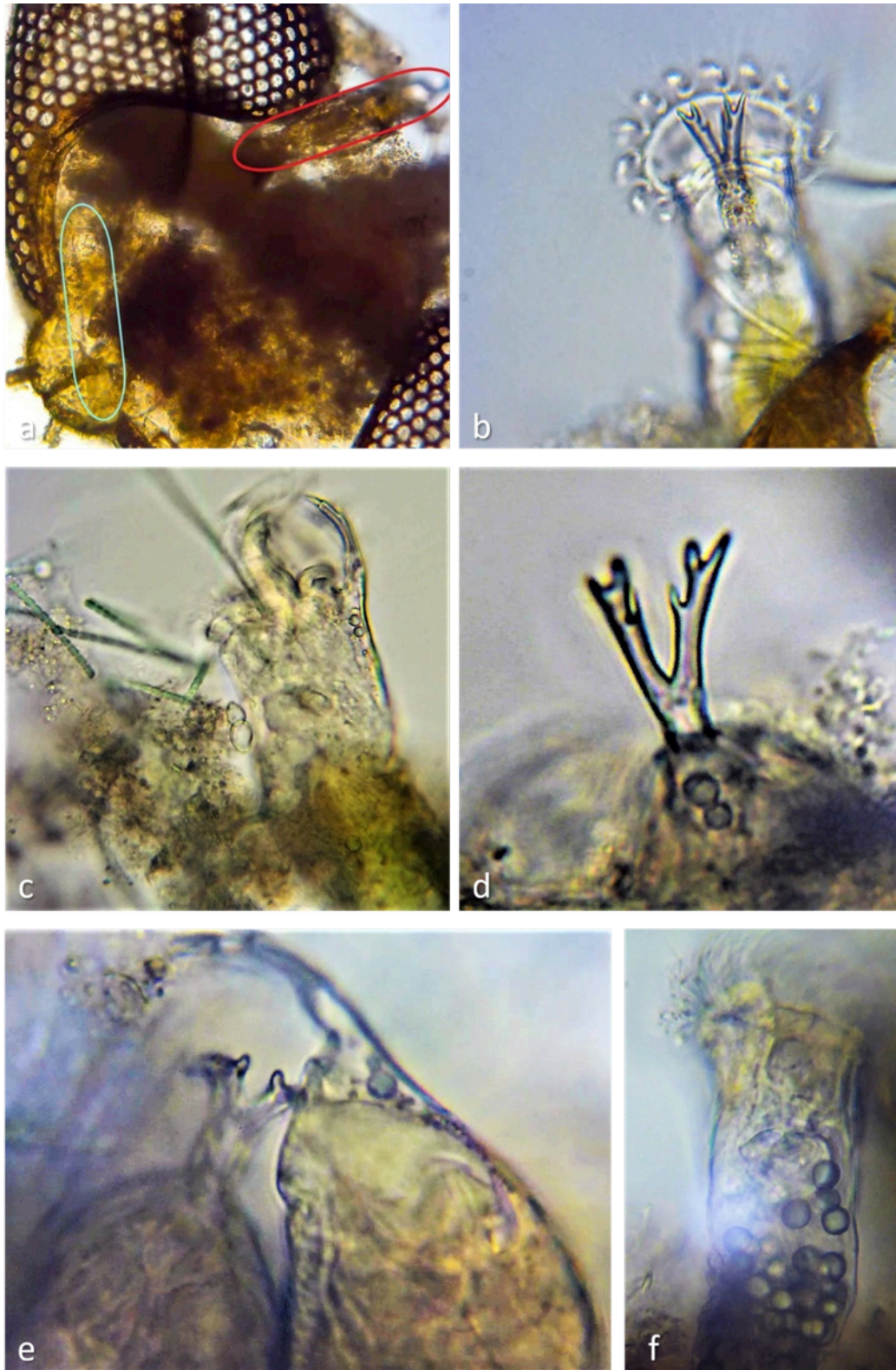


Figure 3. *Ptygura innominata* n. sp. in vivo. a) Paratype 1 (red oval) and another specimen (blue oval) in the decaying head of an insect. b) Paratype 1, dorsal view, corona open, c) Paratype 2, lateral view, corona open, feeding cyanobacteria trichomes. d) Paratype 5, cervical hooks, closeup, e) Paratypes 3 and 4 contracted in ventral and lateral view, f) Paratype 5 showing oil droplets as spherical bodies filling the trunk, ventro-lateral view.

All specimens were deeply anchored in fragments of insect heads or in masses of organic matter, so I did not achieve complete isolation in any case. One specimen was macerated with 4% NaOH, trying to obtain clear images of the trophi and dorsal plate as they only become visible with that treatment (Segers and Shiel 2008), but unfortunately the presence of organic matter in the preparation prevented obtaining images with the 100x immersion objective. With this sample, I was also unable to prepare a specimen on permanent slides as a holotype.

The second water sample, without *Lemna*, was taken by scraping the inner walls of the pond to a depth of about 20 cm. Water variables measured with a portable multifunction AQOTER RT-PH meter were: temperature 21,5 °C, pH 7,06, and salinity 548 ppt. For transport to the usual laboratory on the continent (Murcia, Spain), it was partially air-dried, and the wet sediment was kept in covered plastic dishes to avoid total desiccation. Forty-eight hours after sampling and 24 hours after partial desiccation, the sample was placed in a glass crystallizer and deionized water was added. This vessel was also exposed to indirect sun light; evaporative loss was periodically replenished with deionized water.

A few days after the second sampling, I observed only two specimens. On October 21, one specimen accidentally detached from its substrate, and on October 22, another specimen was found fixed within a fold of the chitinous exoskeleton of an indeterminate insect, together with a specimen of *Ptygura ctenoida*. In this sample, I also found *Ptygura stygis* (Gosse, 1886) and *Ptygura longicornis* (Davis, 1867), the latter almost always inside shells of decomposed Cladocera.

With that specimen, I made a permanent preparation as a holotype. I isolated it, fixed it with glutaraldehyde 2.5% at 80 °C (Örstan 2015), mounted it with glycerol 40% on a slide with a cavity, covered it with a circular cover slide and sealed it with DPX.

Preparations were observed with an Olympus microscope, model CX43, equipped with an Olympus camera EP50. Observations were video recorded, selecting the frames from which the images were obtained. Images were lightly processed with Adobe software.

I followed the systematics and nomenclature of José de Paggi et al. (2020).

## RESULTS

Based on the distinct morphology, ecology, and biogeographic pattern of the morphotype “unnamed *Ptygura*”, I propose this form as a new species separating it from *Ptygura elsteri*.

Phylum Rotifera Cuvier, 1817

Class Eurotatoria De Ridder, 1957

Subclass Monogononta Plate, 1889

Superorder Gnesiotrocha Kutikova, 1970

Order Flosculariacea Haring, 1913

Family Flosculariidae Ehrenberg, 1838

Genus *Ptygura* Ehrenberg, 1832

*Ptygura innominata*, n. sp.

LSID: urn:lsid:zoobank.org:act:A82B4AC9-C336-47E1-BF21-61D6A9137C2E

Synonyms:

*Ptygura* sp., after Stokes (1881)

*Oecistes melicerta* Ehrenberg, after Jennings (1900)

unnamed *Ptygura*, after Edmondson (1949)

Type series: One holotype in permanent preparation and images of six non-conserved paratypes from which descriptions have been made. Data and images of them are in the supplementary material section. In addition, another five specimens were identified.

Holotype: A specimen attached on a chitinous fragment of an indetermined insect, mounted on a permanent slide deposited in the

Museum of Zoology of the University of Navarra: MZNA-750471. It is an adult female in the lower range of size, collected on October 10, 2021, and observed and prepared on October 22, 2021.

Paratypes: Photographs of six adult female specimens designated as Paratypes 1 to 6.

Type locality: An irrigation pond, located at coordinates 39° 41' 27.504" N, 2° 38' 10.320" E (WGS84 reference system), in a place known as S'Estret, next to the Torrent of Valldemossa, island of Mallorca, Spain (Fig. 1 and 2).

Differential diagnosis: *Ptygura innominata* n. sp. is distinguished from other dorsal forked hooks-bearing *Ptygura* species by the morphology of the dorsal hooks. It has a forked hook with three spines in each branch, two terminals and one in the middle position directed inwards (Fig. 4, a). *Ptygura furcillata* has similar hooks, but it lacks spines (Fig 6, b). On the other hand, *P. elsteri* possesses two additional small hooks in the median position and branches of the largest hooks have spines in different places, one terminal, one intermediate and one basal (Fig. 4, c). *P. kostei* has four robust hooks that are similar in size and slightly incurved (Fig. 4, d).

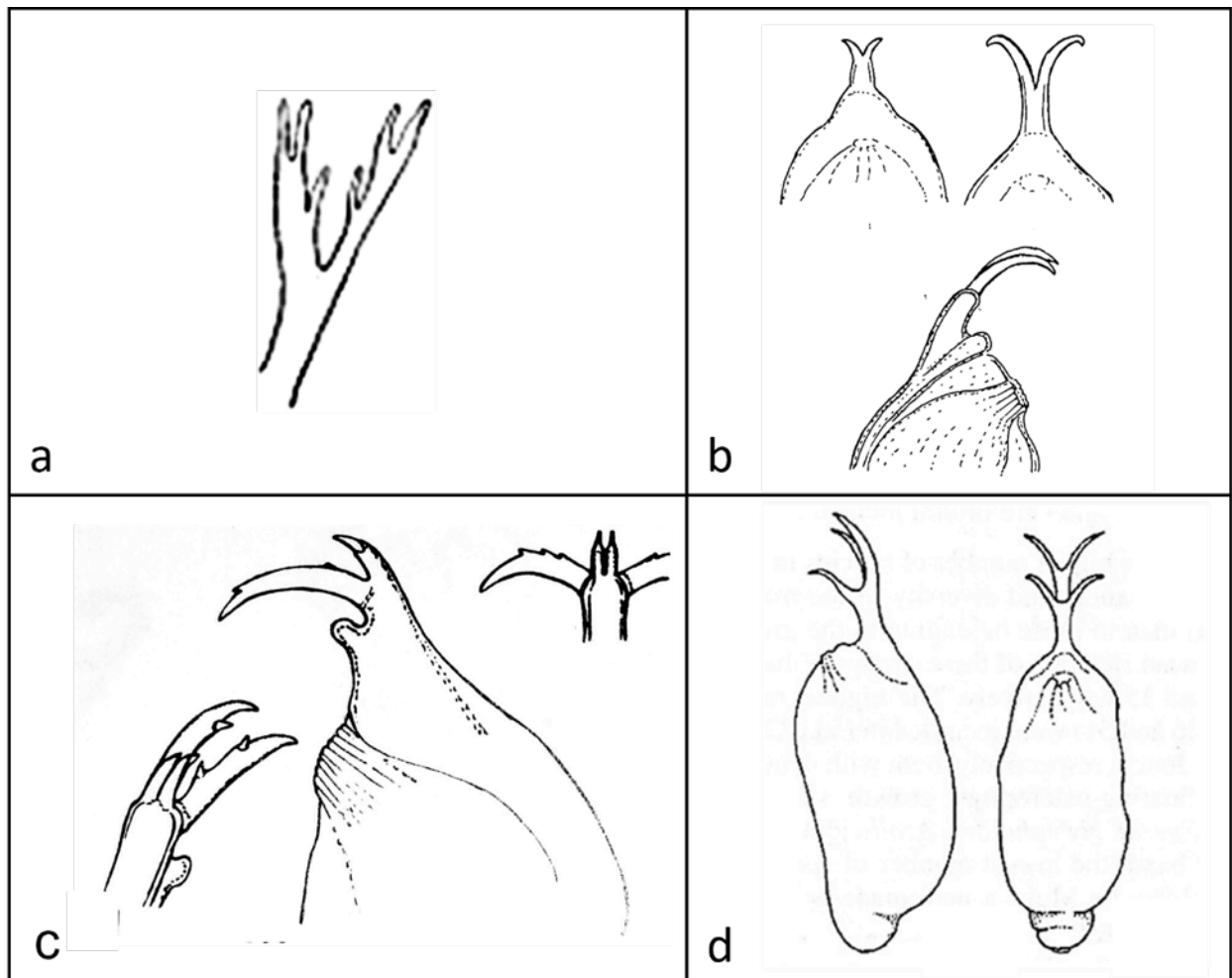


Figure 4. Cervical hooks morphology of the *P. elsteri* group species. a) *P. innominata* n. sp., after Jennings (1900) as *Oecistes melicerta* Ehr. b) *P. furcillata* after Koste (1975). c) *P. elsteri* after Koste (1972) d) *P. kostei*. after José de Paggi (1996).



Description: Long, cylindrical body, progressively narrowing toward the foot but lacking a clear separation between head and trunk. Corona ellipsoidal, diameter slightly larger than the trunk, without folds; cilia extending out of a thick perimeter except at the base, where it is protected by a lip directed backward. Corona located on the front plane of the animal, perpendicular to the mouth (Fig. 5 b3), but bent backward. The mouth leads to a funnel-shaped, ciliated pharynx (Fig. 5 b2). Antennae are not conspicuous. Possessing a semi-rigid, oval-shaped dorsal plate where processes in the form of cervical hooks begins (Fig. 5 a3, b1 and d), but whether the posterior limit of the plate has a net limit, or it is a progressive hardening of the integument is unclear. Large oil droplets may be present in the trunk and foot (Fig. 3, f).

The antler-like, cervical hooks begin with a forward-facing stem forked at an angle of 60° forming two branches (Fig. 5 a3, a4 and b1). Each branch curves ventrally describing an arc of about 70° (Fig. 5 a1, a2 and b3) with three spines, two terminals and one median. The medial spine is directed inward and slightly upward. The stem is somewhat rough on the outside, while the branches are smooth with a slight thickening at the point of origin of the spines (Fig. 5 a3 and b1). Cervical hooks are hollow (Fig. 5 d). Malleoramate trophi with 11 teeth, but it is uncertain whether there may be one more (Fig. 5 e) because they were counted from several frames taken from specimens *in vivo*. Cloaca is delimited by a very marked fold. When defecating, the animal elongates itself so that the material is released outside of the cavities in which the animal is fixed (Fig. 5 a1 and 2). The foot has a similar length to the trunk (Fig. 5 c).

Males, resting eggs, and free-swimming larvae have not been observed.

Measurements: (in  $\mu\text{m}$ ). Some factors make measurements difficult. First, the fact that, in almost all cases, part of the animal's body is hidden by the material to which it is

attached. Secondly, the peculiar curvature of the cervical hooks and the corona presents different aspects depending on the point of view. Finally, the degree of contraction. The measurements have been taken from different specimens and indicate a range among them. Total length: 160-400. Trunk: length 80-150, width at the trophy level 22-43. Foot: length 80-200, width 15-20. Corona: major axis 45-60, minor axis 25-30. Cervical hooks: length in lateral view as a straight line from the ventral base of the hook to the tips 20-30, separation between the furthest apart tips 20-30. Subitaneous embryo: length 100, width 50.

Etymology: The species name *innominata* represents the adjective "unnamed" to which rotiferologists have historically referred to it (Edmondson 1949, Koste 1972, Koste 1978).

Ecology: Animal sessile, either solitary or in groups of up to four individuals, colonizing small masses of decomposing material; often found in the cavity of decaying insect bodies. They build a hyaline tube that is difficult to distinguish. All the specimens of the first sample were feeding on short cyanobacteria trichomes, probably *Pseudanabaena* sp. Temperature ranged from 10–22° C and pH 7.06-8.00.

Distribution: Found in the northern hemisphere in temperate climates, usually with a slightly alkaline pH (Stokes 1881, Kellicott 1889, Jennings 1894, Wulfert 1966, Shoemaker and Williams 1986).

## DISCUSSION

Clearly, the taxonomic position of specimens resembling the *P. innominata* rotifer have been uncertain from the beginning.

The first description of this rotifer made by Stokes (1881) comes from samples probably located in New Jersey (USA), but the drawing included in his work did not distinguish details of the cervical hooks. He assigned his specimens to the genus *Ptygura*, but without

establishing a species name. A few years later, Kellicott (1889), describing *Cephalosiphon furcillatus* (= *P. furcillata*) in Ohio (USA), indicated that some specimens had hooks (“claws”) that possessed spines, but in others, they were smooth. Thus, it might be that he observed both *P. furcillata* and Stokes’ *Ptygura*.

A similar observation was made by Jennings (1894) in Saint Clair Lake, Michigan (USA). Some of the specimens he described had antler-like hooks, while others only had simple hooks, including both types of specimens in *Ptygura melicerta*, which he considered synonymous with *P. furcillata*. Although in his first publication Jennings did not provide a drawing of the hooks, he did in a later work (Jennings 1900), but he identified it with the specimen described by Stokes (1881). This drawing was included in later works referring to this rotifer: Edmondson 1949 (Plate 1), Koste 1972 (Abb 5), and Koste 1978 (Tafel 206).

In the absence of further observations, the systematics of this form became quite confusing. Thus, Harring (1913) included *C. furcillatus*, *Oecistes melicerta* Jennings, and *O. ptygura* Hudson and Gosse, 1886, as synonyms of *Ptygura melicerta*. In his formula key, Edmondson (1949) referred to this species as “unnamed *Ptygura*” and quoted Stokes (1881) for a description using Jennings’ drawing (1900).

Koste referred to this species in several of his contributions. In the description of *P. elsteri* as a new species, Koste (1972) included the “unnamed *Ptygura*” of Edmondson (1949), but he recognized that the hooks were distinct. In a later work, Koste (1974), in the reference bibliography for *P. elsteri* included Kellicott’s (1889) paper on *P. furcillata*; thus, suggesting that specimens with “branched antler-like structures” actually belong to *P. elsteri*.

Koste (1978, plate 206) included Jennings’ drawing to illustrate the “unnamed

species” (number 5). On the same plate he reproduced the drawings from Wulfert (1966) to illustrate *P. furcillata* (numbers 8 a–h), some of which Koste suggested would correspond to *P. elsteri*, specifically 8f, 8g, and 8h, and in my opinion perhaps also 8e. In the original text of these drawings, Wulfert (1966) explained that drawings c, d, e, and g came from “Herr Wright” (possibly H.G.S. Wright), who in turn received them from “Herr Rumford” in New Hampshire (USA), based on live specimens. Drawings 5h and 8h show the same morphology of the cervical hooks that I observed. Therefore, this would be another of the few observations of this morphotype, but unfortunately the original place of the samples is unknown.

I posit that my observations help resolve this issue. José de Paggi (1996) raised the taxonomic range of *P. elsteri* var. *thailandis* to species with the name of *P. kostei* and considered the structure of the cervical hooks as a valid characteristic for the identification of *Ptygura* species. Similarly, Segers and Shiel (2008) elevated *P. ctenoida* to species, separating it from *P. melicerta*. I separate the rotifer observed from *P. elsteri* into the new species *Ptygura innominata* n. sp., following this same criterion. Furthermore, other ecological and geographical differences reinforce this separation: its association with insect exoskeletons, its presence in waters with a slightly basic pH and its location in temperate climates of the Nearctic and Palearctic regions.

Having found this species in the same pond over a two-year period, indicates that the population is stable but, since at the second observation the *Lemna* layer had been eliminated, it appears not to be strictly dependent on duckweed. Moreover, the association with the decaying insect’s exoskeleton may be related to duckweed as I have often seen live or dead insects and chironomid eggs located among the leaves and roots of *Lemna*.

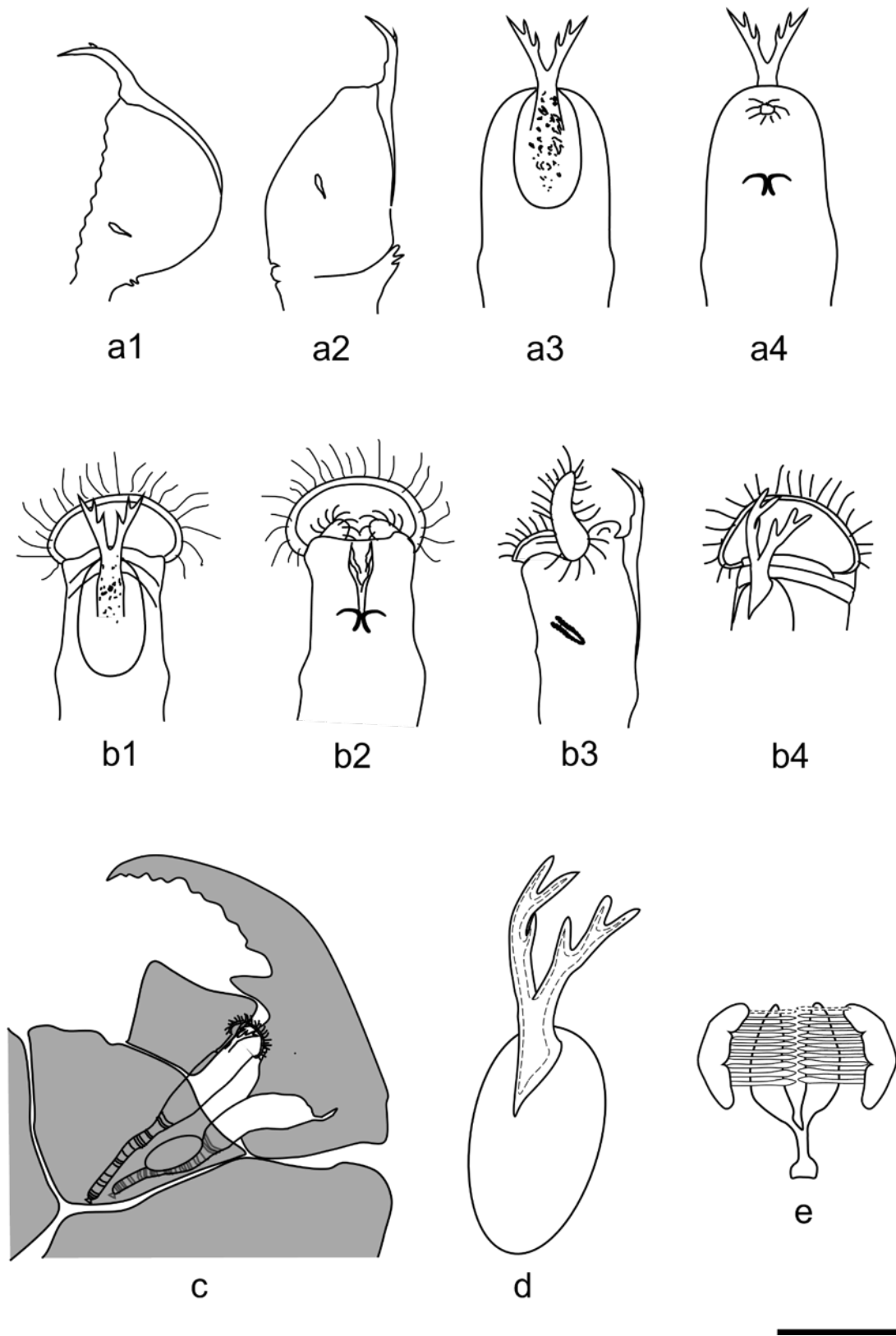


Figure 5. *Ptygura innominata* n. sp. a) contracted: a1) maximum contraction, lateral view, a2) lateral view, a3) dorsal view, a4) ventral view. b) open corona: b1) dorsal view, b2) ventral view, b3) lateral view, b4) dorsolateral view. c) habit in head of an insect larva, from paratypes 3 and 4. d) dorsal plate and cervical hooks, dorsolateral view. e) trophi. Bar: a-b = 50  $\mu$ m, c = 200  $\mu$ m, d = 25  $\mu$ m, e = 10  $\mu$ m.

This may indicate a relationship of metabiosis whereby this species is using dead insects as a habitat. Several rotifers are known to have an epizootic or endozootic relationship with other animals, including insect larvae (May 1989, Fontaneto et al 2004). But no sessile rotifers are found among them. While some sessile rotifers utilize specific hydrophytes as substrata (Wallace 1980, Wallace et al. 2006, Fontaneto and De Smet 2015), metabiosis with insects has not been recognized as a specific habitat for them. Whether this association is occasional or is linked to a substrate selection by the free-swimming larvae of *Ptygura innominata* n. sp. is not proven.

Until now, *Ptygura innominata* n. sp. has certainly only been observed in the North American central region, which might suggest some endemism. However, this new observation in Mallorca suggests that it really could be a cosmopolitan species of temperate climates, like *P. furcillata* with which it has been observed on different occasions (Kellicott 1889, Jennings 1894). *P. kostei* also has a wide distribution, but in tropical and subtropical regions. On the contrary, *P. elsteri* has only been observed once in the Amazon River, a habitat with a high level of diversity and endemism (Martens and Segers 2009), which suggests that it could be a true case of endemism (Fig. 6).

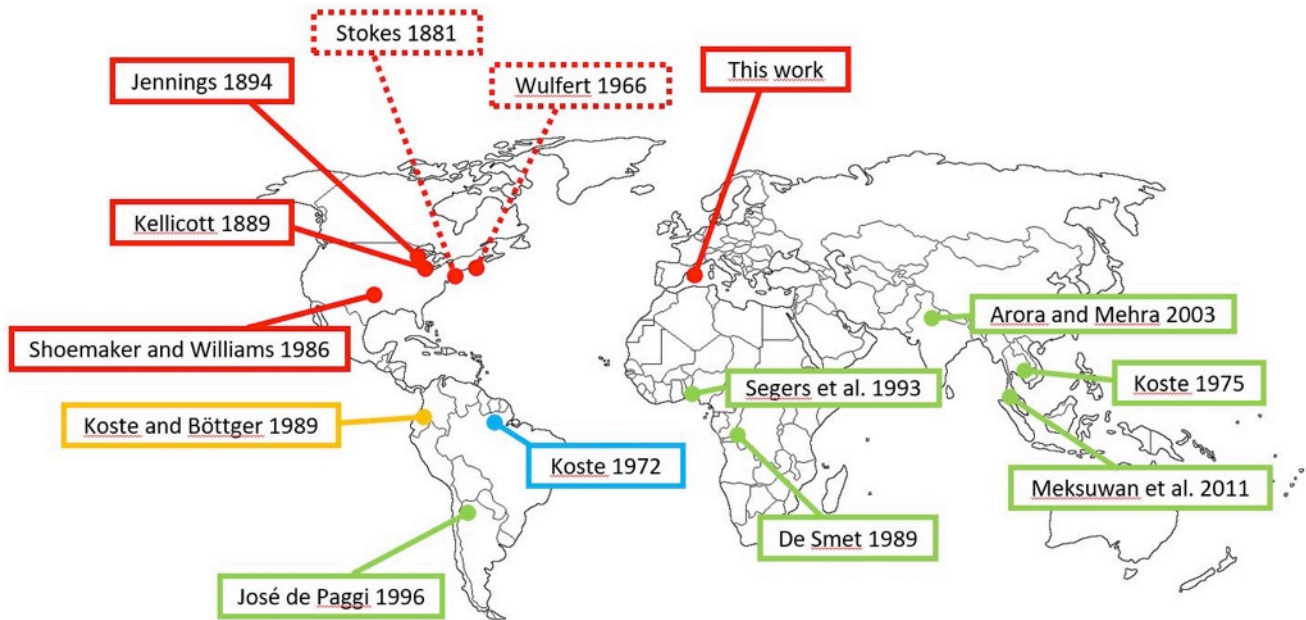


Figure 6. Distribution of *Ptygura elsteri* group species. Red boxes solid line, *P. innominata* n. sp. Red boxes dotted line, *P. innominata* n. sp. uncertain location. Orange box, doubtful *P. innominata* n. sp. or *P. elsteri*. Blue boxes, *P. elsteri*. Green boxes, *P. kostei* (Meksuwan et al. 2001 refers to *P. elsteri*, but a personal communication with P. Meksuwan notes that it actually was *P. kostei*).

Table 1 shows the main anatomical, ecological, and biogeographic features of the *Ptygura* species bearing cervical hooks. The table does not show other anatomical details, such as the shape of the corona, as in some cases this is unknown, because fully or partially contracted specimens have only been studied, such as *P. elsteri* or *P. kostei*.

Species with well-developed hooks (*P. furcillata*, *elsteri*, *kostei*, and *innominata*) appear to follow a different morphological pattern than species with poorly developed hooks (*P. melicerta*, *ctenoida*, and *mucicola*). When the hooks are well developed, they always start from a stem seated on a semi-rigid, oval-shaped dorsal plate.

Table 1. Species of *Ptygura* possessing cervical hooks, ordered by complexity of their morphology. Unreferred data in the table come from Jersabek and Leitner (2013). Biogeography acronyms as in Segers (2007).

Taxa	Dorsal hooks morphology	Dorsal plate	Tube	Macrohabitat Microhabitat	Temp. pH	Biogeography
<i>Ptygura mucicola</i> (Kellicott 1888)	One small, dorsal denticle just below the corona (Kellicott 1888)	No	Yes? (Kellicott 1888)	Lake, pond, freshwater. Gelatinous blue-green algae colonies ( <i>Gleotrichia</i> ). Macrophytes ( <i>Utricularia</i> ).	20 - 30°C 6.4 – 6.5	AFR, AUS, NEA, NEO, ORI, PAL
<i>Ptygura melicerta</i> Ehrenberg, 1832	Two neck hooks behind the corona (Leutbecher and Koste 1998)	No	Yes	Lake, freshwater (eutrophic). Leaves of <i>Chara</i> , <i>Ceratophyllum</i> . <i>Gleotrichia</i> .	No information	AFR, ANT, AUS, NEA, NEO, ORI, PAL
<i>Ptygura ctenoida</i> Koste & Tobias, 1990	Two hooks on the neck, one behind the other in the midline. The dorsally directed one was larger than the previous one (Koste and Tobias 1990)	No	No	Freshwater lakes, ponds, billabongs, reservoirs, and streams; occasionally in athalassic oligosaline waters. Fixosessile in colonies of cyanobacteria and among macrophytes; probably prefers warmer temperatures and higher trophic levels.	16 - 19 °C 8.95	AFR, AUS, PAL, ORI
<i>Ptygura elsteri</i> Koste, 1972	Stalk terminates in a small median furca. On both sides, symmetrical, long hooks are curved downwards, which show up to three rungs on their inner edge (Koste 1972)	Yes	Yes (Koste 1974)	Lagoon, freshwater. Roots of <i>Eichornia</i> and <i>Salvinia</i>	No information	NEO
<i>Ptygura furcillata</i> (Kellicott, 1889)	Stalk with two stout, down-curved, smooth hooks (Kellicott 1889)	Yes	Yes	Freshwater ponds, lakes, reservoirs, streams, and rivers. Leaves of <i>Myriophyllum</i> and <i>Ceratophyllum</i> . Sessile on macrophytes; warm stenothermic (Jersabek and Bolortsetseg, 2010)	19 - 20 °C 8.95 – 8.99	AFR, AUS, NEA, NEO, ORI, PAL
<i>Ptygura innominata</i> n. sp.	Stalk with two down-curved hooks, which show three spines on each branch, two terminals as a small fork, and one in the middle position directed inwards (This work)	Yes	Yes	Pond. Trabeculae of dead insects head and flocculus masses around roots of <i>Lemna minor</i> . Feeding trichomes of <i>Pseudanabaena</i> .	11 - 22 °C 7.06 – 8.00	NEA, PAL, (NEO)
<i>Ptygura kostei</i> José de Paggi, 1996	Stalk with four robust hooks, almost similar in size, curved downwards (José De Paggi 1996)	Yes	No information	Swamp, freshwater. Water ferns ( <i>Salvinia</i> , <i>Azolla</i> ), roots of <i>Eichornia</i> , interrhizon.	26 °C pH 5.8 range 3.2 – 9.9 (Meksuwan et al. 2011)	AFR, NEO, ORI

The adaptive meaning of these traits is unclear. Regarding hooks, Kellicott (1889) already raised it in his first description of *Ptygura furcillata*, wondering himself about several functions: sensitive, defensive, or as a rake for organic material, the latter being the most likely in my opinion. Its objective would be to separate the waste material from the opening of the tube, accumulating it in the ventral area, especially in the position of maximum contraction (Fig. 5a1). This not only helps to keep the tube clean but also clears the dorsal area of the animal where the feed comes from. Regarding the dorsal plate, in addition to serving as a support for the hooks, it can function as a protective shield when the animal contracts. In this position, the animal is protected by the tube or the insect's exoskeleton, the plate acting as a cover that closes the cavity.

Although the macrohabitat of all species appears to be similar, there are some differences (Table 1). For example, the relationship with cyanobacteria in the case of *P. ctenoida*, *P. innominata*, *P. melicerta*, and *P. mucicola*. The range of temperatures in which the different species have been found does not show striking differences, with *P. mucicola* being the one found at higher temperatures (30 °C) and *P. innominata* at lower temperatures (11 °C). On the other hand, differences do appear in the pH range. *Ptygura mucicola* and *P. elsteri* have been found in acidic waters while the others seem to inhabit slightly alkaline waters.

A detailed analysis of the morphological features of some genetically discriminated cryptic species has allowed them to be identified as new species (Cyros-Pérez et al 2001, Fontaneto et al. 2007, Schroder and Wash 2007, Michaloudi et al. 2015). Based on the fine morphology of the cervical hooks, the *P. elsteri* group has so far been split into three species: *P. elsteri*, *P. kostei*, and *P. innominata* n. sp. Since the genomes of this group has not been analyzed, it remains to be determined how

much genetic diversity these differences represent.

However, specific diversity within the group could be even higher, if one considers that the cryptic species of a complex may also present ecological and behavioral diversity (Obertegger et al 2014, Gabaldón et al 2017, Mills et al 2017). As hypothesized by Kordbacheh et al (2018), the lower dispersal capacity of sessile rotifers compared to planktonic rotifers would limit gene flow between populations, generating a more pronounced genetic diversity. All the populations of *P. innominata* n. sp. prior to this work were found in central North America, presenting some ecological features coinciding with those found in Mallorca, such as the presence of duckweed and slight alkalinity of the water. However, other traits have not previously been reported, such as fixation on an insect exoskeleton and feeding on the trichomes of cyanobacteria. Furthermore, some sessile rotifers show substrate selection (Wallace et al 2006) so that fixation in the chitinous trabeculae of insects could indicate specific diversity.

Furthermore, geographical separation of the populations of *P. innominata* n. sp. between America and Europe and their differences in behavior could suggest an additional intraspecific genetic diversity. But unfortunately, the verification of this hypothesis is especially difficult in the case of rare species, as is our case, with only a dozen observations of the *Ptygura elsteri* group reported in the last 150 years. For this reason, I consider that, after the identification of some rare rotifer, it should be a priority to obtain material preserved in ethanol for genetic analysis, which would facilitate biodiversity studies.

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