

## New Species of the Genus *Richtersius* Pilato & Binda, 1989 (Tardigrada: Eutardigrada: Richtersiusidae) from Uzbekistan

Yevgen KIOSYA<sup>ID</sup> and Daniel STEC<sup>ID</sup>

Accepted October 13, 2022

Published online November 15, 2022

Issue online December 06, 2022

Original article

KIOSYA Y., STEC D. 2022. New species of the genus *Richtersius* Pilato & Binda, 1989 (Tardigrada: Eutardigrada: Richtersiusidae) from Uzbekistan. *Folia Biologica* (Kraków) **70**: 141-150.

A new tardigrade species from the family Richtersiusidae, *Richtersius mazepi* sp. nov., from Uzbekistan, is described and illustrated. A comparison to all three of the previously described congeners revealed a unique set of morphological characteristics of the egg ornaments, which makes the new species clearly distinct from the others. The unique characteristics are as follows: (i) the presence of light-refracting dots or pores and a crown of thickenings around the base of the egg processes; (ii) the presence of dark dots on the egg surface between the processes; (iii) a labyrinthine layer seen as light-refracting dots in the wall of the proximal portion of the egg processes; and (iv) egg processes comprised of a wide dome-shaped proximal portion and an elongated slender distal portion. The new species discovered in this study constitutes the fourth formally described species within the genus *Richtersius* Pilato & Binda, 1989. The issue concerning the use of a classical taxonomic description based on morphological data is also discussed.

Key words: egg ornamentation, morphology, morphometrics, tardigrade, taxonomy.

Yevgen KIOSYA, School of Biology, V. N. Karazin Kharkiv National University, Svobody Sq., 4, 61022, Kharkiv, Ukraine.

Daniel STEC<sup>✉</sup>, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland.  
E-mail: daniel.stec@isez.pan.krakow.pl

Richtersiusidae Guidetti, Schill, Giovannini, Massa, Goldoni, Ebel, Förschler, Rebecchi & Cesari, 2021 is a family of tardigrades with only seven nominal taxa that are classified and divided into two genera: *Diaforobiotus* Guidetti, Rebecchi, Bertolani, Jönsson, Kristensen & Cesari, 2016; and *Richtersius* Pilato & Binda, 1989. For more than 30 years, *Richtersius* was a monotypic genus, with its sole formally described species being *Richtersius coronifer* (Richters, 1903). However, several studies to date have demonstrated that *R. coronifer* is an umbrella name that includes more than one species (REBECCHI *et al.* 2003; FAURBY *et al.* 2008; GUIDETTI *et al.* 2016). The main reason for this was an inaccurate and highly general original description of the species, which led to a false impression about its ubiquity. The taxonomic obstacle presented by this ambiguity was overcome by an integrative re-description of the species, followed by a new neotype designation (STEC *et al.* 2020a; STEC & MICHALCZYK 2020). The genus should now be considered to be

oligotypic, since two other species were subsequently integratively described, namely: *Richtersius ziemowiti* Kayastha, Berdi, Mioduchowska, Gawlak, Łukasiewicz, Goldyn, Jędrzejewski & Kaczmarek, 2020; and *Richtersius tertius* Pogwizd & Stec, 2022.

In this study, we provide a description of a new *Richtersius* species from Uzbekistan, based on a detailed morphological and morphometric examination using phase contrast light microscopy (PCM). A comparison with all the other nominal taxa reveals peculiar characteristics of the egg ornamentation in the new species, which make it unique and distinct within the genus.

### Materials and Methods

#### Specimen preparation and microscopy

A moss sample containing the new species was collected in April 2010 from soil in Uzbekistan, near a bor-

der post with Tajikistan, by Glib Mazepa. The sample was examined for tardigrades using the protocol developed by DASTYCH (1980). Together with the new species, representatives of three other tardigrade genera were found: *Echiniscus* C.A.S. Schultze, 1840; *Hypsibius* Ehrenberg, 1848; and *Isohypsibius* Thulin, 1928. Except for the new species, no other macrobiotid taxa that lay ornamented eggs have been recorded. In order to perform the taxonomic analysis, animals and eggs were extracted from the sample and prepared for a morphological analysis in PCM (for details, see 'Materials examined' below). The specimens were mounted on microscope slides in a small drop of Faure's medium (composition: 30 g of gum arabic, 50 ml of distilled water, 20 ml of glycerol, 150 mg of chloral hydrate; mixed without heating) and secured with a cover slip. The slides were examined under a Leica DMLB light microscope with phase contrast (PCM), associated with a digital camera. All the figures were assembled in Corel Photo-Paint X8. For structures where the focus could not be achieved in a single photograph, a stack of 2 to 10 images was taken with an equidistance of approximately 0.2  $\mu\text{m}$  and were manually assembled into a single deep focus image.

#### Morphometrics and morphological nomenclature

All measurements are given in micrometres ( $\mu\text{m}$ ). Structures were measured only if their orientation was suitable. The body length was measured from the anterior to the posterior extremity of the body, excluding the hind legs. The types of bucco-pharyngeal apparatuses follow PILATO & BINDA (2010). The terminology used to describe the armature of the oral cavity and the morphology of the eggshells follows GUIDETTI *et al.* (2016) and STEC *et al.* (2020a,b). The macroplacoid length sequence is given according to KACZMAREK *et al.* (2014). The length of the buccal tube and level of the insertion point of the stylet support were measured according to PILATO (1981). The *pt* index, which is the ratio of the length of a given structure to the length of the buccal tube, was calculated and expressed as a percentage (PILATO 1981). The width of the buccal tube was measured as the external and internal diameter at the level of the stylet support insertion point. The heights of the claw branches were measured from the base of the claw (*i.e.* excluding the lunulae) to the top of the branch, including the accessory points. The claw common tract index (*cct*), which is the proportion of the height of the common tract of the claw (measured from the base of the claw to the separation point between the primary and secondary branch) to the total height of the claw, was calculated and expressed as a percentage (GUIDETTI *et al.* 2016). The description of the cuticular bars on the legs follows KIOSYA *et al.* (2021). The distance between the egg processes was measured as the shortest distance between the base edges of the two closest processes. Following STEC *et al.* (2020a), we measured six additional characteristics: cuticular

pore density (PD – the number of pores per 2500  $\mu\text{m}^2$  counted within a rectangle in the dorsal cuticle between legs III and IV), pore size (PS – measured as the largest diameter; ten pores per measured specimen), number of teeth in the external and internal lunules III (ExtT and IntT, respectively), and number of teeth in the anterior and posterior lunules IV (AntT and PosT, respectively). The morphometric data were handled using the 'Parachela' ver. 1.8 template, which is available from the Tardigrada Register (MICHALCZYK & KACZMAREK 2013) and is provided as Supplementary Material (SM.01). The tardigrade taxonomy follows BERTOLANI *et al.* (2014), STEC *et al.* (2020b) and GUIDETTI *et al.* (2021).

## Results

### Taxonomic account of the new species

Phylum: Tardigrada Doyère, 1840

Class: Eutardigrada Richters, 1926

Order: Macrobiotidea Thulin, 1928

Family: Richtersiidae Guidetti, Schill,

Giovannini, Massa, Goldoni, Ebel,

Förschler, Rebecchi, Cesari, 2021

Genus: *Richtersius* Pilato & Binda, 1989

*Richtersius mazepi* sp. nov.

Figures 1-5, Tables 1-2

ZooBank:

urn:lsid:zoobank.org:act:14060392-82DC-484E-9A-53-4827CBEE2D52

**Etymology:** The name 'mazepi' was chosen in reference to a gifted batrachologist and a friend of the first author, Glib Mazepa, who kindly provided the sample.

**Materials examined:** 11 animals and 5 eggs mounted on microscope slides in Faure's medium.

### Description of the new species

Animals (measurements and statistics are included in Table 1)

Body is yellow; all specimens became transparent after the fixation in Faure's medium (Fig. 1A). Eyes were present in five of the 11 specimens mounted in Faure's medium. Body and leg cuticle is without granulation in all life stages and with pores present only in hatchlings (Figs 1A-B, 2A-B). Hatchlings are similar in appearance to adults, except for a smaller body size and roundish pores (0.8-1.6  $\mu\text{m}$  in diameter) with smooth edges, clearly visible under PCM, scattered randomly throughout the body cuticle, with a mean pore density of  $31 \pm 5$  per 2500  $\mu\text{m}^2$  of the dorsal cuticle (Fig. 1B).

Table 1

Measurements [in  $\mu\text{m}$ ] and *pt* values of selected morphological structures of the specimens of *Richtersius mazepi* sp. nov. Specimens were mounted in Faure's medium; N – number of specimen/structures measured, Range refers to the smallest and the largest structure among all the measured specimens; SD – standard deviation

Character	N	Range		Mean		SD		Holotype	
		$\mu\text{m}$	<i>pt</i>	$\mu\text{m}$	<i>pt</i>	$\mu\text{m}$	<i>pt</i>	$\mu\text{m}$	<i>pt</i>
Body length	11	391 - 897	729 - 1155	652	951	180	160	708	1069
Buccal tube									
Buccal tube length	11	51.1 - 79.5	-	67.6	-	10.1	-	66.2	-
Stylet support insertion point	11	36.4 - 58.6	71.0 - 73.9	48.9	72.2	7.6	0.9	47.6	71.9
Buccal tube external width	11	4.0 - 7.5	7.2 - 9.5	5.6	8.3	1.1	0.8	5.0	7.6
Buccal tube internal width	11	1.0 - 2.1	1.9 - 3.1	1.6	2.4	0.3	0.4	1.6	2.4
Ventral lamina length	6	24.5 - 36.9	37.6 - 47.9	31.4	43.1	5.4	4.4	25.2	38.1
Placoid lengths									
Macroplacoid 1	10	7.5 - 9.5	11.4 - 14.7	8.4	12.2	0.8	0.9	7.8	11.8
Macroplacoid 2	10	5.4 - 8.6	8.7 - 11.7	7.1	10.2	1.1	1.0	6.3	9.5
Macroplacoid row	10	14.5 - 19.6	22.9 - 28.4	17.3	25.2	1.9	1.9	16.1	24.3
Claw I heights									
External base	9	6.9 - 14.7	12.7 - 18.5	10.0	15.0	2.6	1.9	10.5	15.9
External primary branch	10	17.5 - 31.2	29.6 - 42.0	23.7	35.3	5.5	4.2	23.3	35.2
External secondary branch	9	8.1 - 17.4	15.8 - 22.7	12.6	18.9	3.5	3.0	15.0	22.7
External base/primary branch ( <i>cct</i> )	9	38.0 - 49.1	-	43.3	-	3.6	-	45.1	-
Internal base	8	6.1 - 12.0	11.9 - 18.1	8.9	13.9	2.2	2.0	8.8	13.3
Internal primary branch	10	17.2 - 31.0	29.0 - 43.3	23.4	34.7	5.7	4.7	22.0	33.2
Internal secondary branch	9	8.1 - 16.1	14.6 - 24.7	11.4	17.3	2.9	3.1	11.2	16.9
Internal base/primary branch ( <i>cct</i> )	8	35.5 - 45.8	-	41.3	-	3.5	-	40.0	-
Claw II heights									
External base	8	6.9 - 16.1	13.2 - 20.3	11.5	17.0	3.0	2.5	11.2	16.9
External primary branch	9	18.6 - 34.6	31.6 - 46.3	26.6	38.8	6.0	5.1	25.5	38.5
External secondary branch	9	7.9 - 18.8	15.1 - 24.5	14.2	20.6	3.5	3.1	13.3	20.1
External base/primary branch ( <i>cct</i> )	8	37.1 - 48.9	-	44.2	-	3.7	-	43.9	-
Internal base	8	6.3 - 13.2	11.8 - 20.2	9.4	14.6	2.5	2.7	9.4	14.2
Internal primary branch	10	18.2 - 34.0	29.9 - 48.2	25.2	37.6	6.2	5.5	24.7	37.3
Internal secondary branch	6	8.4 - 16.4	16.1 - 25.2	13.5	19.8	3.1	3.1	12.1	18.3
Internal base/primary branch ( <i>cct</i> )	8	34.6 - 45.3	-	39.2	-	3.3	-	38.1	-
Claw III heights									
External base	8	6.1 - 14.8	11.7 - 22.7	10.8	16.6	3.5	4.0	10.5	15.9
External primary branch	9	18.4 - 34.4	32.5 - 48.8	25.4	38.6	6.4	5.5	25.0	37.8
External secondary branch	8	7.9 - 18.2	15.1 - 27.3	13.8	20.3	3.5	3.7	13.7	20.7
External base/primary branch ( <i>cct</i> )	8	33.2 - 58.1	-	43.7	-	8.3	-	42.0	-
Internal base	10	6.0 - 13.3	11.5 - 18.1	10.2	15.1	2.6	2.2	9.9	15.0
Internal primary branch	10	18.1 - 35.4	31.7 - 48.5	26.0	38.7	6.7	5.6	25.2	38.1
Internal secondary branch	8	8.2 - 16.8	15.7 - 25.5	14.0	20.1	3.1	3.0	12.7	19.2
Internal base/primary branch ( <i>cct</i> )	10	32.8 - 45.5	-	39.3	-	4.5	-	39.3	-
Claw IV heights									
Anterior base	8	8.6 - 15.9	14.9 - 23.2	11.8	18.3	2.8	2.7	13.6	20.5
Anterior primary branch	8	24.3 - 45.0	38.7 - 60.0	31.6	49.5	7.2	7.6	32.9	49.7
Anterior secondary branch	7	10.3 - 19.6	16.6 - 26.3	14.4	22.4	3.6	3.3	17.4	26.3
Anterior base/primary branch ( <i>cct</i> )	8	32.1 - 44.0	-	37.3	-	4.2	-	41.3	-
Posterior base	6	10.0 - 17.6	16.0 - 27.0	13.2	20.7	3.1	4.0	15.4	23.3
Posterior primary branch	6	27.6 - 41.4	42.6 - 63.5	31.9	50.6	5.2	7.7	33.1	50.0
Posterior secondary branch	5	11.7 - 19.7	18.3 - 30.2	15.6	24.5	3.6	4.5	17.6	26.6
Posterior base/primary branch ( <i>cct</i> )	6	34.7 - 46.5	-	40.9	-	4.3	-	46.5	-
Number of teeth in external lunula III	4	6 - 10	-	8	-	1	-	-	-
Number of teeth in internal lunula III	4	5 - 10	-	8	-	2	-	-	-
Number of teeth in anterior lunula IV	5	8 - 10	-	9	-	1	-	-	-
Number of teeth in posterior lunula IV	5	7 - 11	-	9	-	2	-	-	-
Pore density	2	26 - 36	-	31	-	5	-	-	-
Pore size	2	0.8 - 1.6	-	1.1	-	0.3	-	-	-

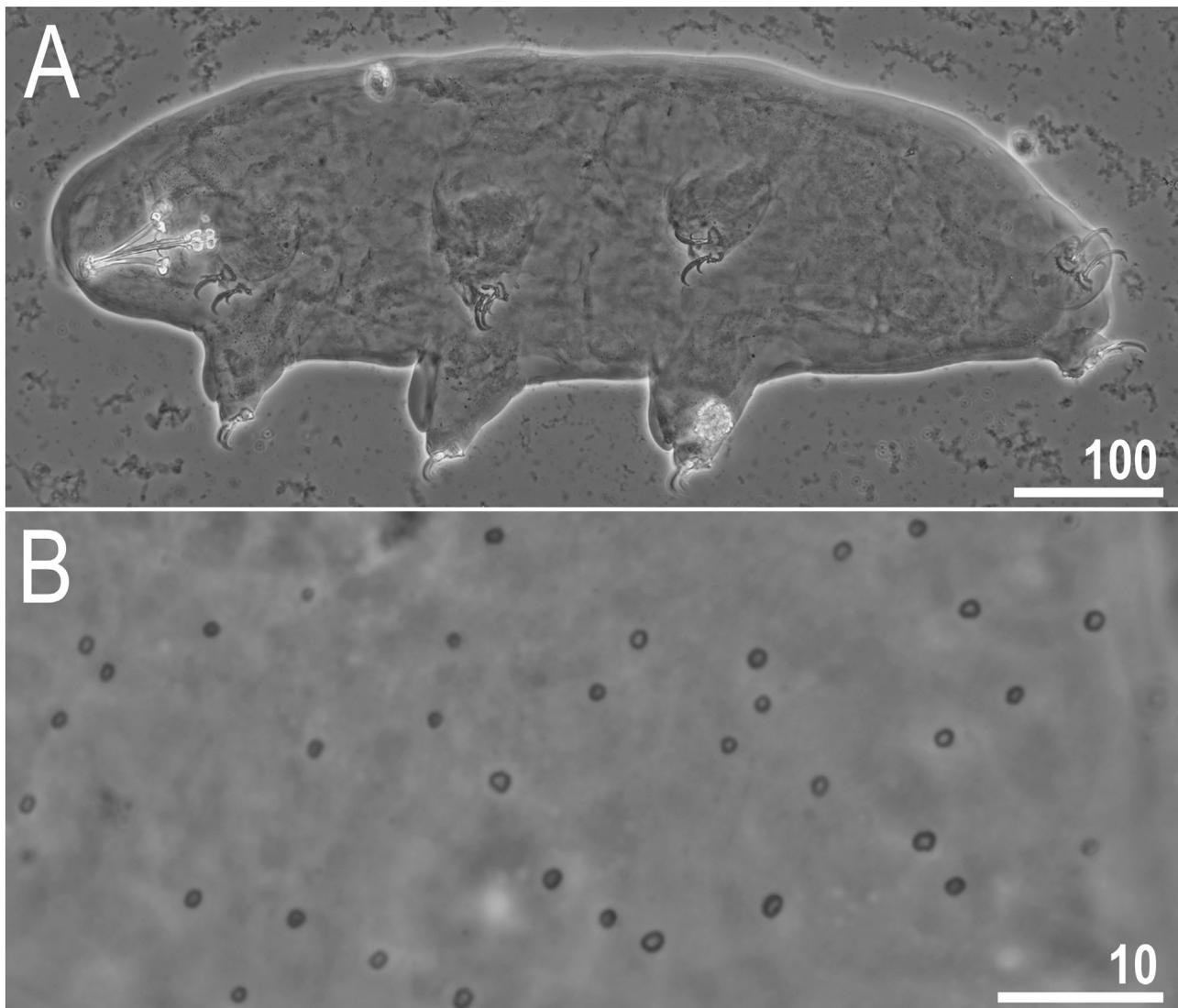


Fig. 1. *Richtersius mazepi* sp. nov. – habitus and cuticular pores. A – adult, dorso-ventral view (holotype); B – pores on the hatchling dorsal cuticle. Scale bars are in  $\mu\text{m}$ .

Claws are slender, primary branches with distinct accessory points (Fig. 2A-B) and an internal system of septa as described for *Richtersius coronifer* s.l. by LISI *et al.* (2020). The claw common tract index is always below 50%, meaning that the basal portion of the claw is shorter than half the total length of the primary branch. An evident stalk system connecting the claws to the lunulae is visible under PCM (Fig. 2A-B). The stalk system consists of a thin laminar stalk connecting the claw to the lunula and two posterior lateral extensions, whose distal tips under PCM appear to be connected to the stalk where it contacts the lunula (Fig. 2A-B). Lunulae are large, with a crown of long, numerous and densely arranged spikes (1.9-3.4  $\mu\text{m}$  long) (Fig. 2A-B). All the lunulae are more or less trapezoidal (Fig. 2A-B). Double muscle attachments in legs I-III and horseshoe structures in legs IV are visible in PCM, whereas cuticular bars are absent (Fig. 2A-B).

Mouth is antero-ventral. The oral cavity armature is faintly visible under PCM, with only the second band

of teeth visible mainly in the larger specimens, and the third band of teeth being sometimes visible in the lateral projection of the anterior portion of the buccal apparatus (Fig. 3B-C). Under PCM, the second band of teeth is visible as several irregular rows of densely packed and faint dark dots (Fig. 3B-C). The discontinuous third band of teeth is situated between the second band of teeth and the opening of the buccal tube, and is divided into a dorsal and a ventral portion, both in the form of a single large tooth resembling a beak (Fig. 3A). The buccal apparatus is of the *Richtersius* type (Fig. 3A). The oral cavity is followed by a system of large apophyses that form a buccal crown (Fig. 3A-C). Anteriorly, the system consists of dorso-lateral and ventro-lateral triangular apophyses (Fig. 3A, C). The dorsal and ventral apophyses are composed of anteriorly positioned large cuticular hooks, followed by longitudinal crests (Fig. 3B). The hook in the ventral apophyses is smaller than the dorsal hook (Fig. 3B). The wall of the buccal tube exhib-

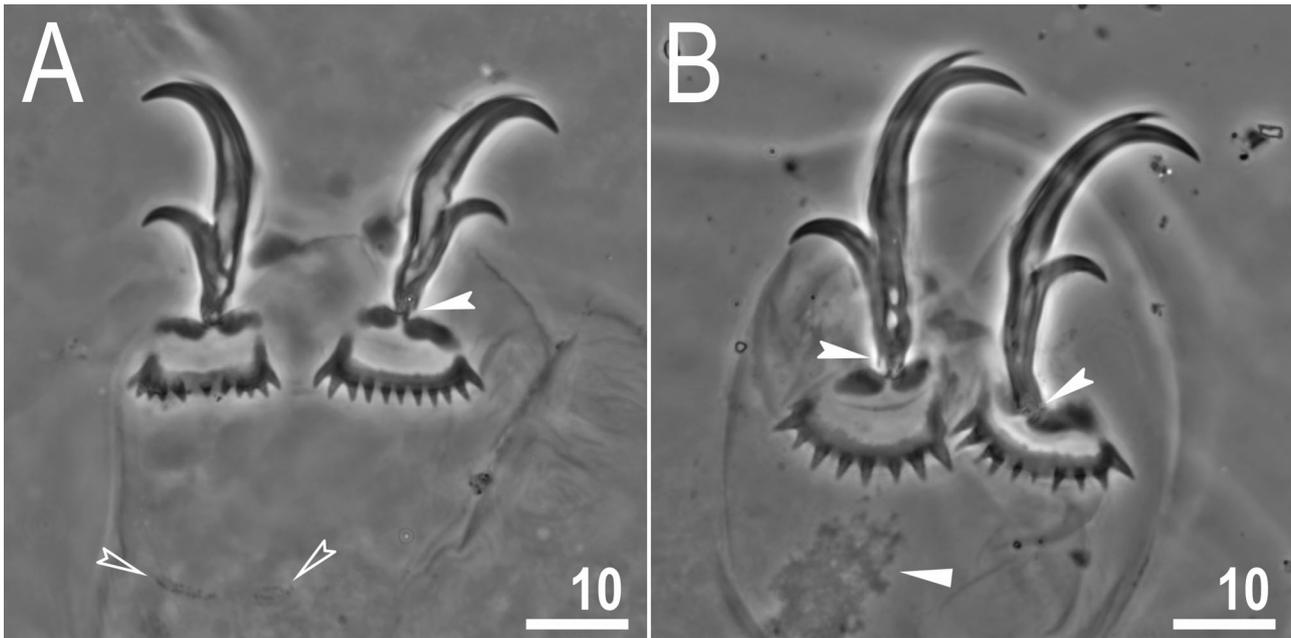


Fig. 2. *Richtersius mazepi* sp. nov. – claws. A – claws II; B – claws IV (holotype). Filled indented arrowheads indicate lateral expansions positioned posteriorly to the laminar stalk connecting the claw to the lunula, empty indented arrowheads indicate the double muscle attachments under the claws, and the filled flat arrowhead indicates the horseshoe structure. Scale bars are in  $\mu\text{m}$ .

its a variable thickness, but the internal diameter of the buccal tube is almost uniformly narrow (Fig. 3A). From the mouth opening to the stylet support insertion point, the thickness of the buccal tube wall increases only slightly, while below this point the evident posterior thickness is clearly visible (Fig. 3A). The pharynx is spherical, with bilobed apophyses, three anterior cuticular spikes (typically only two are visible in any given plane) and two granular macroplacoids ( $2 < 1$ ). The first and second macroplacoids have a faint constriction positioned centrally and subterminally, respectively (Fig. 3D-E).

Eggs (measurements and statistics are included in Table 2)

The eggs are large, oval, light yellow and laid freely (Fig. 4A-I). Under PCM, the surface between the processes is smooth, but with evident refracting dots or pores and a crown of thickenings distributed around the bases of the processes (Fig. 4A, D, G). The egg surface between the processes is also covered by irregularly distributed dark dots (Fig. 4A, D, G). The processes are conical, with a wide proximal portion being dome-shaped and a distal portion constituted of a long slender ending (Figs 4A-I, 5A-F). The walls of the egg process in their proximal portion contain light refracting dots that are probably caused by the labyrinthine layer (Fig. 4B, E, H). There are flexible distal portions of the egg processes, sometimes divided into two or three short filaments, and rarely bifurcated into two longer arms (Figs. 4A-I, 5A-F). The proximal and

distal portions of the egg processes are separated by at least one internal septum that is not always clearly visible (Figs 4C, F, I, 5A-F). Sometimes, additional internal septa and bubble-like structures can be observed in the distal portion (Figs 4C, F, I, 5A-F). Terminal discs or spatulas are absent.

**Reproduction:** The type of reproduction characteristic of the new species is unknown.

**Locality:**  $39^{\circ}46'08.3''\text{N}$ ,  $68^{\circ}36'52.2''\text{E}$ ; Uzbekistan, near a border post with Tajikistan; moss from soil; coll. 04.2010 by Glib Mazepa.

**Slide depositories:** The holotype (Slide UZ.001.02 with 5 paratypes), 5 paratypes (Slides UZ.001.01 and UZ.001.03) and 5 eggs (Slides UZ.001.01 and UZ.001.03) are deposited at the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences (Kraków, Poland).

#### Differential diagnosis

As mentioned above, *Richtersius* is oligotypic. The genus includes the nominal species, *R. coronifer*, which is known only from Norway and Greenland (STEC *et al.* 2020a), as well as two other previously described species: *R. ziemowiti*, known only from Nepal (KAYASTHA *et al.* 2020a,b), and *R. tertius*, known only from Greece (POGWIZD & STEC 2022). The new species is clearly distinct from all three of the previously known species, mainly due to a different morphology of the egg ornamentation. The distinctive egg morphology of the new species includes: (i)

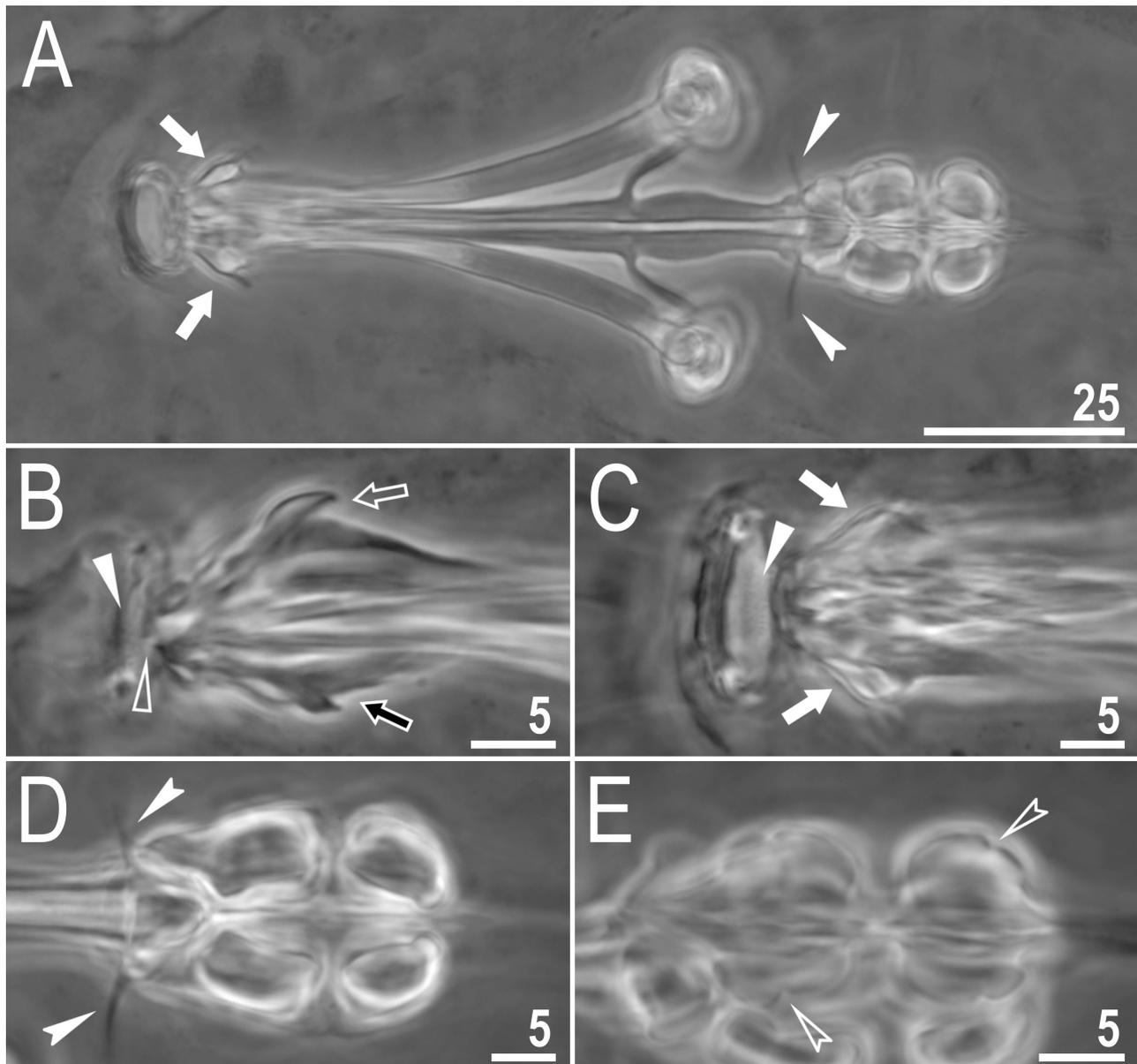


Fig. 3. *Richtersius mazepi* sp. nov. – buccal apparatus. A – dorsal projection of the entire buccal apparatus; B – lateral view of the buccal crown; C – dorso-ventral view in the midsection of the buccal crown; D – macroplacoid morphology visible in the dorsal view; E – macroplacoid morphology visible in the ventral view. Filled arrows indicate the dorso-lateral triangular apophysis, filled indented arrowheads indicate dorsal spikes, empty indented arrowheads indicate constrictions in the macroplacoids, the empty arrow indicates the cuticular hook on the dorsal apophysis, the black filled arrow indicates the cuticular hook on the ventral apophysis, filled flat arrowheads indicate the second band of teeth in the oral cavity, and the empty flat arrowhead indicates the dorsal and ventral portion of the third band of teeth (the so-called ‘beak’). Scale bars are in  $\mu\text{m}$ .

the presence of light refracting dots or pores and a crown of thickenings around the bases of the egg processes (absent in all previously described species); (ii) the presence of dark dots on the egg surface between the processes (absent in all the previously described species); (iii) the presence of light refracting dots in the wall of proximal portion of the egg processes (absent in all the previously described species); and (iv), a different shape of the egg processes (the processes have a wide dome-shaped proximal portion and an

elongated slender distal portion in the new species vs. simple elongated, thin and conical spikes in all of the previously described species). The new species also differs specifically from:

*Richtersius coronifer* due to: the visibility of the second band of teeth under a light microscope (the second band is not visible in *R. coronifer*), smaller eggs (egg bare and full diameter: 77.6-91.4 and 107.1-129.5  $\mu\text{m}$  in the new species vs. 173.2-233.4 and 201.5-263.7  $\mu\text{m}$  in *R. coronifer*), a larger process

Table 2

Measurements [in  $\mu\text{m}$ ] of the eggs of *Richtersius mazepi* sp. nov. Eggs were mounted in Faure's medium; the process base/height ratio is expressed as a percentage; N – number of eggs/structures measured, Range refers to the smallest and the largest structure among all the measured specimens; SD – standard deviation

Character	N	Range	Mean	SD
Egg bare diameter	4	77.6 - 91.4	86.0	6.0
Egg full diameter	4	107.1 - 129.5	117.1	9.4
Process height	15	8.3 - 19.5	14.6	3.7
Process base width	15	6.6 - 10.4	8.0	1.1
Process base/height ratio	15	42% - 83%	57%	12%
Inter-process distance	15	2.1 - 4.1	2.9	0.6
Number of processes on the egg circumference	5	32 - 36	33.6	1.7

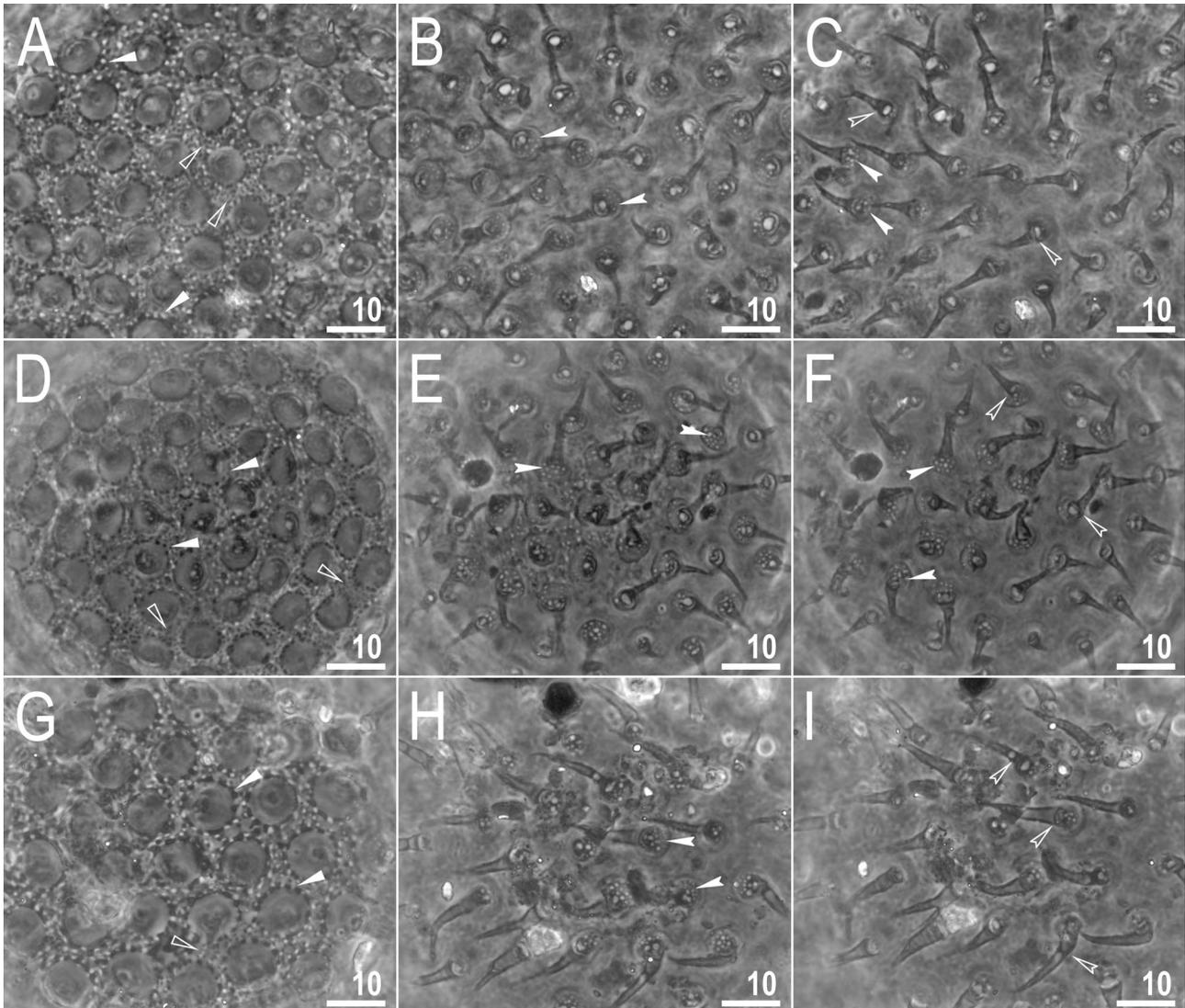


Fig. 4. *Richtersius mazepi* sp. nov. – egg chorion morphology (each row represents a different egg). A, D, G – egg surface under 1000 $\times$  magnification, focusing on the surface between the egg processes; B, E, H – egg surface under 1000 $\times$  magnification, focusing on the proximal (basal) part of the egg processes; C, F, I – egg surface under 1000 $\times$  magnification, focusing on the distal (apical) part of the egg processes. Filled flat arrowheads indicate light refracting dots/pores and the crown of thickenings surrounding the bases of the egg processes, empty flat arrowheads indicate dark dots on the egg surface between the processes, filled indented arrowheads indicate light refracting dots in the walls of the proximal (basal) portion of the egg processes, and empty indented arrowheads indicate internal septa in the egg processes. Scale bars are in  $\mu\text{m}$ .

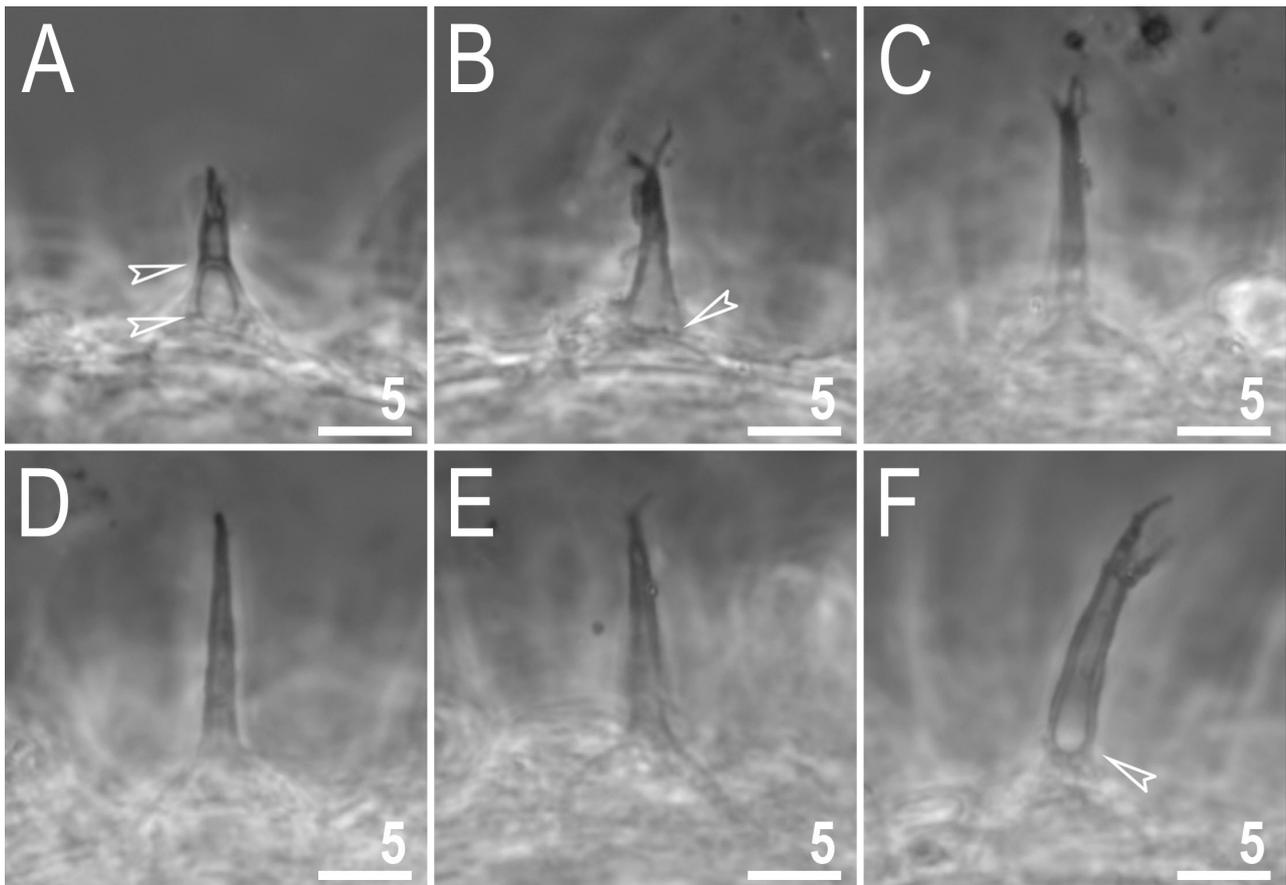


Fig. 5. *Richtersius mazepi* sp. nov. – egg chorion morphology – details of the egg processes (the processes from three different eggs). Empty indented arrowheads indicate internal septa in the egg processes. Scale bars are in  $\mu\text{m}$ .

base/height ratio (42–83% in the new species vs. 12–33% in *R. coronifer*), a smaller inter process distance (2.1–4.1  $\mu\text{m}$  in the new species vs. 4.4–13.2  $\mu\text{m}$  in *R. coronifer*), a smaller number of processes in the egg circumference (32–36 in the new species vs. 60–77 in *R. coronifer*), a smaller pore density count on 2500  $\mu\text{m}^2$  of the dorsal cuticle (26–36 in the new species vs. 66–88 in *R. coronifer*), and a slightly smaller number of teeth in the lunulae in legs III and IV (5–11 in the new species vs. 11–22 in *R. coronifer*).

*Richtersius ziemowiti* due to: smaller eggs (egg bare and full diameter: 77.6–91.4 and 107.1–129.5  $\mu\text{m}$  in the new species vs. 125.4–172.3 and 155.6–203.5  $\mu\text{m}$  in *R. ziemowiti*), slightly wider bases of the processes (6.6–10.4  $\mu\text{m}$  in the new species vs. 3.5–6.6  $\mu\text{m}$  in *R. ziemowiti*), a larger process base/height ratio (42–83% in the new species vs. 17–40% in *R. ziemowiti*), a smaller inter process distance (2.1–4.1  $\mu\text{m}$  in the new species vs. 5.5–13.4  $\mu\text{m}$  in *R. ziemowiti*), and a larger pore density count on 2500  $\mu\text{m}^2$  of the dorsal cuticle (26–36 in the new species vs. 20–24 in *R. ziemowiti*);

*Richtersius tertius* due to: the shape of the cuticular pores (roundish pores with smooth edges in the new species vs. roundish pores with wavy edges in *R. tertius*), the absence of cuticular bars in legs I–III (divided cu-

ticular bars are present in *R. tertius*), a strongly developed thickness of the buccal tube wall posterior to the stylet support insertion point (the thickness is poorly developed and much less obvious in *R. tertius*), a more posteriorly positioned stylet support insertion point ( $pt = 71.0\text{--}73.9$  in the new species vs.  $pt = 65.3\text{--}68.9$  in *R. tertius*), smaller eggs (egg bare and full diameter: 77.6–91.4 and 107.1–129.5  $\mu\text{m}$  in the new species vs. 117.4–155.3 and 149.8–188.2  $\mu\text{m}$  in *R. tertius*), slightly wider bases of the processes (6.6–10.4  $\mu\text{m}$  in the new species vs. 3.0–6.5  $\mu\text{m}$  in *R. tertius*), a slightly larger process base/height ratio (42–83% in the new species vs. 14–42% in *R. tertius*), a smaller inter process distance (2.1–4.1  $\mu\text{m}$  in the new species vs. 5.2–13.1  $\mu\text{m}$  in *R. tertius*), and a larger pore density count on 2500  $\mu\text{m}^2$  of the dorsal cuticle (26–36 in the new species vs. 3–6 in *R. tertius*).

## Discussion

*Richtersius mazepi* sp. nov., discovered in this study, is the fourth species to be formally described within the genus *Richtersius*. Its peculiar egg ornamentation morphology clearly distinguishes the new

species from its congeners. In their recent paper in which the type species was redescribed, STEC *et al.* (2020a) expressed the hope that any further descriptions of the *Richtersius* taxa would be integrative, with phenotypic data tightly linked to the DNA sequences of the given species. In this case, we have gone against those recommendations by providing a classical taxonomic description based on morphology and morphometry. We argue that all species are working hypotheses with assigned names (DE QUEIROZ 2007). To define and delineate a species, multiple forms of evidence can be used, such as morphological, genetic, ecological, reproductive and geographical analyses, as well as their combinations. In our case, the establishment of a new species is supported solely by morphological and morphometric data that explicitly shows *Richtersius mazepi* sp. nov. to be different from all other species within the genus. In other words, the line of phenotypic evidence is sufficient to delineate the species from the other known taxa. Nevertheless, we strongly support integrative studies in tardigrade taxonomy and recommend that these be preferred over classical morphology-based taxonomic studies where possible, especially when cryptic or pseudocryptic species complexes are involved. Importantly, however, situations in which some types of evidence are omitted from the taxonomic descriptions (e.g. DNA, SEM, physiology) will surely occur. In itself, the inability to include all possible forms of evidence does not automatically invalidate the establishment of a new species. The prohibition of sufficient and reliably formulated species hypotheses based only on some types of evidence, as was proposed in a recent paper by GAŚCIOREK *et al.* (2021), is not warranted. Indeed, inaccurate and outdated species descriptions constitute a considerable obstacle in taxonomical studies; however, such obstructions cannot be overcome through the establishment of a single rule that indicates some integrative configurations of data as the only correct solution. Furthermore, revisions and redescriptions constitute readily available tools for appropriate actions when species descriptions turn out to be insufficient. Prohibiting species descriptions that do not include some arbitrarily chosen types of data could seriously hamper our understanding of biodiversity. Taking this study as an example, without the new species description, knowledge about the morphological diversity of egg ornamentation in the genus *Richtersius* would remain limited. Naming a species allows it to be catalogued and ensures that it will be considered in future taxonomic studies (SEIFERT 2017). The danger of impeding the completion of the inventory of living biota is even more alarming now in the era of the so-called sixth mass extinction and the very slow tempo of new species descriptions (FONTAINE *et al.* 2012). As there is no one universal solution and rule, the responsibility for pinning a name to a given organism lies solely with the authors, who should always try to provide the

best possible evidence when testing species hypotheses. The issue is not trivial, as species play a central role in biology, and species names greatly influence how we evaluate these elements of biodiversity, their conservation and their evolution.

## Acknowledgments

In naming the new species, we honour Glib MAZEPA for his huge commitment to the local community in Kharkiv and the supply of materials necessary to protect and support the people in East Ukraine during the brutal war in 2022. DS would like to thank to his friend Erica DEMILIO for her comments on an early version of the manuscript. The study received support from the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences. During this study, DS was supported by the Foundation for Polish Science (FNP).

## Author Contributions

Research concept and design: Y.K., D.S.; Collection and/or assembly of data: D.S.; Data analysis and interpretation: D.S.; Writing the article: D.S.; Critical revision of the article: Y.K., D.S.; Final approval of article: Y.K., D.S.

## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Materials

Supplementary Materials to this article can be found online at:

<http://www.isez.pan.krakow.pl/en/foIiabiologica.html>

SM.01. Raw morphometric data for the animals and eggs of *Richtersius mazepi* sp. nov.

## References

- BERTOLANI R., GUIDETTI R., MARCHIORO T., ALTIERO T., REBECCHI L., CESARI M. 2014. Phylogeny of Eutardigrada: New molecular data and their morphological support lead to the identification of new evolutionary lineages. *Mol. Phylogenet. Evol.* **76**: 110-126.  
<https://doi.org/10.1016/j.ympev.2014.03.006>
- DASTYCH H. 1980. Niesporczaki (Tardigrada) Tatrzańskiego Parku Narodowego [Water bears (Tardigrada) from the Tatra National Park]. *Monografie Fauny Polski* **9**: 1-232. (In Polish with English Summary).
- DE QUEIROZ K. 2007. Species concepts and species delimitation. *Syst. Biol.* **56**: 879-886.  
<https://doi.org/10.1080/10635150701701083>

- DOYÈRE P.L.N. 1840. Memoire sur les Tardigrades. *Ann. Sci. Nat.* **2**: 269-362.
- EHRENBERG C.G. 1848. Fortgesetzte Beobachtungen über jetzt herrschende atmosphärische mikroskopische etc. mit Nachtrag und Novarum specierum Diagnosis. Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlichen Preussischen Akademie der Wissenschaften zu Berlin **13**: 370-381.
- FAURBY S., JÖNSSON K.I., REBECCHI L., FUNCH P. 2008. Variation in anhydrobiotic survival of two eutardigrade morphospecies: a story of cryptic species and their dispersal. *J. Zool.* **275**: 139-145. <https://doi.org/10.1111/j.1469-7998.2008.00420.x>
- FONTAINE B., PERRARD A., BOUCHET P. 2012. 21 years of shelf life between discovery and description of new species. *Curr. Biol.* **22**: R943-R944. <https://doi.org/10.1016/j.cub.2012.10.029>
- GAŚIOREK P., VONČINA K., NELSON D.R., MICHALCZYK Ł. 2021. The importance of being integrative: a remarkable case of synonymy in the genus *Viridiscus* (Heterotardigrada: Echiniscidae). *Zool. Lett.* **7**: 13. <https://doi.org/10.1186/s40851-021-00181-z>
- GUIDETTI R., REBECCHI L., BERTOLANI R., JÖNSSON K.I., KRISTENSEN R.M., CESARI M. 2016. Morphological and molecular analyses on *Richtersius* (Eutardigrada) diversity reveal its new systematic position and lead to the establishment of a new genus and a new family within Macrobiotioidea *Zool. J. Linn. Soc.* **178**: 834-845. <https://doi.org/10.1111/zoj.12428>
- GUIDETTI R., SCHILL R.O., GIOVANNINI I., MASSA E., GOLDONI S.E., EBEL C., FÖRSCHLER M.I., REBECCHI L., CESARI M. 2021. When DNA sequence data and morphological results fit together: Phylogenetic position of *Crenubiotus* within Macrobiotioidea (Eutardigrada) with description of *Crenubiotus ruhesteni* sp. nov. *J. Zool. Syst. Evol. Res.* **59**: 576-587. <https://doi.org/10.1111/jzs.12449>
- KACZMAREK Ł., CYTAN J., ZAWIERUCHA K., DIDUSZKO D., MICHALCZYK Ł. 2014. Tardigrades from Peru (South America), with descriptions of three new species of Parachela. *Zootaxa* **3790**: 357-379. <https://doi.org/10.11646/zootaxa.3790.2.5>
- KAYASTHA P., BERDI D., MIODUCHOWSKA M., GAWLAK M., ŁUKASIEWICZ A., GOLDYN B., JĘDRZEJEWSKI S., KACZMAREK Ł. 2020a. Description and molecular characterization of *Richtersius ziemowiti* sp. nov. (Richtersiidae) from Nepal (Asia) with evidence of heterozygous point mutation events in the 28S rRNA. *Ann. Zool.* **70**: 381-396. <https://doi.org/10.3161/00034541ANZ2020.70.3.010>
- KAYASTHA P., BERDI D., MIODUCHOWSKA M., GAWLAK M., ŁUKASIEWICZ A., GOLDYN B., KACZMAREK Ł. 2020b. Some tardigrades from Nepal (Asia) with integrative description of *Macrobiotus wandae* sp. nov. (Macrobiotidae: *hufelandi* group). *Ann. Zool.* **70**: 121-142. <https://doi.org/10.3161/00034541ANZ2020.70.1.007>
- KIOSYA Y., POGWIZD J., MATSKO Y., VECCHI M., STEC D. 2021. Phylogenetic position of two *Macrobiotus* species with a revisional note on *Macrobiotus sottilei* Pilato, Kiosya, Lisi & Sabella, 2012 (Tardigrada: Eutardigrada: Macrobiotidae). *Zootaxa* **4933**: 113-135. <https://doi.org/10.11646/zootaxa.4933.1.5>
- LISI O., LONDOÑO R., QUIROGA S. 2020. Description of a new genus and species (Eutardigrada: Richtersiidae) from Colombia, with comments on the family Richtersiidae. *Zootaxa* **4822**: 531-550. <https://doi.org/10.11646/zootaxa.4822.4.4>
- MICHALCZYK Ł., KACZMAREK Ł. 2013. The Tardigrada Register: a comprehensive online data repository for tardigrade taxonomy. *J. Limnol.* **72**: 175-181. <https://doi.org/10.4081/jlimnol.2013.s1.e22>
- PILATO G., BINDA M.G. 1989. *Richtersius*, nuovo nome generico in sostituzione di *Richtersia* Pilato e Binda 1987 (Eutardigrada). *Animalia* **16**: 147-148.
- PILATO G., BINDA M.G. 2010. Definition of families, subfamilies, genera and subgenera of the Eutardigrada, and keys to their identification. *Zootaxa* **2404**: 1-52. <https://doi.org/10.11646/zootaxa.2404.1.1>
- PILATO G. 1981. Analisi di nuovi caratteri nello studio degli Eutardigradi. *Animalia* **8**: 51-57.
- POGWIZD J., STEC D. 2022. An integrative description of a new *Richtersius* species from Greece (Tardigrada: Eutardigrada: Richtersiidae). *Acta Zool. Acad. Sci. Hung.* **68**: 1-21. <https://doi.org/10.17109/AZH.68.1.1.2022>
- REBECCHI L., ROSSI V., ALTIERO T., BERTOLANI R., MENOZZI P. 2003. Reproductive modes and genetic polymorphism in the tardigrade *Richtersius coronifer* (Eutardigrada, Macrobiotidae). *Invertebr. Biol.* **122**: 19-27. <https://doi.org/10.1111/j.1744-7410.2003.tb00069.x>
- RICHTERS F. 1903. Nordische Tardigraden. *Zool. Anz.* **27**: 168-172.
- RICHTERS F. 1926. Tardigrada. In: KÜKENTHAL W., KRUMBACH T. (Eds). *Handbuch der Zoologie* **3**: 58-61.
- SCHULTZE C.A.S. 1840. *Echiniscus* Bellermanni, Animal Crustaceum, *Macrobiotus hufelandii* Affine. Apud G. Reimer, Berlin, pp. 1-8.
- SEIFERT K.A. 2017. When should we describe species? *IMA Fungus* **8**: A37-A39. <https://doi.org/10.1007/BF03449459>
- STEC D., KRZYWAŃSKI Ł., ARAKAWA K., MICHALCZYK Ł. 2020a. A new redescription of *Richtersius coronifer*, supported by transcriptome, provides resources for describing concealed species diversity within the monotypic genus *Richtersius* (Eutardigrada). *Zool. Lett.* **6**: 2. <https://doi.org/10.1186/s40851-020-0154-y>
- STEC D., MICHALCZYK Ł. 2020. *Macrobiotus coronifer* Richters, 1903 (type species for *Richtersius* Pilato & Binda, 1989): designating a new neotype from the original type locality described within the integrative taxonomy framework. *Zootaxa* **4858**: 292-294. <https://doi.org/10.11646/zootaxa.4858.2.10>
- STEC D., VECCHI M., MACIEJOWSKI W., MICHALCZYK Ł. 2020b. Resolving the systematics of Richtersiidae by multilocus phylogeny and an integrative redescription of the nominal species for the genus *Crenubiotus* (Tardigrada). *Sci. Rep.* **10**: 19418. <https://doi.org/10.1038/s41598-020-75962-1>
- THULIN G. 1928. Über die Phylogenie und das System der Tardigraden. *Hereditas* **11**: 207-266. <https://doi.org/10.1111/j.1601-5223.1928.tb02488.x>