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An integrative description of *Mesobiotus dilimanensis*, a new tardigrade species from the Philippines (Eutardigrada: Macrobiotidae: *furciger* group)

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Abstract. In this study, a new species of the *Mesobiotus furciger* group from Diliman, Quezon City, Philippines was identified using an integrative taxonomy approach. Morphological and morphometric data obtained through phase and Nomarski contrast microscopy and scanning electron microscopy along with molecular analyses of the DNA sequences of four molecular markers (18S rRNA, 28S rRNA, ITS-2, and COI) support the erection of *Mesobiotus dilimanensis*, new species. The new species is the third limno-terrestrial tardigrade species from the Philippines described as new to science and is the first member of the *Mesobiotus furciger* species complex described using an integrative taxonomy approach in the country.

Keywords. Asia, egg ornamentation, integrative taxonomy, *Mesobiotus dilimanensis*, new species, Tardigrada

INTRODUCTION

Over 1,200 species of tardigrades have been discovered worldwide (Guidetti & Bertolani, 2005; Degma & Guidetti, 2007; Degma et al., 2019). However, data on tardigrade species in certain regions of the world are still lacking. In the Philippines, only six tardigrade species have been reported thus far in peer-reviewed scientific publications, two of which are limno-terrestrial (Mapalo et al., 2016; Mapalo et al., 2017) while the remaining four are marine (Chang & Rho, 1997; Chang & Rho, 1998). The two limno-terrestrial tardigrades, *Mesobiotus philippinicus* Mapalo, Stec, Mirano-Bascos & Michalczyk, 2016, and *Mesobiotus insanis* Mapalo, Stec, Mirano-Bascos & Michalczyk, 2017, were both found in the University of the Philippines, Diliman, Quezon City. Both species belong to the *harmsworthi* species complex within the genus *Mesobiotus* Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti, 2016.

The cosmopolitan genus *Mesobiotus* was erected in 2016 from the genus *Macrobiotus* C.A.S. Schultze, 1834, after combined morphological and molecular analyses within an integrative taxonomy approach. *Mesobiotus* species are characterised by Y-shaped double claws with an internal septum defining a distal part, three macroplacoids and a microplacoid positioned close to the third macroplacoid, and freely laid eggs with conical or hemispherical processes (Vecchi et al., 2016). Based on egg shell morphology, two species groups are currently recognised within *Mesobiotus*: the *harmsworthi* group (Kaczmarek et al., 2011) and the *furciger* group (Binda et al., 2005). The *harmsworthi* group (58 spp.) is characterised by eggs with conical or hemispherical processes (Kaczmarek et al., 2011) whereas the *furciger* group (8 spp.) is composed of species with branched egg processes (Binda et al., 2005). The nominal species for the genus and the *harmsworthi* group, *Mesobiotus harmsworthi* (Murray, 1907a), has recently been redescribed by Kaczmarek et al. (2018b), but *Mesobiotus furciger* (Murray, 1907b), the nominal species for the *furciger* group, still awaits a modern redescription.

In this study, we describe a limno-terrestrial tardigrade species isolated from moss collected, similarly to the two *Mesobiotus* species mentioned above, from the University of the Philippines Diliman. Isolated tardigrades were cultured under laboratory conditions to establish an isofemale strain. The species was confirmed to be new to science through an integrative taxonomic approach, combining molecular and morphological data. Here, we provide detailed morphological and morphometric data obtained with contrast light microscopy (LCM) and scanning electron microscopy (SEM). Alongside the phenotypic description, we also present DNA sequences of four molecular markers (18S rRNA, 28S rRNA, ITS-2, and COI) of the new species. The new species belongs to the genus *Mesobiotus* and represents the *Mesobiotus furciger* species complex.

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MATERIAL AND METHODS

Sample and specimens. Moss was collected from the surface of a rock located at A. Roces St., University of the Philippines, Diliman, Quezon City, Philippines in September 2015. Anisofemale culture was established and maintained following the methods in Mapalo et al. (2016). The moss was soaked in tap water for 24 hours and continuously monitored for the presence of tardigrades. A single tardigrade was found and transferred to a 60-mm Petri plate lined with 2% agar in KCM solution covered with a thin layer of distilled deionised water (ddH_2O). A small fragment of the moss as well as 200 µL of the water in which the moss was soaked was added to the plate. Two hundred microliters of *Chlorella* Beijerinck, 1890, sp. (Aquatic Biotechnology Laboratory, National Institute of Molecular Biology and Biotechnology [NIMBB], Philippines) were added as a food source. The culture was then continuously observed for progeny. Four weeks later, a clutch of eggs from a single female was transferred from the plate into a separate culture plate in order to establish an isofemale culture. One more week later, two females with mature oocytes from the original culture were transferred into separate culture plates to establish two more isofemale cultures. These cultures are currently being maintained at the NIMBB, UP Diliman. In order to perform the taxonomic analysis, animals and eggs were isolated from the cultures and split randomly into three groups destined for specific analyses: morphological and morphometric analysis with light contrast microscopy (LCM), morphological analysis with scanning electron microscopy (SEM), and DNA sequencing (for details please see section "Material examined" provided below).

Microscopy and imaging. Specimens for light microscopy were mounted on glass microscope slides in a small drop of Hoyer's medium and secured with a cover slip, following the protocol by Morek et al. (2016). Slides were then dried for five to seven days at 60°C. Dried slides were sealed with transparent nail polish and examined under an Olympus BX53 light microscope with phase and Nomarski interference contrast (PCM and NCM, respectively), associated with an Olympus DP74 digital camera. In order to obtain clean eggs for SEM, eggs were processed according to the protocol by Stec et al. (2015). Briefly, eggs were first subjected to a water/ethanol and an ethanol/acetone series, then to CO_2 critical point drying, and finally sputter coated with a thin layer of gold. Specimens were examined under high vacuum in a Versa 3D DualBeam Scanning Electron Microscope at the ATOMIN facility of the Jagiellonian University, Kraków, Poland.

All figures were assembled in Corel Photo-Paint X6, ver. 16.4.1.1281. For structures that could not be satisfactorily focused in a single LCM photograph, a stack of 2–6 images were taken with an equidistance of ca. 0.2 µm and assembled manually into a single deep-focus image using Corel.

Morphometrics and morphological nomenclature. All measurements are given in micrometres (µm). Sample size was adjusted following recommendations by Stec et al. (2016). Structures were measured only if their orientation was suitable. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs. The buccal apparatus and claws were classified according to Pilato & Binda (2010) and Vecchi et al. (2016), respectively. The terminology used to describe oral cavity armature and egg shell morphology follows the convention established by Michalczyk & Kaczmarek (2003), Kaczmarek et al. (2011), and Kaczmarek & Michalczyk (2017). Macroplacoid length sequence is given according to Kaczmarek et al. (2014). Buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). The *pt* index is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato, 1981). All other measurements and nomenclature follow Kaczmarek & Michalczyk (2017). Specifically, buccal tube width was measured as the external and internal diameter at the level of the stylet support insertion point. Heights of the claw branches were measured from the base of the claw (i.e., excluding the lunula) to the top of the branch, including accessory points. Distance between egg processes was measured as the shortest distance between the base edges of the two closest processes. Morphometric data were handled using the "Parachela" ver. 1.7 template available from the Tardigrada Register (Michalczyk & Kaczmarek, 2013) and row measurements are provided as Supplementary Materials (SM.1). Tardigrade taxonomy follows Vecchi et al. (2016) and Guil et al. (2019).

Comparative material. The new species was compared to original descriptions of all nine species of the *M. furciger* complex published so far: *M. aradasi* Binda, Pilato & Lisi, 2005, *M. creber* Pilato & Lisi, 2009, *M. divergens* Binda, Pilato & Lisi, 2005, *M. furciger* Murray, 1907b, *M. kovalevi* Tumanov, 2004, *M. orcadensis* Murray, 1907c, *M. pilatoi* Binda & Rebecchi, 1992, *M. siamensis* Tumanov, 2006, and *M. sicheli* Binda, Pilato & Lisi, 2005.

DNA extraction and genetic marker amplification. Individual tardigrades from the three isofemale cultures were starved in distilled deionised water for 2 days prior to DNA extraction to remove contaminating microorganisms and food particles. Genomic DNA was extracted using the Nucleospin® *Tissue genomic DNA Extraction Kit* (Macherey-Nagel) following the manufacturer's protocol. Four markers were amplified from the DNA extracts. These were composed of three nuclear markers, namely the small ribosome subunit (18S rRNA), the large ribosome subunit (28S rRNA), and the internal transcribed spacer-2 (ITS-2); and one mitochondrial gene, the cytochrome oxidase subunit I (COI). The sequences of the primers used to amplify these markers are listed in Table 1. The PCR cocktails and profiles are provided in the Supplementary Materials (SM.2). The amplicons were sent to 1st Base Asia or Macrogen Korea for sequencing. The sequences were then processed using BioEdit ver. 7.1.9 (Hall, 1999) and submitted to GenBank.

Comparative genetic analysis. For molecular comparisons, all published sequences of the four aforementioned markers for species of the genus *Mesobiotus* were downloaded from

Table 1. Primers and references for PCR protocols used in this study.

DNA fragment	Primer name	Primer direction	Primer sequence (5'–3')	Primer source
18S rRNA	18S_Tar_Ff1	forward	AGGCGAAACCGCGAATGGCTC	Stec et al. (2017)
	18S_Tar_Rr2	reverse	CTGATCGCCTTCGAACCTCTAACTTTCG	Gąsiorek et al. (2017)
28S rRNA	28SF0001	forward	ACCCVCYNAATTTAAGCATAT	Mironov et al. (2012)
	28SR0990	reverse	CCTTGGTCCGTGTTTCAAGAC	
ITS-2	Eutar_Ff	forward	CGTAACGTGAATTGCAGGAC	Stec et al. (2018a)
	Eutar_Rr	reverse	TCCTCCGCTTATTGATATGC	
COI	LCO1490	forward	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
	HCOoutout	reverse	GTAAATATATGRTGDGCTC	Prendini et al. (2005)

GenBank (listed in Table 2). The sequences were aligned using the Auto Strategy (in the case of COI and ITS-2) and the Q-INS-I method (in the case of ribosomal markers: 18S rRNA, 28S rRNA) of MAFFT version 7 (Katoh et al., 2002; Katoh & Toh, 2008) and manually checked for non-conservative alignments in BioEdit. The aligned sequences were then trimmed to the shortest available alignment: 717 bp (18S rRNA), 660 bp (28S rRNA), 462 bp (ITS-2), or 565 bp (COI). All COI sequences were translated into protein sequences in MEGA7 version 7.0 (Kumar et al., 2016) to check against pseudogenes. Uncorrected pairwise distances were calculated using MEGA7 and all the matrices are provided in the Supplementary Materials (SM.3).

TAXONOMY

Phylum Tardigrada Doyère, 1840

Class Eutardigrada Richters, 1926

Order Macrobiotoidea Thulin, 1928 (sensu Guil et al., 2019)

Family Macrobiotidae Thulin, 1928

Genus *Mesobiotus* Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti, 2016

Mesobiotus dilimanensis, new species

(Tables 3, 4, Figs. 1–5)

Material examined: 65 animals (including eight simplex), 47 eggs, and 7 empty chorions mounted on microscope slides in Hoyer's medium, 5 eggs fixed on SEM stubs, and 8 specimens processed for DNA sequencing.

Type locality: 14°39′40″N, 121°04′07″E; 76 m asl: Philippines, Quezon City, Diliman, University of the Philippines, A. Roces St.; moss on a rock; September 2015; coll. Lowelyn Itang.

Etymology: The species is named after Diliman, the district in Quezon City, Philippines, where it was discovered.

Type depositories: Holotype: slide PH.006.10 with six paratypes; 58 paratypes (slides: PH.006.*, where the asterisk can be substituted by any of the following numbers: 02–03, 11–16), 47 eggs (slides: PH.006.*: 5–9, 17); seven empty chorions (slides: PH.006.*: 01, 04) are deposited at the Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387, Kraków, Poland.

RESULTS

Description of the new species. Animals (measurements and statistics in Table 3): Body white but after fixation in Hoyer's medium transparent (Fig. 1A). Eyes absent (observed in live animals). Body cuticle smooth with fine granulation

Table 2. Sequences used for molecular comparisons between the *Mesobiotus dilimanensis*, new species, and all other species of the genus *Mesobiotus*, for which homologous DNA sequences are currently available. Sequences newly obtained in this study are bolded whereas underlined GenBank accession numbers indicate that these sequences represent type or neotype populations.

DNA marker	Species	Accession number	Source
18S rRNA	***M. dilimanensis*, new species**	**MN257048**	**this study**
	M. ethiopicus Stec & Kristensen, 2017	MF678793	Stec & Kristensen (2017)
	M. philippinicus Mapalo et al., 2016	KX129793	Mapalo et al. (2016)
	M. insanis Mapalo et al., 2017	MF441488	Mapalo et al. (2017)
	M. datanlanicus Stec, 2019	MK584659	Stec (2019)
	M. hilariae Vecchi et al., 2016	KT226068–71	Vecchi et al. (2016)
	M. polaris (Murray, 1910)	KT226075–8	Vecchi et al. (2016)
	M. cf. *mottai* (Binda & Pilato, 1994)	KT226072	Vecchi et al. (2016)
	M. aff. *harmsworthi*	KT226073–4	Vecchi et al. (2016)
	M. radiatus (Pilato et al., 1991)	MH197153	Stec et al. (2018b)
	M. romani Roszkowska, Stec & Gawlak, 2018	MH197158	Roszkowska et al. (2018)
	M. harmsworthi (Murray, 1907a)	MH197146	Kaczmarek et al. (2018b)
	M. occultatus Kaczmarek et al., 2018b	MH197147	Kaczmarek et al. (2018b)
	M. aff. *furciger*	MH197148	Kaczmarek et al. (2018b)
	M. aff. *harmsworthi*	MH197149	Kaczmarek et al. (2018b)
	M. aff. *harmsworthi*	HQ604967–70	Bertolani et al. (2014)
	M. furciger (Murray, 1907b)	EU266927–8	Sands et al. (2008)
28S rRNA	***M. dilimanensis*, new species**	**MN257049**	**this study**
	M. ethiopicus Stec & Kristensen, 2017	MF678792	Stec & Kristensen (2017)
	M. philippinicus Mapalo et al., 2016	KX129794	Mapalo et al. (2016)
	M. insanis Mapalo et al., 2017	MF441489	Mapalo et al. (2017)
	M. datanlanicus Stec, 2019	MK584658	Stec (2019)
	M. radiatus (Pilato et al., 1991)	MH197152	Stec et al. (2018b)
	M. romani Roszkowska et al., 2018	MH197151	Roszkowska et al. (2018)
	M. harmsworthi s.s. (Murray, 1907a)	MH197264	Kaczmarek et al. (2018b)
	M. aff. *furciger*	MH197265	Kaczmarek et al. (2018b)
	M. aff. *harmsworthi*	MH197266	Kaczmarek et al. (2018b)
ITS-2	***M. dilimanensis*, new species**	**MN257050**	**this study**
	M. ethiopicus Stec & Kristensen, 2017	MN122776	Stec (2019)
	M. philippinicus Mapalo et al., 2016	KX129795	Mapalo et al. (2016)
	M. insanis Mapalo et al., 2017	MF441490	Mapalo et al. (2017)
	M. datanlanicus Stec, 2019	MK584657	Stec (2019)
	M. radiatus (Pilato et al., 1991)	MH197267–8	Stec et al. (2018b)
	M. romani Roszkowska et al., 2018	MH197150	Roszkowska et al. (2018)
	M. harmsworthi s.s. (Murray, 1907a)	MH197154	Kaczmarek et al. (2018b)
	M. occultatus Kaczmarek et al., 2018b	MH197155	Kaczmarek et al. (2018b)
	M. aff. *furciger*	MH197156	Kaczmarek et al. (2018b)
	M. aff. *harmsworthi*	MH197157	Kaczmarek et al. (2018b)
COI	***M. dilimanensis*, new species**	**MN257047**	**this study**
	M. ethiopicus Stec & Kristensen, 2017	MF678794	Stec & Kristensen (2017)
	M. philippinicus Mapalo et al., 2016	KX129796	Mapalo et al. (2016)
	M. insanis Mapalo et al., 2017	MF441491	Mapalo et al. (2017)
	M. datanlanicus Stec, 2019	MK578905	Stec (2019)
	M. hilariae Vecchi et al., 2016	KT226108	Vecchi et al. (2016)
	M. radiatus (Pilato et al., 1991)	MH195147–8	Stec et al. (2018b)
	M. romani Roszkowska et al., 2018	MH195149	Roszkowska et al. (2018)
	M. harmsworthi s.s. (Murray, 1907a)	MH195150–1	Kaczmarek et al. (2018b)
	M. occultatus Kaczmarek et al., 2018b	MH195152	Kaczmarek et al. (2018b)
	M. aff. *furciger*	MH195153	Kaczmarek et al. (2018b)
	"*M. harmsworthi*" [misidentification]	GU113140	unpublished
	M. aff. *harmsworthi*	MH195154	Kaczmarek et al. (2018b)
	M. furciger (Murray, 1907b)	JX865306, JX865308, JX865314	Czechowski et al. (2012)

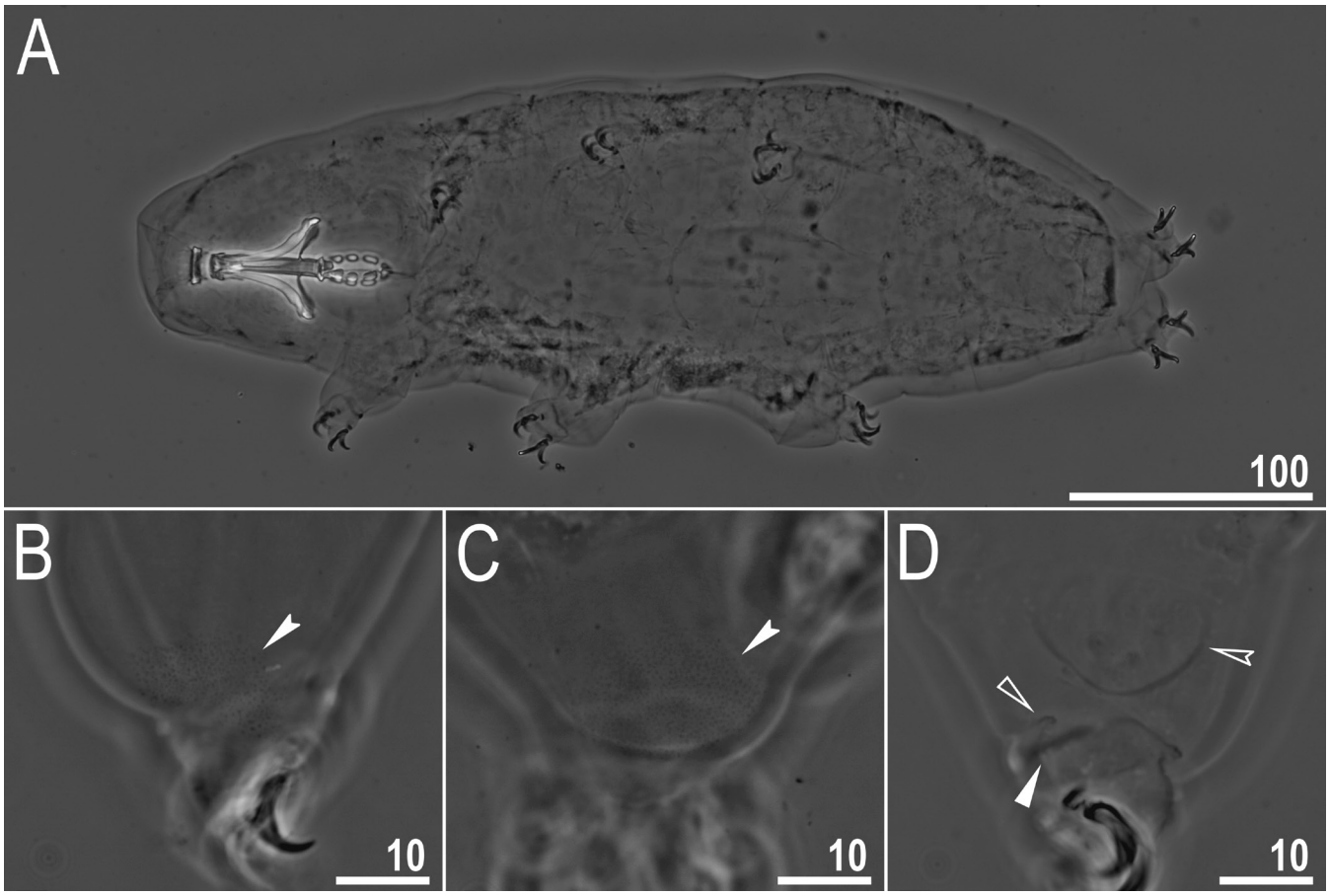

Fig. 1. *Mesobiotus dilimanensis*, new species, PCM image of habitus and leg's cuticle morphology. A, dorso-ventral projection (holotype); B, granulation on the external surface of leg III (paratype); C, granulation on dorsal surface of leg IV (paratype); D, pulvinus on the internal surface of leg II (paratype). Filled indented arrowheads indicate the granulation on the legs, empty indented arrowhead indicates the pulvinus, the filled flat arrowhead indicates the cuticular bar under the claws and the empty flat arrowhead indicated muscle attachment. Scale bar in μm.

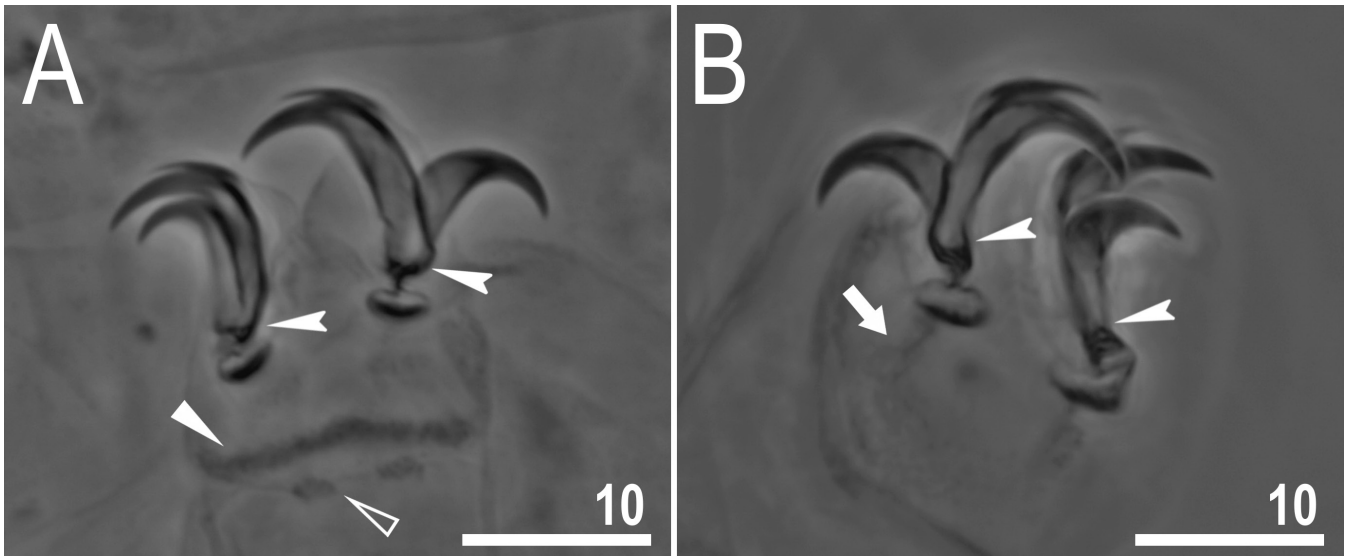

Fig. 2. *Mesobiotus dilimanensis*, new species, PCM images of claws. A, claws III with smooth lunules (holotype); B, claws IV with smooth lunules (paratype). Filled indented arrowheads indicate claws' septa, filled arrowhead indicates the cuticular bar, the empty arrowhead indicates paired muscles attachments and arrow indicates horseshoe structure connecting the anterior and the posterior claw. Scale bars in μm.

Table 3. Measurements (in µm) of selected morphological structures of individuals of *Mesobiotus dilimanensis*, new species, mounted in Hoyer's medium (N, number of specimens/structures measured; RANGE, refers to the smallest and the largest structure among all measured specimens; SD, standard deviation).

CHARACTER	N	RANGE		MEAN		SD		Holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Body length	29	235–426	633–999	345	840	50	105	396	957
Buccopharyngeal tube									
Buccal tube length	30	35.8–44.3	–	41.0	–	2.1	–	41.4	–
Stylet support insertion point	30	28.4–35.2	78.0–81.4	32.6	79.5	1.6	0.9	33.4	80.7
Buccal tube external width	30	5.7–7.0	14.0–17.4	6.4	15.7	0.4	0.7	6.9	16.7
Buccal tube internal width	30	4.4–5.6	11.4–14.0	5.1	12.5	0.3	0.7	5.5	13.3
Ventral lamina length	27	20.8–28.4	51.0–68.4	24.2	59.0	1.9	4.0	28.3	68.4
Placoid lengths									
Macroplacoid 1	30	4.1–6.9	10.1–16.0	5.4	13.0	0.7	1.4	5.5	13.3
Macroplacoid 2	30	3.4–5.0	7.8–11.9	4.2	10.3	0.4	1.0	4.4	10.6
Macroplacoid 3	30	4.1 – 6.4	9.6–15.3	5.3	13.0	0.7	1.4	5.6	13.5
Microplacoid	29	3.7–5.8	8.7–13.1	4.4	10.7	0.5	1.0	5.1	12.3
Macroplacoid row	30	15.2–21.0	38.6–47.6	18.1	44.1	1.6	2.4	19.6	47.3
Placoid row	29	19.9–27.1	51.8–62.2	23.8	58.0	2.0	2.9	24.3	58.7
Claw 1 heights									
External primary branch	28	8.8–12.1	22.9–27.7	10.5	25.7	0.8	1.3	11.0	26.6
External secondary branch	29	6.1–10.5	16.4–24.0	7.9	19.0	1.0	1.6	7.4	17.9
Internal primary branch	28	7.8–12.6	18.2–28.8	10.0	24.7	1.1	2.4	11.2	27.1
Internal secondary branch	27	5.5–10.7	14.5–21.7	7.6	18.2	1.1	1.9	6.8	16.4
Claw 2 heights									
External primary branch	30	10.0–12.9	25.7–31.1	11.5	28.1	0.9	1.5	11.6	28.0
External secondary branch	29	6.7–9.9	16.2–24.3	8.2	20.0	0.9	1.9	6.7	16.2
Internal primary branch	27	9.2–12.0	23.3–28.2	10.5	25.6	0.8	1.4	9.9	23.9
Internal secondary branch	26	6.0–9.8	14.8–22.7	8.0	19.4	0.9	1.8	7.5	18.1
Claw 3 heights									
External primary branch	29	9.2–12.9	22.3–30.0	11.3	27.5	0.9	1.7	10.6	25.6
External secondary branch	27	6.8–11.3	17.1–25.9	8.4	20.5	0.9	1.9	8.1	19.6
Internal primary branch	30	8.0–11.6	18.3–29.0	10.3	25.0	0.9	2.3	9.7	23.4
Internal secondary branch	28	4.8–10.0	11.6–22.5	7.6	18.4	1.0	2.3	4.8	11.6
Claw 4 heights									
Anterior primary branch	27	9.7–14.8	24.2–33.4	11.5	27.9	1.1	2.2	10.4	25.1
Anterior secondary branch	26	6.5–9.6	15.7–22.9	8.5	20.7	0.8	1.8	6.5	15.7
Posterior primary branch	27	10.7–14.8	26.7–36.2	12.3	30.0	1.0	2.1	12.0	29.0
Posterior secondary branch	22	7.4–10.9	19.2–24.7	8.6	21.1	0.7	1.4	8.5	20.5

on the external surface of legs (Fig. 1B, C). Pulvinus (a cuticular bulge) present on the internal surface of the legs I–III (Fig. 1D).

Claws of the *Mesobiotus* type, with a septum and a peduncle connecting the claw to the lunula (Fig. 2A, B). Lunules on all legs smooth (Fig. 2A, B). Well-developed accessory points on the primary branches of all claws (Fig. 2A, B). A cuticular bar is present under claws I–III, with muscle attachments visible below the bar (Figs. 1D, 2A). A horseshoe structure connects anterior and posterior claws on legs IV (Fig. 2B).

Mouth antero-ventral. Bucco-pharyngeal apparatus of the *Mesobiotus* type (Fig. 3A). Oral cavity starts with ten peribuccal lamellae. All three bands of teeth within the oral cavity visible under PCM (Fig. 3B–D). The first band is composed of fine granules located anteriorly within the buccal cavity just at the base of peribuccal lamellae (Fig. 3B–D). The second band is located posteriorly in the cavity and is composed of ridges parallel to the main axis of the buccal tube (Fig. 3B–D). The third band of teeth is discontinuous and divided into a dorsal and ventral portion. Under PCM, dorsal teeth are visible as two lateral and one median transverse ridges/crests (Fig. 3B), whereas ventral teeth consist of two lateral transverse ridges/crests between which two or three roundish and separated ventro-median teeth are present (Fig. 3C, D). Buccal tube rigid, with ventral lamina. Pharyngeal apophyses, three macroplacoids,

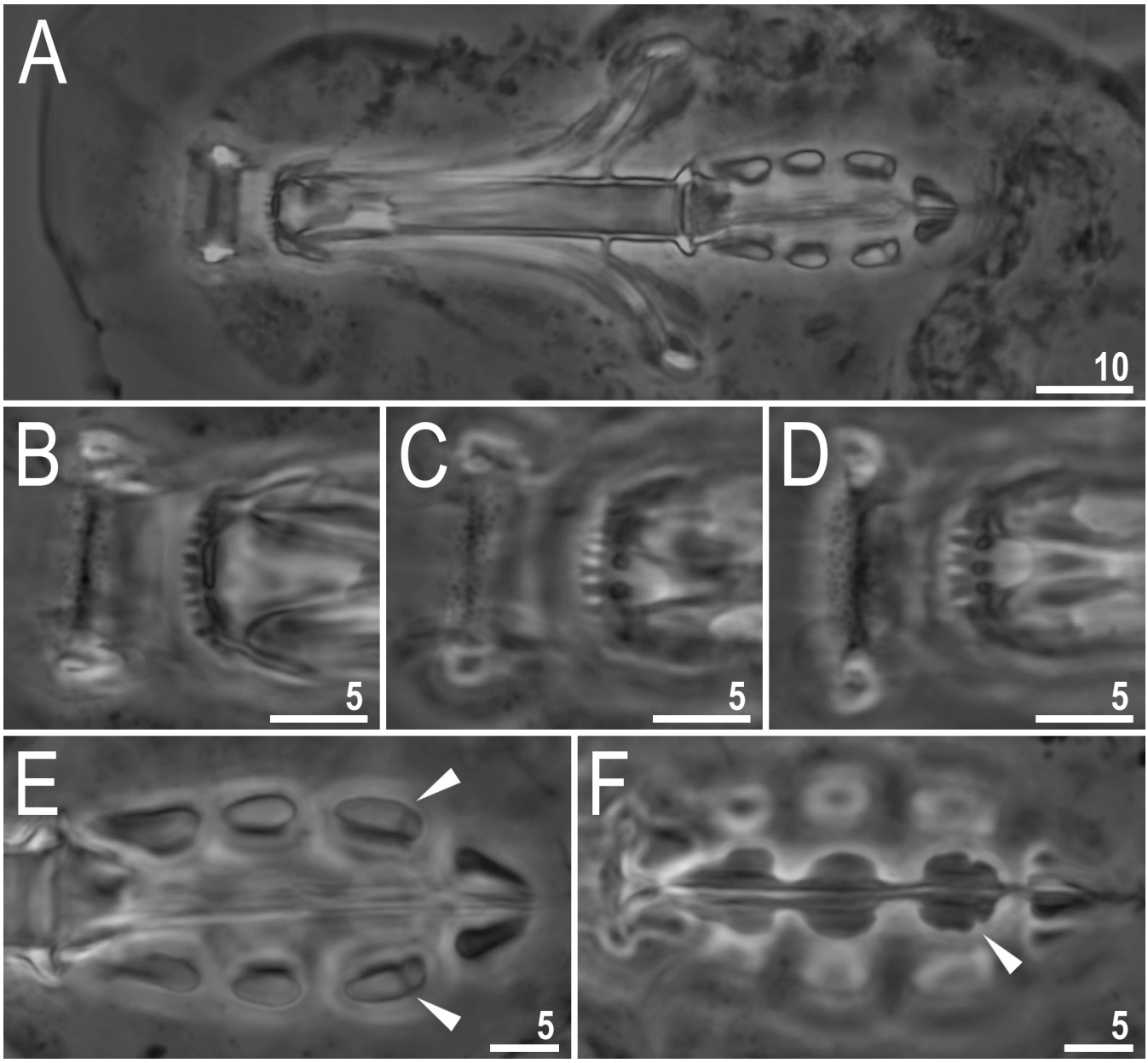

Fig. 3. *Mesobiotus dilimanensis*, new species, PCM images of the buccal apparatus. A, an entire buccal apparatus (paratype); B, C, the oral cavity armature of the paratype, dorsal and ventral teeth respectively; D, the oral cavity armature of another paratype, ventral teeth; E, F, placoid morphology of the paratype, dorsal and ventral placoids respectively. Arrowheads indicate subterminal constrictions in the third macroplacoid. Scale bars in μm.

and a large comma-shaped microplacoid are present in the pharyngeal bulb. The macroplacoids are rod-shaped, with the first macroplacoid narrowed anteriorly and the third macroplacoid constricted subterminally (Fig. 3E, F). The macroplacoid sequence is 2<1=3.

Eggs (measurements and statistics in Table 4). Spherical, laid freely, with processes in the shape of cones with multiple apices (Figs. 4A, 5A). Under LCM, egg surface covered with fine but clearly visible reticulum, typically with three to four meshes between the neighbouring processes (Fig. 4B–D). In contrast to LCM, under SEM, egg surface appears porous rather than reticulated, with pores (0.1–0.4 μm in diameter) being often clearly smaller than mesh walls that can be up to 0.7 μm thick (Fig. 5B, C). Crowns of granular thickenings are present around the base of processes (Figs. 4C, D, 5B, C; flat arrowheads). Small globular tubercles are faintly visible on the process surfaces in PCM (seen as darkenings on the process surface) and clearly visible in SEM (Figs. 4C, D, 5B–D; indented arrowheads). Process apex divided into multiple (typically five to ten) short, nodular, finger-like apices (Figs. 4E–J, 5B–F). Under SEM, apices usually covered with regular microgranulation (Fig. 5B–E) and only rarely smooth (Fig. 5F).

DNA sequences. The obtained sequences for all four analysed molecular markers were represented by single haplotypes. We obtained one sequence for each of the conserved markers (18S rRNA and 28S rRNA), and two and four sequences for the variable markers (ITS-2 and COI, respectively). The **18S rRNA** sequence (GenBank: MN257048), 896 bp long; the **28S rRNA** sequence (GenBank: MN257049), 749 bp long;

Table 4. Measurements (in µm) of selected morphological structures of the eggs of *Mesobiotus dilimanensis*, new species, mounted in Hoyer's medium (N, number of eggs/structures measured; RANGE, refers to the smallest and the largest structure among all measured specimens; SD, standard deviation).

CHARACTER	N	RANGE	MEAN	SD
Diameter of egg without processes	13	57.7 – 75.8	65.5	6.4
Diameter of egg with processes	13	69.1 – 88.5	77.0	6.4
Process height	90	4.8 – 7.8	6.5	0.6
Process base width	90	4.0 – 7.5	5.6	0.7
Process base/height ratio	90	57% – 119%	88%	13%
Distance between processes	90	1.2 – 3.4	2.3	0.5
Number of processes on the egg circumference	13	18 – 24	21.0	1.7

the **ITS-2** sequence (GenBank: MN257050), 450 bp long; the **COI** sequence (GenBank: MN257047), 781 bp long.

DISCUSSION

Phenotypic differential diagnosis. The presence of three macroplacoids and a microplacoid placed closed to the macroplacoids indicates that the species represents the genus *Mesobiotus*, and the branching of the processes at the apex places it in the *Mesobiotus furciger* species group. However, *Mesobiotus dilimanensis*, new species, differs specifically from the known species of the group:

***Mesobiotus aradasi*.** Reported from the type locality in King George Island and from the Argentine Islands (Binda et al., 2005; Kaczmarek et al., 2018a), by: the absence of eyes, the presence of granulation on all legs (granulation on legs absent in *M. aradasi*), the presence of the first band of teeth in the oral cavity under LCM (the first band of teeth absent in *M. aradasi*), more posteriorly positioned stylet supports (*pt*=78.0–81.4 in the new species vs. 72.0–72.8 in *M. aradasi*), a larger microplacoid (3.7–5.8 µm [*pt*=8.7–13.1] in the new species vs. 2.7–3.6 µm [*pt*=7.0–7.6] in *M. aradasi*), a smaller egg bare diameter (57.7–75.8 µm in the new species vs. 83.5–90.5 µm in *M. aradasi*), a smaller egg full diameter (69.1–88.5 µm in the new species vs. 97.5–100 µm in *M. aradasi*), a smaller process base width (4.0–7.5 µm in the new species vs. 8.4–9.5 µm in *M. aradasi*), larger pores/meshes on the egg shell surface, and by a different point of division of the egg process apex (division close to the process tip in the new species vs. division at the half of the process height in *M. aradasi*).

***Mesobiotus creber*.** Reported only from the type locality in the Seychelles Islands (Pilato & Lisi, 2009), by: the presence of granulation on all legs (granulation on legs absent in *M. creber*), relatively smaller buccal tube external width (*pt*=14.0–17.4 in the new species vs. 17.9–19.6 in *M. creber*), a larger egg full diameter (69.1–88.5 µm in the new species vs. 59–66 µm in *M. creber*), and fewer processes on the egg circumference (18–24 in the new species vs. 27–30 in *M. creber*).

***Mesobiotus divergens*.** Reported only from the type locality in New Zealand (Binda et al., 2005), by: the morphology of the stylet sheaths (typical in the new species vs. caudally thickened lateral portions of stylet sheaths in *M. divergens*), more posteriorly positioned stylet supports (*pt*=78.0–81.4 in the new species vs. 75.5–76.2 in *M. divergens*), a relatively longer placoid row (*pt*=51.8–62.2 in the new species vs. 45.4–51.6 in *M. divergens*), a larger microplacoid (3.7–5.8 µm [*pt*=8.7–13.1] in the new species vs. 1.7–3.1 µm [*pt*=7.1–7.4] in *M. divergens*), more processes on the egg circumference (18–24 in the new species vs. 17 in *M. divergens*), and by a different point of division of the egg process apex (division close to the process tip in the new species vs. division at half of the process height in *M. divergens*).

***Mesobiotus furciger*.** Reported from the type locality in South Orkney Islands (Murray, 1906) and many other localities throughout the world but most probably the distribution of this species is restricted to the maritime Antarctic and sub-Antarctic regions (Murray, 1906; McInnes, 1994; Binda et al., 2005; Kaczmarek et al., 2016), by: the absence of eyes, a different morphology of the third macroplacoid (subterminally constricted in the new species vs. not constricted in *M. furciger*), a different morphology of the lunulae IV (smooth in the new species vs. finely indented in *M. furciger*), a different macroplacoid sequence (2<1=3 in the new species vs. 2<1<3 in *M. furciger*), a shorter buccal tube (35.8–44.3 µm in the new species vs. 44.9–48.9 µm in *M. furciger*), more posteriorly positioned stylet supports (*pt*=78.0–81.4 in the new species vs. 76.2–77.1 in *M. furciger*), a shorter macroplacoid row (15.2–21.0 µm [*pt*=38.6–47.6] in the new species vs. ca. 21.5 µm [*pt*=47.9] in *M. furciger*), a shorter second macroplacoid (3.4–5.0 µm [*pt*=7.8–11.9] in the new species vs. ca. 5.8 µm [*pt*=12.9] in *M. furciger*), a shorter third macroplacoid (4.1–6.4 µm [*pt*=9.6–15.3] in the new species vs. ca. 8.3 µm [*pt*=18.5] in *M. furciger*), a smaller egg bare diameter (57.7–75.8 µm in the new species vs. 91.7–100.4 µm in *M. furciger*), a smaller egg

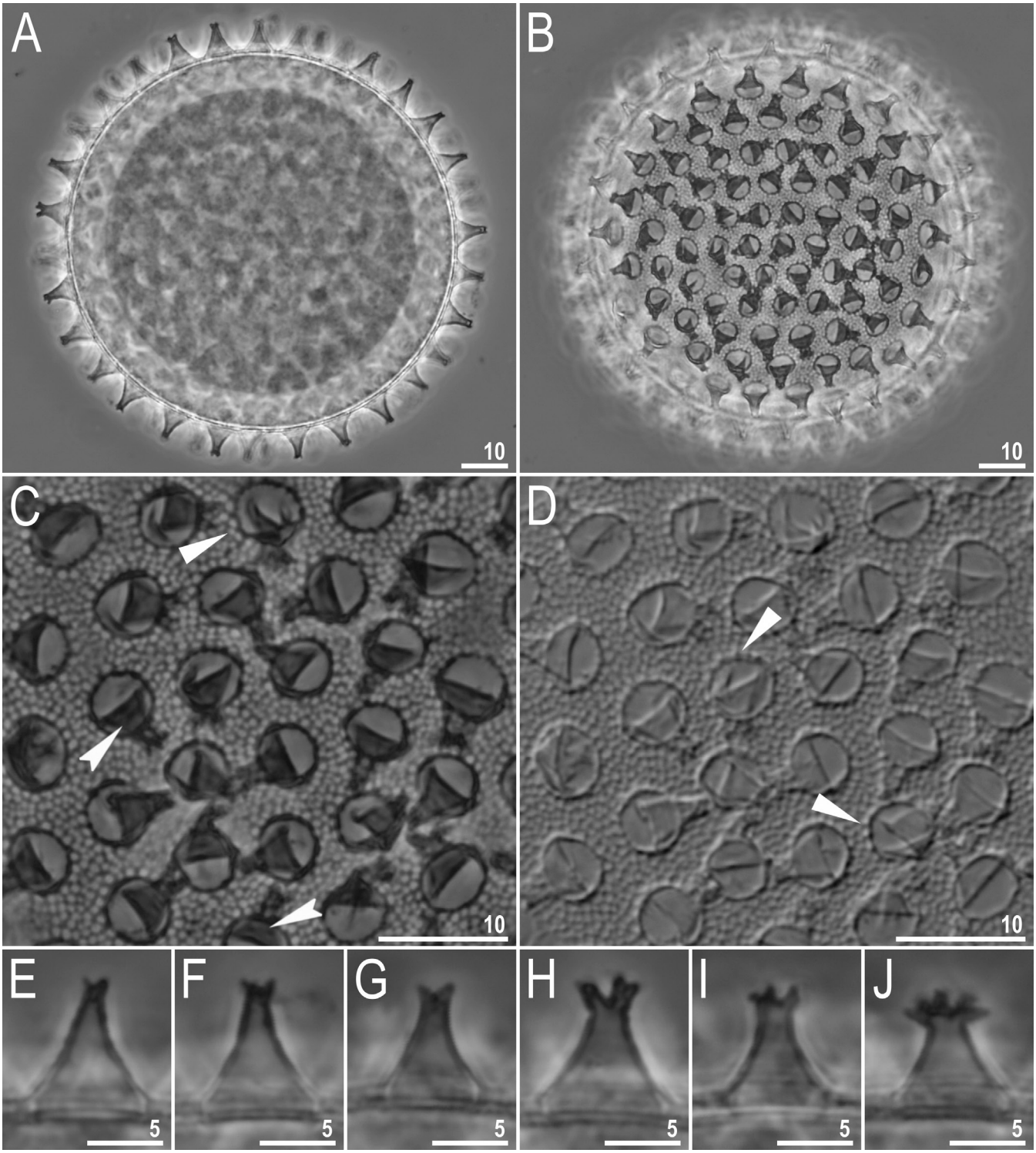

Fig. 4. *Mesobiotus dilimanensis*, new species, images of the eggs under light microscopy. A, midsection (PCM); B, surface (PCM); C, D, surfaces under a ×1000 magnification (PCM and NCM, respectively); E–J, midsections of six different egg processes (PCM). Flat arrowheads indicate faint thickenings around the processes' bases, indented arrowheads indicate small tubercles on the surface of the egg processes. Scale bars in μm.

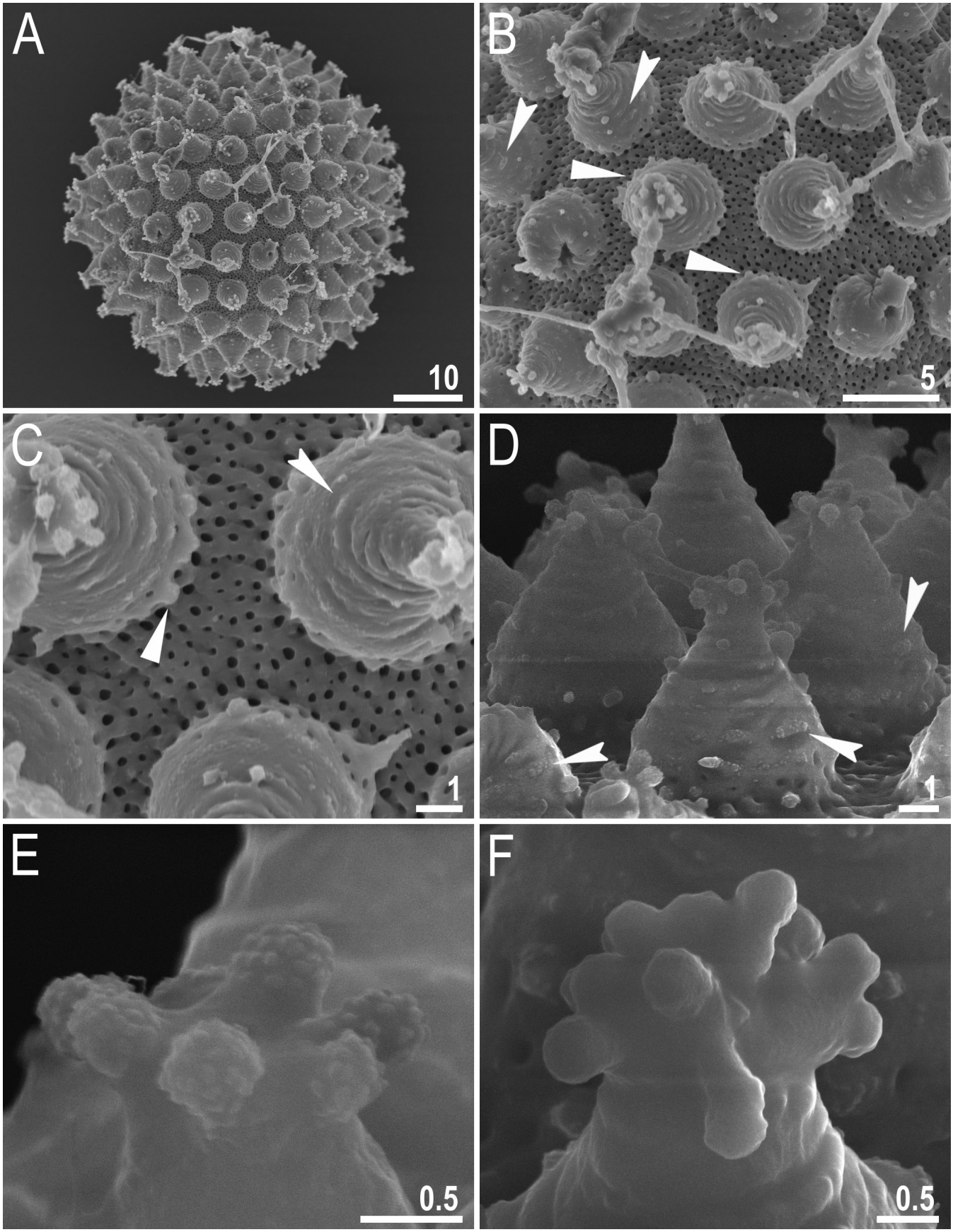

Fig. 5. *Mesobiotus dilimanensis*, new species, images of eggs under SEM. A, entire view of the egg; B, C, details of the egg surface between processes; D, egg processes; E, F, a top part of the processes divided into several short nodular branches. Flat arrowheads indicate faint thickenings around the processes' bases, indented arrowheads indicate small tubercles on the surface of the egg processes. Scale bars in μm.

full diameter (69.1–88.5 µm in the new species vs. 110–123 µm in *M. furciger*), shorter egg processes (4.8–7.8 µm in the new species vs. 12–13 µm in *M. furciger*), narrower egg process bases (4.0–7.5 µm in the new species vs. 11–14.5 µm in *M. furciger*), and by the absence of refracting areas/ bubble-like structures within the walls of the egg processes.

Remarks. Since the original description of *M. furciger* is outdated and incomplete, we used data presented by Binda et al. (2005) for this comparison.

Mesobiotus kovalevi. Reported only from the type locality in New Zealand (Tumanov, 2004), by: the absence of eyes, the presence of granulation on legs (absent in *M. kovalevi*), the presence of three bands of teeth (the first and the second band of teeth absent or invisible under light microscope in *M. kovalevi*), a wider inner buccal tube diameter (4.4–5.6 µm in the new species vs. 2.4–4.4 µm in *M. kovalevi*), a longer microplacoid (3.7–5.8 µm [*pt*=8.7–13.1] in the new species vs. 1.9–3.3 µm [*pt*=3.6–5.8] in *M. kovalevi*), a smaller bare egg diameter (57.7–75.8 µm in the new species vs. 86–95 µm in *M. kovalevi*), shorter egg processes (4.8–7.8 µm in the new species vs. 12–17 µm in *M. kovalevi*), and slightly fewer processes on the egg circumference (18–24 in the new species vs. ca. 25 in *M. kovalevi*).

***Mesobiotus orcadensis*.** Reported from the type locality in Scotland (Murray, 1907c) and many other localities throughout the world but most probably the distribution of this species is restricted to the Palaearctic region (McInnes, 1994; Binda et al., 2005; Kaczmarek et al., 2015), by: the absence of eyes, the presence of the first band of teeth in the oral cavity under LCM (the first band of teeth absent in *M. orcadensis*), a shorter buccal tube (35.8–44.3 µm in the new species vs. 45.1 µm in *M. orcadensis*), a narrower buccal tube external diameter (5.7–7.0 µm [*pt*=14.0–17.4] in the new species vs. ca. 7.9 µm [*pt*=17.5] in *M. orcadensis*), more posterior stylet supports (*pt*=78.0–81.4 in the new species vs. 76.5 in *M. orcadensis*), a shorter first macroplacoid (4.1–6.9 µm in the new species vs. ca. 7.1 µm in *M. orcadensis*), a shorter second macroplacoid (3.4–5.0 µm [*pt*=7.8–11.9] in the new species vs. ca. 6.5 µm [*pt*=14.4] in *M. orcadensis*), a smaller third macroplacoid (4.1–6.4 µm in the new species vs. 6.6 µm in *M. orcadensis*), a smaller egg bare diameter (57.7–75.8 µm in the new species vs. 81 µm in *M. orcadensis*), and by a smaller egg full diameter (69.1–88.5 µm in the new species vs. 91 µm in *M. orcadensis*).

Remarks. The original description of *M. orcadensis* is outdated, thus we used data from Pilato & Lisi (2009) for this comparison.

Mesobiotus pilatoi. Reported from the type locality in Italy and from Canada (Argue, 1972; Binda & Rebecchi, 1992), by: a different morphology of lunulae IV (smooth in the new species vs. serrated in *M. pilatoi*), a shorter body length (235–426 µm in the new species vs. 507–600 µm in *M. pilatoi*), a shorter buccal tube (35.8–44.3 µm in the new species vs. ca. 65.7 µm in *M. pilatoi*), a relatively shorter placoid row (*pt*=51.8–62.2 in the new species vs. 67.96–72.76 in *M. pilatoi*), a relatively shorter macroplacoid row (*pt*=38.6–47.6 in the new species vs. 54.14–57.00 in *M. pilatoi*), a relatively shorter first macroplacoid (*pt*=10.1–16.0 in the new species vs. 16.72–17.77 in *M. pilatoi*), a relatively shorter second macroplacoid (*pt*=7.8–11.9 in the new species vs. 12.54–15.21 in *M. pilatoi*), a relatively shorter third macroplacoid (*pt*=9.6–15.3 in the new species vs. 16.23–18.13 in *M. pilatoi*), a smaller egg bare diameter (57.7–75.8 µm in the new species vs. 90–100 µm in *M. pilatoi*), a smaller egg full diameter (69.1–88.5 µm in the new species vs. 115–130 µm in *M. pilatoi*), shorter egg processes (4.8–7.8 µm in the new species vs. 15 µm in *M. pilatoi*), narrower process bases (4.0–7.5 µm in the new species vs. 15 µm in *M. pilatoi*), and by the absence of refracting points at the base of the process surface.

Remarks. According to Binda & Rebecchi (1992), this species of the *furciger* complex has been suggested to represent many of the Northern Hemisphere *M. furciger* records.

Mesobiotus siamensis. Reported only from the type locality in Thailand (Tumanov, 2006), by: the presence of granulation on all legs (granulation on legs absent in *M. siamensis*), more developed first band of teeth in the oral cavity (always clearly visible under LCM in the new species vs. barely visible even in largest specimens of *M. siamensis*), a different morphology of lunulae IV (smooth in the new species vs. with undulated margins in *M. siamensis*), and by shorter processes (4.8–7.8 µm in the new species vs. 10.7–11.8 µm in *M. siamensis*).

Mesobiotus sicheli. Reported only from the type locality in South Africa (Binda et al., 2005), by: the absence of eyes, the absence of fine granulation on the dorsal cuticle (the granulation present in *M. sicheli*), a relatively shorter second macroplacoid (*pt*=7.8–11.9 in the new species vs. 12.3–13.4 in *M. sicheli*), narrower egg process bases (4.0–7.5 µm in the new species vs. 7.9–12.2 µm in *M. sicheli*), and by the absence of peribasal striae around egg process bases.

Genetic comparison summary. The ranges of uncorrected genetic p-distances between the new species and species of the genus *Mesobiotus*, for which sequences are available from GenBank, are as follows:

18S rRNA: 4.1–8.6% (5.1% on average), with the most similar being *M. furciger* and *M. hilariae* Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti, 2016, from the Antarctic (EU266927 and KT226068–71, respectively) and the least similar being an undetermined *M. furciger* group species from Norway (MH197148);

28S rRNA: 12.2–15.6% (13.6% on average), with the most similar being *M. harmsworthi* s.s. (Murray, 1907a) from Norway (MH197264) and the least similar being *M. radiatus* (Pilato, Binda & Catanzaro, 1991) from Kenya (MH197152);

ITS-2: 38.1–46.0% (41.6% on average), with the most similar being *M. occultatus* Kaczmarek, Zawierucha, Buda, Stec,

Gawlak, Michalczyk & Roszkowska, 2018b, from Norway (MH197155) and the least similar being an undetermined *M. furciger* group species from Norway (MH197156);

COI: 21.2–26.7% (24.7% on average), with the most similar being an undetermined *M. furciger* group species from Norway (MH195153) and the least similar being *M. philippinicus* from the Philippines (KX129796).

CONCLUSIONS

We have identified a tardigrade species new to science using an integrative approach, including phase contrast light microscopy, scanning electron microscopy, and DNA analysis. The characteristic division of the egg process into several finger-like apices indicated that *Mesobiotus dilimanensis*, new species, represents the *Mesobiotus furciger* species complex. To the best of our knowledge, this is only the third limno-terrestrial tardigrade species found in the Philippines reported in a peer-reviewed publication and it is also the first species of the *M. furciger* complex identified using this type of analysis in the country.

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