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Integrative Description of a New Freshwater Tardigrade Species, *Dactylobiotus taiwanensis* (Tardigrada: Eutardigrada: Murrayidae), Discovered Through Social Media

Daniele Camarda[1], Chih-Yu Pai[2], Reinhardt Møbjerg Kristensen[3], and Daniel Stec[4,*]

[1]*University of Catania, Department of Biological, Geological and Environmental Sciences, Section of Animal Biology, Via Androne 81, 95124, Catania, Italy. E-mail: daniele.camarda@phd.unict.it (Camarda)*
[2]*Ruey Long Industry co., LTD, 1 F., No. 247, Sec. 3, Datong Rd., Xizhi Dist., New Taipei City 22178, Taiwan. E-mail: chihyupai@gmail.com (Pai)*
[3]*Natural History Museum of Denmark, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark. E-mail: rmkristensen@snm.ku.dk (Kristensen)*
[4]*Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland.*
**Correspondence: E-mail: daniel_stec@interia.eu (Stec)*

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Two freshwater tardigrade populations belonging to the genus *Dactylobiotus* were investigated using phase contrast microscopy, scanning electron microscopy, and molecular markers commonly employed in tardigrade phylogenetic studies (18S rRNA, 28S rRNA, ITS2, and *COI*). The population from Taiwan, discovered through social media, represents a new species, described here as *Dactylobiotus taiwanensis* sp. nov. This species is most similar to *Dactylobiotus parthenogeneticus* but differs in the presence of singular rings of pores surrounding the egg processes and specific morphometric traits. The second population, from Greenland, was provisionally identified as *D.* cf. *octavi*, and its morphological discrepancies are discussed in detail. A revision of the type material for *Dactylobiotus caldarellai* and *Dactylobiotus lombardoi* raises questions about their validity due to insufficient data. Finally, a phylogenetic analysis incorporating taxa from the family Murrayidae, along with the newly sequenced populations, is presented. An updated dichotomous key for the genus *Dactylobiotus* is also provided.

Key words: Meiofauna, Taxonomy, Freshwater, Egg ornamentation, New species

BACKGROUND

Tardigrades, commonly known as water bears or moss piglets, belong to a phylum of micrometazoans (50–1000 µm) with approximately 1500 described species (Degma and Guidetti 2007 2024; Guidetti and Bertolani 2005). The Phylum is represented by two classes, Heterotardigrada comprising armoured-bodied tardigrades (marine and limno-terrestrial) and the soft-bodied Eutardigrada (mostly limno-terrestrial), which inhabit nearly all limno-terrestrial environments on Earth (Nelson et al. 2015 2020).

Within Eutardigrada, the most species-rich superfamily is *Macrobiotoidea*, which currently includes 399 nominal taxa, 38 of which (approximately 10%) are considered doubtful. This superfamily accounts for 27% of all known tardigrade species. However, knowledge of some of its families remains limited due to the relatively small number of recognized and studied taxa. One such family is Murrayidae Guidetti, Rebecchi and Bertolani, 2000, which includes 37 nominal taxa grouped into four genera: *Dactylobiotus* Schuster, 1980 (in Schuster et al. 1980), the monospecific genus *Macroversum* Pilato and Catanzaro, 1988, *Murrayon* Bertolani and Pilato, 1988,

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and *Paramurrayon* Guidetti et al., 2022. Interestingly, sexual reproduction has never been observed in this family, and all its taxa are commonly recognized as parthenogenetic (Nelson et al. 2015 2020). Among the four recognized genera within Murrayidae, the cosmopolitan genus *Dactylobiotus* contains the majority of taxa (19 species), which are considered strictly freshwater inhabitants. However, the genus currently includes several species with vague diagnoses, four of which have already been designated as *nomina dubia*: *Dactylobiotus aquatilis* Yang, 1999, *Dactylobiotus henanensis* Yang, 2002, *Dactylobiotus kansae* Beasley, Miller and Shively, 2009, and *Dactylobiotus macronyx* (Dujardin, 1851) (Dastych et al. 2015; Kaczmarek et al. 2012; Pogwidz and Stec 2020).

When referring to the latest taxonomic key, the genus *Dactylobiotus* can be divided into two groups of species, distinguished by the presence or absence of papillae on the dorso-caudal region of the animals' body (Kaczmarek et al. 2012). Although this division currently lacks molecular confirmation due to limited genetic data, it remains a useful framework for species classification during morphological analyses. Currently, only a small amount of molecular data is available for species of this genus, as well as for other members of the family Murrayidae. This scarcity likely arises from the fact that the majority of taxa within the family were described prior to 2000, a time when genetic data were rarely used for the delineation and characterization of tardigrade species. At present, genetic data are available for six nominal species within the genus *Dactylobiotus*: *Dactylobiotus ambiguus* (Murray, 1907) (in Guil et al. 2019), *Dactylobiotus parthenogeneticus* Bertolani, 1982 (in Guidetti et al. 2022; Pogwidz and Stec 2020), *Dactylobiotus grandipes* (Schuster, Toftner and Grigarick, 1978) (in Guidetti et al. 2022), *Dactylobiotus octavi* Guidetti, Altiero and Hansen, 2006 (in Jorgensen et al. 2010), *Dactylobiotus ovimutans* Kihm, Kim, McInnes, Zawierucha, Rho, Kang and Park, 2020 (in Kihm et al. 2020), and *Dactylobiotus selenicus* Bertolani, 1982 (in Stec et al. 2020).

In this study, newly discovered populations of the genus *Dactylobiotus* from Taiwan and Greenland were investigated using an integrative approach. Morphological, morphometric, and phylogenetic analyses revealed that the Taiwanese population represents a species new to science, which is formally described here. The phenotypic discrepancies between the Greenlandic population and other similar taxa are discussed in detail. Additionally, the type material of two older species, *Dactylobiotus caldarellai* Pilato and Binda, 1994, and *Dactylobiotus lombardoi* Binda and Pilato, 1999, was re-examined, leading to further discussion on the validity of these taxa. Finally, an updated taxonomic key to the valid species of the genus *Dactylobiotus* is provided.

MATERIALS AND METHODS

Sample processing

In autumn 2023, the second author posted photos on Facebook of their freshwater tardigrade culture, showing hundreds of chunky, whitish tardigrades clustering together. Following a brief private conversation, a decision was made to collaborate on identifying the species. A sample of debris from a *Limnophila* sp. leaf was collected from a lotus pond in Xinzhuang Touqian Sports Park, New Taipei City, Taiwan, on December 1, 2022 (25.0506021, 121.4628704; leg. Chih-Yu Pai). Approximately 20 live tardigrades were observed and extracted from the sample under a stereomicroscope. These animals were transferred to a six-well plate containing a medium of spring water supplemented with algae and rotifers as food. The medium was changed every three days, with a fresh portion of food added to the culture, which was maintained at room temperature. After about one year of maintaining this culture, a batch of live animals and eggs was extracted and divided into three groups for specific analyses: (1) morphological analysis using phase contrast microscopy (PCM) and scanning electron microscopy (SEM), and (2) molecular analyses of DNA sequences targeting ribosomal markers (18S rRNA, 28S rRNA, ITS-2) and the mitochondrial marker *COI*. For details, please see the "Material examined" section below in the description. Additionally, a mixed sample of moss and algae was collected in Greenland from a wet stone in a river outlet of Tasersuaq (Lake) in Qaqortoq (Julianehåb) on July 30, 2022 (GPS: 60.7192427, -46.0409180; leg. Lars Engberg Hansen). The sample was examined for tardigrades using standard methods described by Stec et al. (2015). It contained animals and eggs belonging to the genus *Dactylobiotus*. An embryonated egg was used to obtain DNA sequences for this species, while other specimens were mounted on permanent slides or prepared for SEM analysis as described below.

Microscopy and imaging

Specimens for light microscopy were mounted on microscope slides in a small drop of Hoyer's medium and secured with a cover slip, following the protocol by Morek et al. (2016). The dried slides were sealed with transparent nail polish and examined under a Leica DMLB phase contrast microscope (PCM) equipped

with a digital camera. Specimens of the new species prepared for SEM were processed according to the "A2" protocol described by Camarda et al. (2024) and sputter-coated with gold. The SEM imaging was conducted using a Phenom XL-G2 SEM at the University of Catania, Sicily, Italy (voltage 15.00 kV, working distance 5.5 mm). Eggs of the Greenlandic population designated for SEM analyses were prepared following the protocol of Stec et al. (2015). Briefly, the eggs underwent a water/ethanol and ethanol/acetone series, followed by CO_2 critical point drying, and were subsequently sputter-coated with a thin layer of gold. These specimens were examined under high vacuum using a Versa 3D DualBeam Scanning Electron Microscope at the ATOMIN facility of the Jagiellonian University, Kraków, Poland (voltage 10.00 kV, working distance 10.2 mm). All figures were assembled in Corel Photo-Paint X6. For structures that could not be satisfactorily focused in a single photograph, a stack of 2–6 images was captured at an equidistance of approximately 0.2 µm and manually combined into a single deep-focus image.

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 and Binda (2010). The terminology used to describe oral cavity armature and egg shell morphology follows Michalczyk and Kaczmarek (2003). Macroplacoid length sequence is given according to Kaczmarek et al. (2014) whereas morphological states of cuticular bars on legs follow Kiosya et al. (2021). 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). Claws were measured according to Binda and Pilato (1999). Buccal tube width was measured as the external and internal diameter at the level of the stylet support insertion point. Distance between egg processes was measured as the shortest distance between the base edges of the two closest processes. Morphometric data underlying the new species description were handled using the "Parachela" ver. 1.8 template available from the Tardigrada Register (Michalczyk and Kaczmarek 2013) and are given in Supplementary Materials (SM. 1). Tardigrade taxonomy follows Bertolani et al. (2014) and Stec et al. (2020a).

DNA sequencing

The DNA was extracted from individual animals following a *Chelex® 100* resin (*Bio-Rad*) extraction method by Casquet et al. (2012) with modifications described in detail in Stec et al. (2020b). After DNA extraction, the exoskeletons of the new species were recovered from *Chelex®* beads and mounted on permanent slides as described above. This procedure was not successful for the embryonated egg of the Greenlandic population. Four DNA fragments differing in mutation rates were sequenced. Namely: the small ribosome subunit (18S rRNA, nDNA), the large ribosome subunit (28S rRNA, nDNA), the internal transcribed spacer (ITS-2, nDNA), and the cytochrome oxidase subunit I (*COI*, mtDNA). All fragments were amplified and sequenced according to the protocols described in Stec et al. (2020b); primers are listed in table 1. Sequencing products were read with the *ABI 3130xl* sequencer at the Genomed company (Warsaw, Poland). Sequences were processed in *BioEdit* ver.

Table 1. Primers with their original references used for amplification of the four DNA fragments sequenced in the study

DNA marker	Primer name	Primer direction	Primer sequence (5'-3')	Primer source
18S rRNA	18S_Tar_Ff1	forward	AGGCGAAACCGCGAATGGCTC	Stec et al. (2017)
	18S_Tar_Rr1	reverse	GCCGCAGGCTCCACTCCTGG	
28S rRNA	28SF0002	forward	GRCRAGAKTACCCGCTGAAC	Stec (2022)
	28SR0990	reverse	CCTTGGTCCGTGTTTCAAGAC	Mironov et al. (2012)
ITS-2	ITS2_Eutar_Ff	forward	CGTAACGTGAATTGCAGGAC	Stec et al. (2018)
	ITS2_Eutar_Rr	reverse	TCCTCCGCTTATTGATATGC	
COI	LCO1490-JJ	forward	CHACWAAYCATAAAGATATYGG	Astrin and Stüben (2008)
	HCO2198-JJ	reverse	AWACTTCVGGRTGVCCAAARAATCA	

7.2.5 (Hall 1999) and submitted to GenBank. Prior submission all obtained *COI* sequences were translated into protein sequences in *MEGA11* (Tamura et al. 2021) to check against pseudogenes.

Phylogenetic analysis

In order to investigate phyletic position of the new species and the Greenlandic population a phylogenetic tree was constructed. For this purpose a data set was compiled from taxa/specimens for which DNA sequences of at least two (out of all four analysed in this study) molecular markers are available and suitable for concatenation (Table 2). The DNA sequences of *Adorybiotus* cf. *granulatus* and *Crenubiotus salishani* Vecchi, Choong and Calhim, 2022 were used as the outgroup. The sequences were aligned using the AUTO method (for *COI* and ITS-2) and the Q-INS-I method (for ribosomal markers: 18S rRNA and 28S rRNA) of MAFFT version 7 (Katoh et al. 2002; Katoh and Toh 2008) and manually checked against non-conservative alignments in BioEdit. Then, the aligned sequences were trimmed to: 831 (18S rRNA), 725 (28S rRNA), 466 (ITS-2), 658 (*COI*) bp and concatenated using SequenceMatrix (Vaidya et al. 2011). Before partitioning, the concatenated alignment was divided into 6 data blocks constituting three separate blocks of ribosomal markers and three separate blocks of three codon positions in *COI* data set. Using PartitionFinder under the Akaike Information Criterion (AIC), the best scheme of partitioning and substitution models were chosen for Bayesian phylogenetic analysis. Bayesian inference (BI) marginal posterior probabilities were calculated for the concatenated (18S rRNA+28S rRNA+ITS-2+*COI*) data set using MrBayes v3.2 (Ronquist and Huelsenbeck 2003). Random starting trees were used and the analysis was run for fifteen million generations, sampling the Markov chain every 1000 generations. An average standard deviation of split frequencies of < 0.01 was used as a guide to ensure the two independent analyses had converged. The program Tracer v1.7 (Rambaut et al. 2018) was then used to ensure Markov chains had reached stationarity, and to determine the correct 'burn-in' for the analysis which was the first 10% of generations. The ESS values were greater than 200 and the consensus tree was obtained after summarising the resulting topologies and discarding the 'burn-in'. ModelFinder

Table 2. Sequences used for phylogenetic analysis. Bold font indicates sequences obtained in this study, while taxa annotated with quotation marks indicate possible misidentifications

Taxon	18S rRNA	28S rRNA	*COI*	ITS-2	Source
D. grandipes V1	OP380711		OP379718	OP390261	Guidetti et al. (2022)
D. grandipes V2	OP380712		OP379719	OP390262	Guidetti et al. (2022)
D. cf. *octavi*	**PV211928**	**PV211931**	**PV213452**	**PV211934**	**this study**
D. ovimutans	MT136805		MT132333		Kihm et al. (2020)
D. parthenogeneticus FR	MT373694	MT373700	MT373804	MT374191	Pogwizd and Stec (2020)
D. parthenogeneticus GB	MT373693	MT373699	MT373803	MT374190	Pogwizd and Stec (2020)
D. parthenogeneticus PL	MT373695	MT373701	MT373805	MT374192	Pogwizd and Stec (2020)
D. parthenogeneticus V3	OP380708		OP379716	OP390258	Guidetti et al. (2022)
D. parthenogeneticus V7	OP380710		OP379717	OP390260	Guidetti et al. (2022)
D. selenicus	MT812476	MT812466	MT808076	MT812602	Stec et al. (2020a)
Dactylobiotus sp. (1)	EF632436		EF632524		Sands et al. (2008)
Dactylobiotus sp. (2)	EF632439		EF632525		Sands et al. (2008)
D. taiwanensis sp. nov. (1)	**PV211926**	**PV211929**	**PV213453**	**PV211932**	**this study**
D. taiwanensis sp. nov. (2)	**PV211927**	**PV211930**	**PV213454**	**PV211933**	**this study**
Paramurrayon cf. *stellatus*	OQ029312		OQ029486		Massa et al. (2024)
M. cf. *pullari* IT.338	MT812477	MT812465	MT808080	MT812603	Stec et al. (2020a)
M. cf. *pullari* US1	OP380713		OP379720	OP390263	Guidetti et al. (2022)
M. cf. *pullari* V1	OP380714		OP379721	OP390264	Guidetti et al. (2022)
"*Paramurrayon dianeae*"	FJ435737	FJ435762	FJ435801		Guil and Giribet (2012)
P. meieri 13	OP380715		OP379723	OP390265	Guidetti et al. (2022)
P. meieri A14	OP380718		OP379726	OP390268	Guidetti et al. (2022)
P. meieri A3	OP380716		OP379724	OP390266	Guidetti et al. (2022)
P. meieri A4	OP380717		OP379725	OP390267	Guidetti et al. (2022)
A. cf. *granulatus* JP.008	MT812475	MT812464	MT808075	MT812600	Stec et al. (2020a)
C. salishani S1916_1	ON062322	ON062305	ON059359	ON062326	Vecchi et al. (2022)
C. salishani S1916_2	ON062323	ON062306	ON059360	ON062327	Vecchi et al. (2022)

(Kalyaanamoorthy et al. 2017) was used to choose the best-fit models according to the AIC for Maximum Likelihood (ML) analysis. Than ML reconstruction was conducted using W-IQ-TREE (Nguyen et al. 2015; Trifinopoulos et al. 2016). One thousand ultrafast bootstrap (UFBoot) replicates were applied to provide support values for branches (Hoang et al. 2018). The consensus tree was viewed and visualised by FigTree v.1.4.3 available from http://tree.bio.ed.ac.uk/software/figtree. The best evolutionary models of sequence evolution selected for BI and ML analyses as well as respective raw trees are given in supplementary materials (SM. 2).

Examined type material

To facilitate a morphological comparison with the new species, the holotype and paratypes of *D. caldarellai* and *D. lombardoi* (species for which eggs have not yet been found or described) were also examined. These specimens are preserved at the University of Catania (Slide Nos. 4299, 4300, and 4333). Microphotographs of the type material are provided in the supplementary materials (SM. 3).

Morphometric comparison

According to the taxonomic key provided by Kaczmarek et al. (2012), the new species is most similar to *D. parthenogeneticus*. To investigate this similarity, we conducted a morphometric comparison of the eggs and animals of the new species and *D. parthenogeneticus* using Principal Component Analysis (PCA). All analyses were performed in R v.4.3.3. For eggs, absolute values (raw measurements in μm) were used for the analysis, whereas for the animals, relative (*pt*) and absolute values were analysed. The PCA was performed using the NIPALS algorithm (which allows for the presence of missing data; Wold 1966) using the R package "pcaMethods" (Stacklies et al. 2007). The PCAs were visualized with the packages "ggplot2 ver. 3.3.2", "plyr ver. 1.8.6" and "gridExtra ver. 2.3" (Wickham 2011; Wickham et al. 2016). The R code and input data are given in supplementary materials (SM. 4 and SM. 5), respectively.

RESULTS

Phylogeny

The phylogenetic trees generated using Maximum Likelihood (ML) and Bayesian Inference (BI) methods showed very similar topologies (Fig. 1 and SM. 2). These analyses illustrated the evolutionary relationships among taxa within the family Murrayidae. However, due to limited taxonomic and phylogenetic coverage, the exact relationships between *Dactylobiotus*, *Murrayon*, and *Paramurrayon* could not be confidently resolved (nodal support < 90; Fig. 1). The analysis clearly indicates that the new species belongs to the genus *Dactylobiotus* and clusters with *D. parthenogeneticus*, *D. selenicus*, and *D. grandipes*. The DNA sequences labeled in GenBank as *Dactylobiotus* sp. (GenBank accession numbers: EF632436, EF632439, EF632524, EF632525) and included in our study cluster closely with *D. ovimutans*, indicating that these three terminals represent a single species. A similar result is observed for sequences of *Paramurrayon dianeae* (Kristensen, 1982) (GenBank accession numbers: FJ435737, FJ435762, FJ435801), which form a uniform, species-level clade together with sequences of the recently described *Paramurrayon meieri* Guidetti, Giovannini, Del Papa, Ekrem, Nelson, Rebecchi and Cesari, 2022.

TAXONOMIC ACCOUNT

Phylum: Tardigrada Doyère, 1840
Class: Eutardigrada Richters, 1926
Superfamily: Macrobiotoidea Thulin, 1928 (in Marley et al. 2011)
Family: Murrayidae Guidetti et al., 2000
Genus: *Dactylobiotus* Schuster, 1980 (in Schuster et al. (1980))

***Dactylobiotus taiwanensis* sp. nov.**

(Figs. 2–9, Tables 3–4)
urn:lsid:zoobank.org:act:2F270225-1415-4690-B8E8-7673F0FF4131

Material examined: 82 animals, 73 eggs mounted on microscope slides in Hoyer's medium (some of the eggs were embryonated), six animals and two eggs examined in SEM and two specimens processed for DNA sequencing.

Type locality: 25.0506021, 121.4628704; 3 m asl: Xinzhuang Touqian Sports Park, New Taipei City, Taiwan; debris from the leaf of *Limnophila* sp.; coll. Chih-Yu Pai; 1 December 2022.

Etymology: The species is named after the country in which it was discovered.

Type depositories: Holotype: slide TW.001.11 and 46 paratypes (slides: TW.001.*, where the asterisk can be substituted by any of the following numbers: 04, 05, 07–10, 12, 13) and 59 eggs (slides: TW.001.*: 01, 14–17) are deposited at the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences,

Sławkowska 17, 31-016, Kraków, Poland, whereas 35 paratypes (slides: TW.001.*: 03, 06) and 14 eggs (slide: TW.001.02) deposited in the Biodiversity Research Center of Academia Sinica. 6 animals and 3 eggs prepared for SEM (UNICT-Stub N.67) are deposited at the University of Catania, Italy.

DNA voucher: Two exoskeletons mounted on permanent slides, labelled Dac.tai._TW.001.01 and Dac.tai._TW.001.02, are deposited at the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland.

Description of the new species

Animals (measurements and statistics in Table 3): Body transparent in juveniles and whitish in adults, but transparent after fixation in Hoyer's medium (Fig. 2A). In live specimens, eyes are present but they dissolve in Hoyer's medium (out of 21 measured animals). In the dorso-lateral head region an area with minute pores (probably chemosensory function) can be identified in both sides of the head, but only with SEM (Fig. 3A–B). Other than that cuticle is without typical pores but wrinkled with two flat, oval papillae present on the dorsum between legs III and IV in adults and juveniles (Figs. 2B–C and 3C–D). Granulation absent on all legs.

Claws of the *Dactylobiotus* type with short basal portion and primary branches with distinct accessory points (Figs. 4 and 5). Lunules absent, but under PCM a robust semilunar cuticular connection is present between external/posterior and internal/anterior claws (Fig. 4). Under SEM this connection is visible as

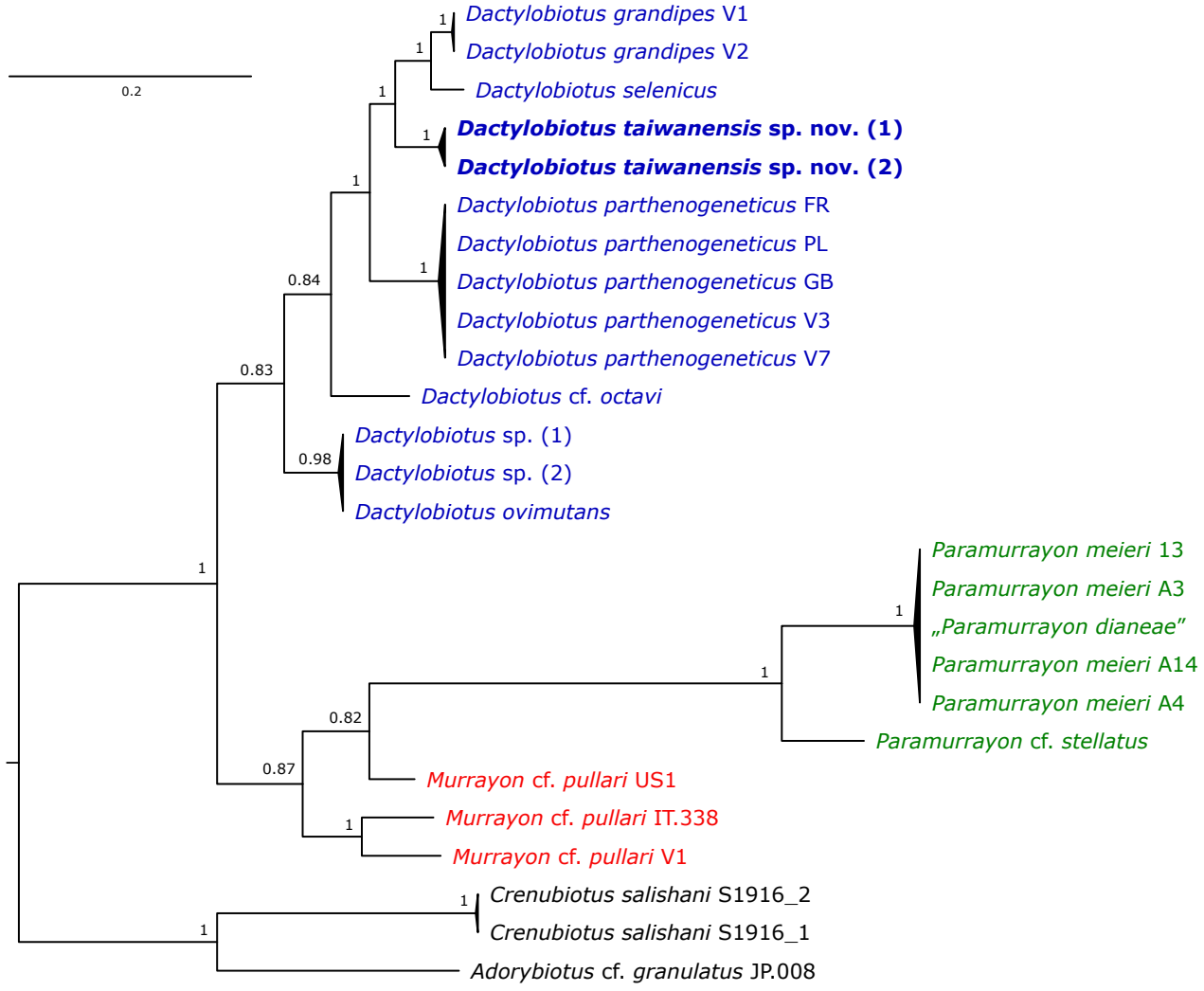

Fig. 1. Bayesian phylogeny (BI) constructed from concatenated sequences (18S rRNA, 28S rRNA, ITS-2, and *COI*) of the family Murrayidae. Numbers above the branches indicate Bayesian posterior probabilities (pp). Nodes with pp < 0.80 were collapsed. The new species sequenced in this study is highlighted in bold. Taxa from the genera *Dactylobiotus*, *Paramurrayon*, and *Murrayon* are shown in blue, green, and red fonts, respectively. For details on the taxa included in the tree, refer to table 2. The outgroup is shown in black. The scale bar represents substitutions per site.

discontinuous, being composed of extended lunule-like thickenings under the claws on the lateral sides whereas its median portion is located within or under cuticle (Fig. 5). Claws on the first three pairs of legs similar in size but obviously larger on the hind legs. A cuticular thickening is present above claws I–III (Fig. 4C and 5A), which under PCM is visible a darkened continuous cuticular bar (Fig. 4C). Under PCM the area above claws IV is darkened (4D), being similar to the darkened area present in horseshoe structure connecting the anterior and the posterior claw in many species of the family Macrobiotidae. The cuticle of this area under SEM appears smooth when compared to the surrounding cuticle (Fig. 5C).

Mouth antero-ventral followed by ten short peribuccal lamellae, bucco-pharyngeal apparatus of the

Table 3. Measurements [in µm] of selected morphological structures of individuals of *Dactylobiotus taiwanensis* sp. nov. mounted in Hoyer's medium

Character	N	Range		Mean		SD		Holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Body length	21	243–640		511		122		575	
Buccal tube									
Buccal tube length	21	32.3–68.6	–	59.0	–	11.3	–	68.6	–
Stylet support insertion point	21	23.3–49.8	*71.0–73.3*	42.6	*72.2*	8.2	*0.7*	49.8	*72.5*
Buccal tube external width	21	3.9–9.7	*11.1–15.1*	7.5	*12.7*	1.6	*0.8*	7.8	*11.4*
Buccal tube internal width	21	2.6–7.2	*7.6–11.1*	5.1	*8.6*	1.2	*0.8*	5.4	*7.9*
Ventral lamina length	21	13.5–30.4	*36.9–44.9*	24.7	*41.8*	4.9	*2.0*	30.4	*44.3*
Placoid lengths									
Macroplacoid 1	20	7.0–27.0	*21.7–39.4*	19.2	*31.9*	5.6	*4.7*	22.1	*32.2*
Macroplacoid 2	20	4.2–15.5	*13.0–24.0*	11.8	*19.5*	3.6	*3.2*	13.4	*19.5*
Macroplacoid row	21	12.5–44.0	*38.6–66.5*	33.7	*55.8*	9.5	*8.0*	38.5	*56.1*
Claw 1 heights									
External primary branch	20	14.3–33.5	*40.2–49.0*	26.3	*44.8*	5.5	*2.3*	30.3	*44.2*
External secondary branch	20	4.8–13.7	*13.6–21.3*	10.3	*17.3*	2.8	*2.1*	11.3	*16.4*
External secondary/primary branch	20	29.2–45.6	–	38.6	–	4.2	–	37.2	–
Internal primary branch	18	14.1–32.6	*29.9–47.7*	24.4	*42.1*	5.7	*4.2*	29.6	*43.1*
Internal secondary branch	19	4.6–13.4	*13.4–19.7*	9.9	*16.6*	2.7	*2.0*	11.1	*16.2*
Internal secondary/primary branch	18	30.0–64.0	–	39.7	–	7.0	–	37.6	–
Claw 2 heights									
External primary branch	20	14.5–33.9	*37.3–51.6*	26.6	*45.2*	5.8	*3.0*	31.5	*45.9*
External secondary branch	20	4.5–13.4	*13.0–20.8*	10.5	*17.6*	2.9	*2.2*	12.0	*17.4*
External secondary/primary branch	20	29.8–46.2	–	38.9	–	4.5	–	38.0	–
Internal primary branch	17	13.4–32.3	*37.3–50.0*	25.7	*43.4*	5.8	*3.2*	28.0	*40.8*
Internal secondary branch	17	4.3–13.1	*12.7–20.4*	10.1	*16.7*	2.9	*2.2*	11.7	*17.1*
Internal secondary/primary branch	17	29.5–45.1	–	38.6	–	4.5	–	41.9	–
Claw 3 heights									
External primary branch	17	14.5–33.6	*39.6–49.7*	27.3	*45.9*	5.6	*2.8*	33.6	*49.0*
External secondary branch	16	4.7–13.8	*14.4–21.3*	10.8	*18.1*	2.7	*1.9*	?	*?*
External secondary/primary branch	16	32.2–45.6	–	39.6	–	3.4	–	?	–
Internal primary branch	17	13.3–31.8	*38.6–48.6*	26.2	*44.2*	5.3	*2.8*	29.6	*43.1*
Internal secondary branch	17	4.1–13.8	*12.8–20.3*	10.2	*17.0*	2.7	*2.1*	11.4	*16.6*
Internal secondary/primary branch	17	30.9–43.3	–	38.3	–	3.5	–	38.6	–
Claw 4 heights									
Anterior primary branch	11	16.0–41.3	*49.4–65.3*	33.0	*58.2*	8.9	*5.2*	38.8	*56.5*
Anterior secondary branch	13	6.6–18.2	*19.1–29.3*	14.7	*25.1*	4.0	*2.8*	17.2	*25.1*
Anterior secondary/primary branch	11	37.7–47.1	–	42.6	–	2.7	–	44.4	–
Posterior primary branch	15	16.3–43.0	*50.4–66.5*	34.1	*60.0*	8.9	*4.7*	39.8	*57.9*
Posterior secondary branch	15	7.0–19.7	*20.7–30.6*	15.2	*26.3*	4.5	*3.3*	17.3	*25.3*
Posterior secondary/primary branch	14	36.6–50.4	–	43.5	–	3.7	–	43.6	–

N, number of specimens/structures measured; Range refers to the smallest and the largest structure among all measured specimens; SD, standard deviation).

Macrobiotus type (Fig. 3A, 6A and 7). Under PCM, only the second and third bands of teeth are visible in the oral cavity armature (Fig. 6B–C). However, in SEM three bands of teeth are clearly visible with the first band being situated at the base of peribuccal lamellae and composed of several rows of scattered small conical teeth arranged around the oral cavity (Fig. 7). The second band of teeth is situated below the ring fold, and comprises 4–6 rows of small cone-shaped teeth which are larger than those of the first band and increase in size towards the third band of teeth (Figs. 6B–C and 7). The teeth of the third band are located within the posterior portion of the oral cavity, between the second band of teeth and the buccal tube opening (Figs. 6B–C and 7B–C). The third band of teeth is discontinuous and divided into dorsal and the ventral portions. Under PCM, the dorsal teeth are seen as three distinct transversal ridges whereas the ventral teeth appear as two separate lateral transverse ridges, between which a roundish median tooth is visible (Fig. 6B–C). In SEM, both dorsal and ventral teeth are also clearly distinct (Fig. 7B–C). Under PCM, in the lateral view of the buccal apparatus, a strengthening bar (ventral lamina) with an incision determining a ventral hook is clearly visible (Fig. 6D).

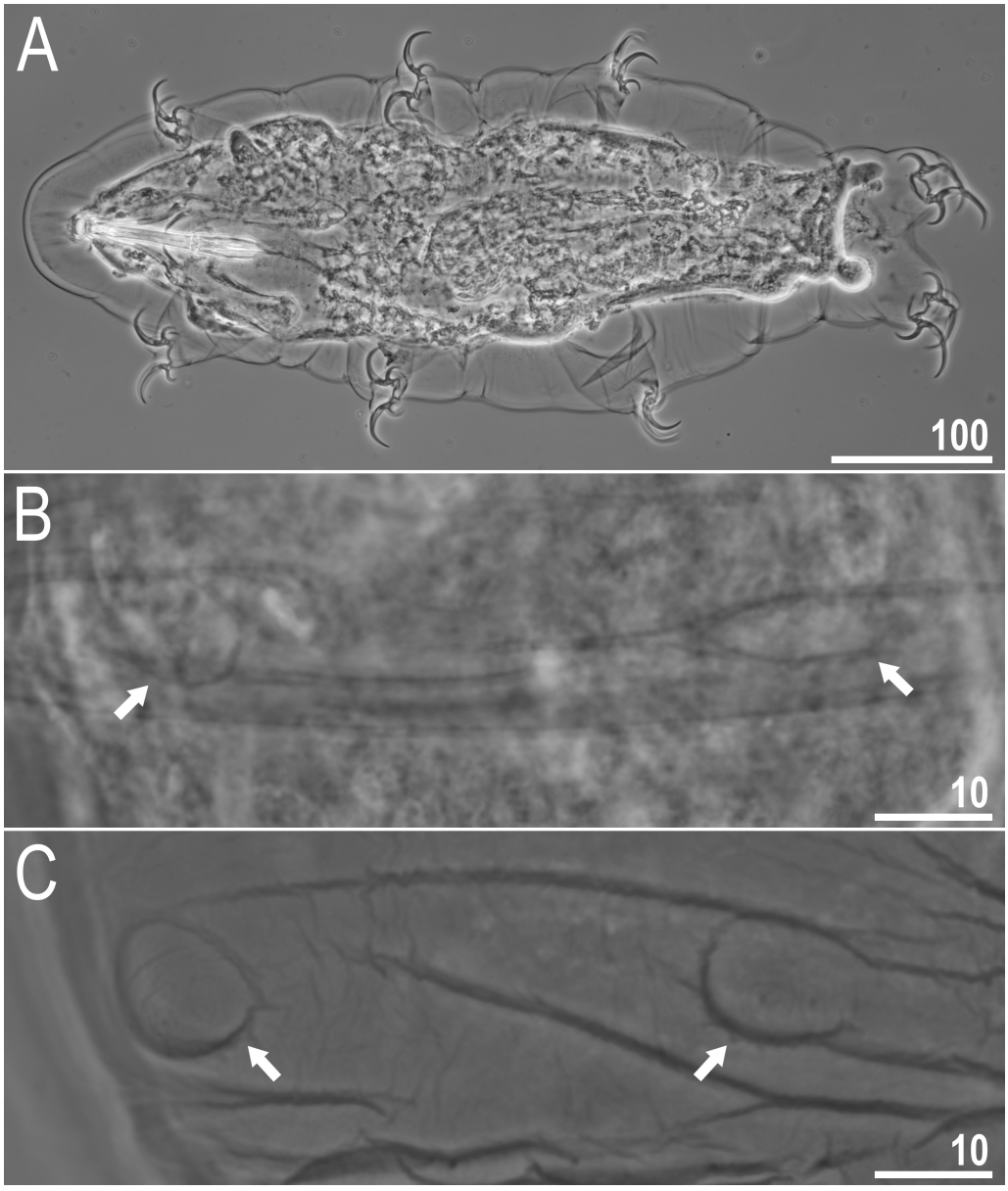

Fig. 2. *Dactylobiotus taiwanensis* sp. nov. – habitus and dorsal cuticle (PCM): A, dorso-ventral view (holotype); B–C, dorsal cuticle showing two flat, oval papillae on the dorsum between legs III and IV (holotype and paratype, respectively). Arrows indicate the dorsal papillae. Scale bars in μm.

Pharyngeal bulb spherical, with triangular apophyses, two rod-shaped macroplacoids which sometimes have jagged edges (Fig. 6E–G). The macroplacoid length sequence 2 < 1. The first macroplacoid has a central constriction, whereas the second macroplacoid is only gently constricted sub-terminally (Fig. 6E–G).

Eggs (measurements and statistics in Table 4): Laid freely, whitish, spherical (Fig. 9A). Processes in the shape of short and wide cones with apexes usually divided into multiple (typically three to six) short, nodular, finger-like apices (Figs. 8 and 9). Under SEM, apices usually covered with microgranulation (Fig. 9C). The egg surface between the processes appears wrinkled; however, this is barely visible under PCM (Fig. 8C, D), where most often the surface appears to be smooth (Fig. 8A, B), whereas wrinkles are clearly distinguishable under SEM imaging (Fig. 9). Under PCM, the margins of the process bases appear serrated and are surrounded by a crown of faint, small thickenings/projections, usually with faintly visible pores (Fig. 8A–D). Under SEM these dark projections are clearly visible as vertical thickenings present on basal portions of processes walls and each process base is surrounded by a line of around 25 small, but evident

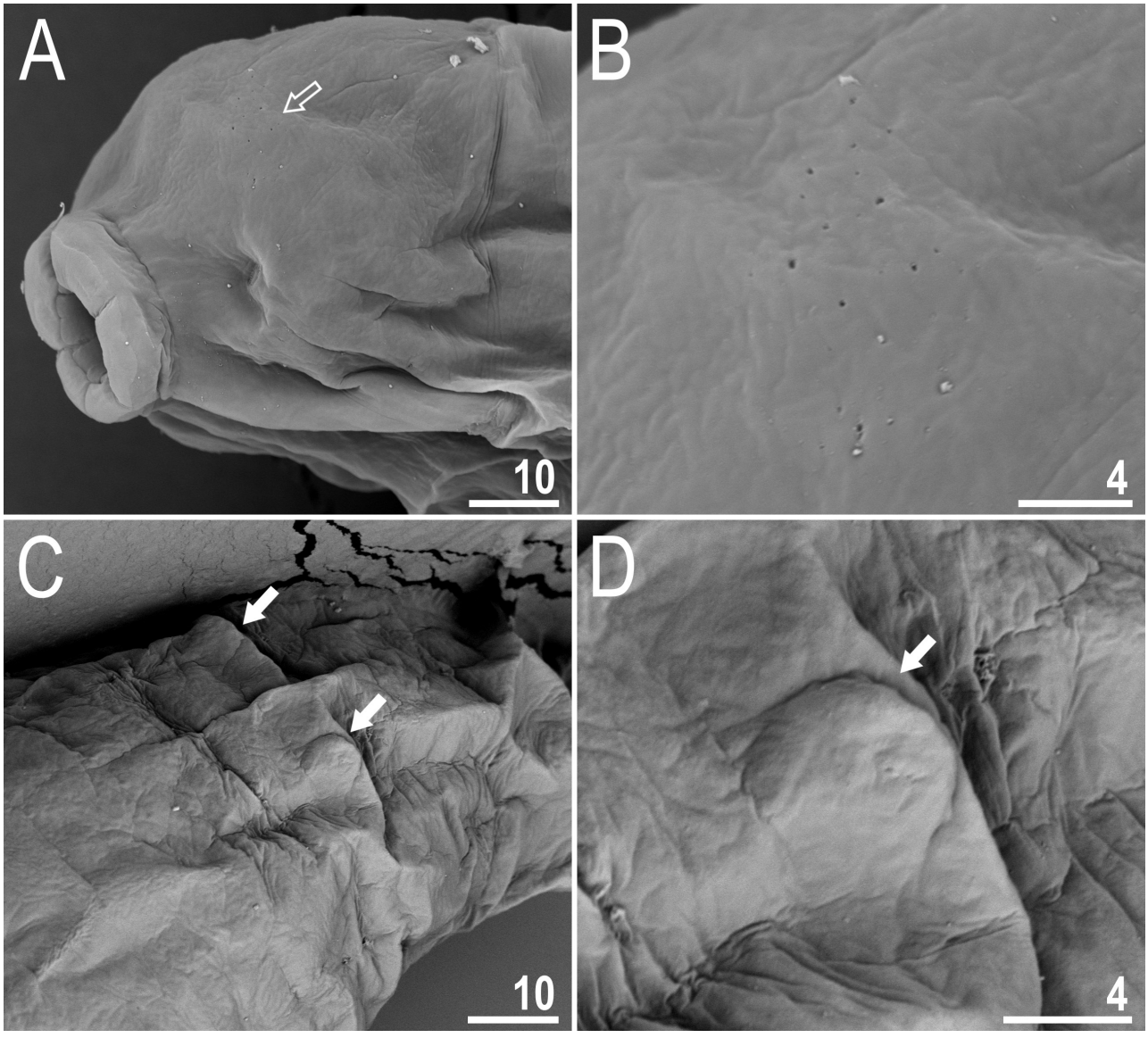

Fig. 3. *Dactylobiotus taiwanensis* sp. nov. – cuticle of the head and dorso-caudal body regions (SEM, all paratypes): A, head and mouth opening; B, detail of the porous area in the dorso-lateral cuticle of the head; C, dorsal cuticle with two flat, oval papillae on the dorsum between legs III and IV; D, detail of the dorsal papilla. Filled arrows indicate dorsal papillae, while the empty arrow indicates the porous area in the head cuticle. Scale bars in μm.

pores (Fig. 9). Eggs are sticky because they are covered by mucus which most likely enhances their adhesion to the substrate and maybe has also a protective function. This mucus is sometimes visible under SEM as a web of flexible filaments that cover the egg surface (Fig. 9C).

Differential diagnosis and PCA results

Currently, there are four other species of *Dactylobiotus* that possess dorsal papillae: *D. selenicus*, *D. dispar* (Murray, 1907), *D. parthenogeneticus*, and *D. grandipes*. However, the new species differs specifically from these taxa as follows:

Dactylobiotus dispar: The new species has two flat dorso-caudal papillae, whereas *D. dispar* exhibits two large conical papillae in the caudal region of the body. The new species also shows less pronounced constriction in the first macroplacoid. While *D. dispar* was described as having three macroplacoids, it is more likely to have only two, with the first one being profoundly constricted. Additionally, the eggs of the new species have interprocess distances smaller than the widths of the process bases, whereas in *D. dispar*, the interprocess distances are equal to the widths of the process bases.

Dactylobiotus grandipes: The new species differs by having two flat dorso-caudal papillae compared to the two large conical papillae in *D. grandipes*. The posterior primary claws on leg IV have lower *pt* values in the new species (*pt 50.4–66.5*) than in *D. grandipes* (*pt 70.7–89.2*). The eggs of the new species have fewer processes around the circumference (38–42 compared to 50–57 in *D. grandipes*) and possess process bases surrounded by a crown of faint, small thickenings or projections, usually with faintly visible pores. These projections and pores are absent or not visible in *D. grandipes*.

Dactylobiotus parthenogeneticus: The new species has egg process bases surrounded by numerous pores (approximately 25) that are faintly visible under light microscopy, but well-visible with SEM. In contrast, *D. parthenogeneticus* exhibits scarce, small, singular pores visible only with SEM. The egg processes of the new species have generally wider bases (5.0–7.2 µm compared to 3.1–5.2 µm in *D. parthenogeneticus*) and a smaller interprocess distance (1.2–2.7 µm compared

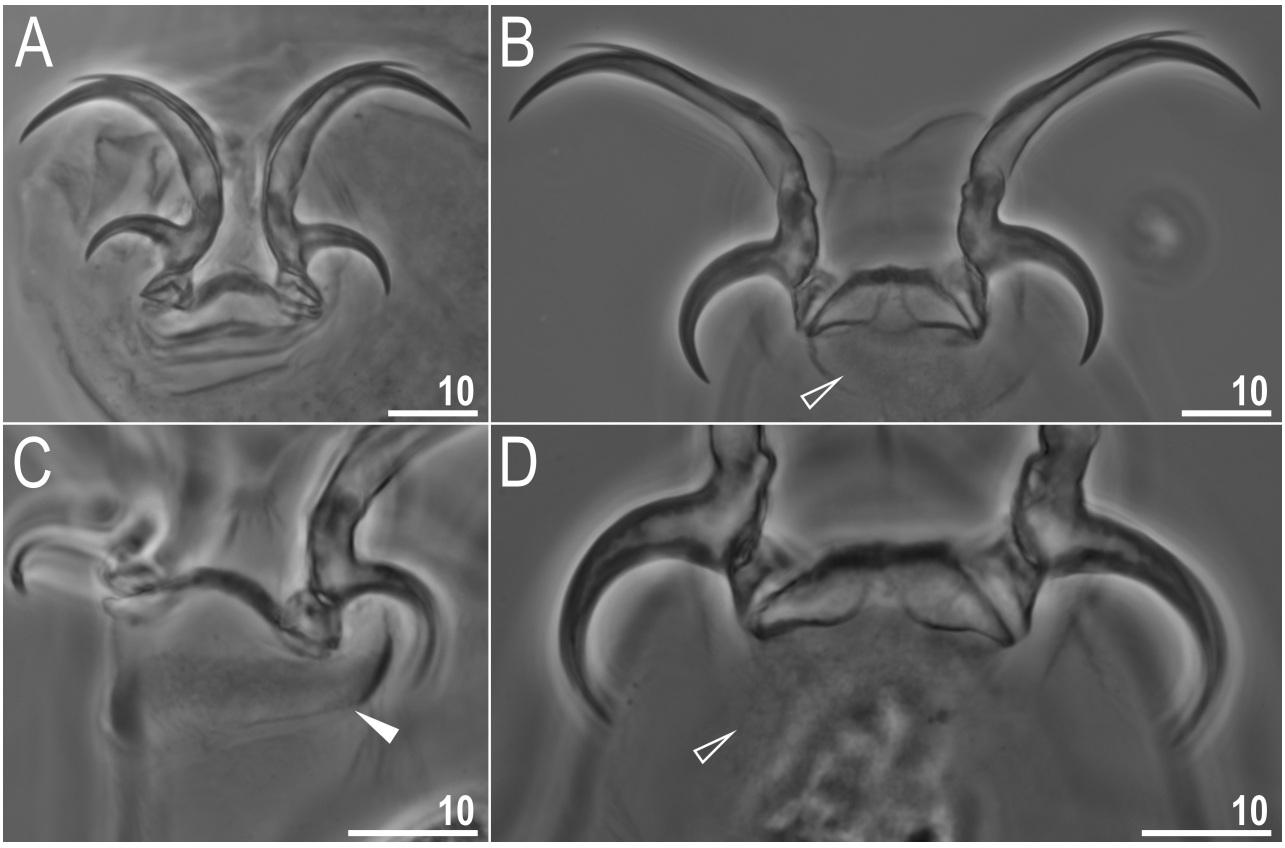

Fig. 4. *Dactylobiotus taiwanensis* sp. nov. – claws (PCM; all paratypes): A–B, claws II (A) and IV (B); C, single continuous cuticular bar/thickening above claws III; D, detail of the area above claws IV. Filled arrowheads indicate the cuticular bar/thickening, while empty arrowheads indicate the darkened area above the hind legs. Please note that the claws in figure are oriented upwards. Scale bars in µm.

to 2.0–4.8 μm in *D. parthenogeneticus*). Although the ranges of egg measurements slightly overlap, these measurements differentiate the species most effectively (see the PCA results section below).

Dactylobiotus selenicus: The new species has conical egg processes, whereas *D. selenicus* has trunco-conical or crater-shaped processes. The eggs of the new species also have fewer processes around the circumference (38–42 compared to approximately 60 in *D. selenicus*). Additionally, the claws of the new species exhibit evident accessory points, which are absent or not visible under PCM in *D. selenicus*.

Among the four species, *D. parthenogeneticus* is the most similar to the new species. Given that an abundant population of *D. parthenogeneticus* was recently studied by Pogwizd and Stec (2020), we utilized their data to perform more detailed comparisons using PCA analysis.

The PCA analysis revealed partial overlap in absolute and relative (*pt*) morphometric data for animals of *D. taiwanensis* sp. nov. and *D. parthenogeneticus*. However, it showed a clear separation (no overlap) in egg morphometric data between the two species (Fig. 10). For the absolute measurement dataset, PC1 explained 87.89% of the variance, while PC2 explained 3.63%. Almost all morphometric traits contributed similarly to PC1, except for body length and ventral lamina length, which contributed more to PC2. For relative measurements (*pt* values), the loadings of the traits were more dispersed compared to absolute

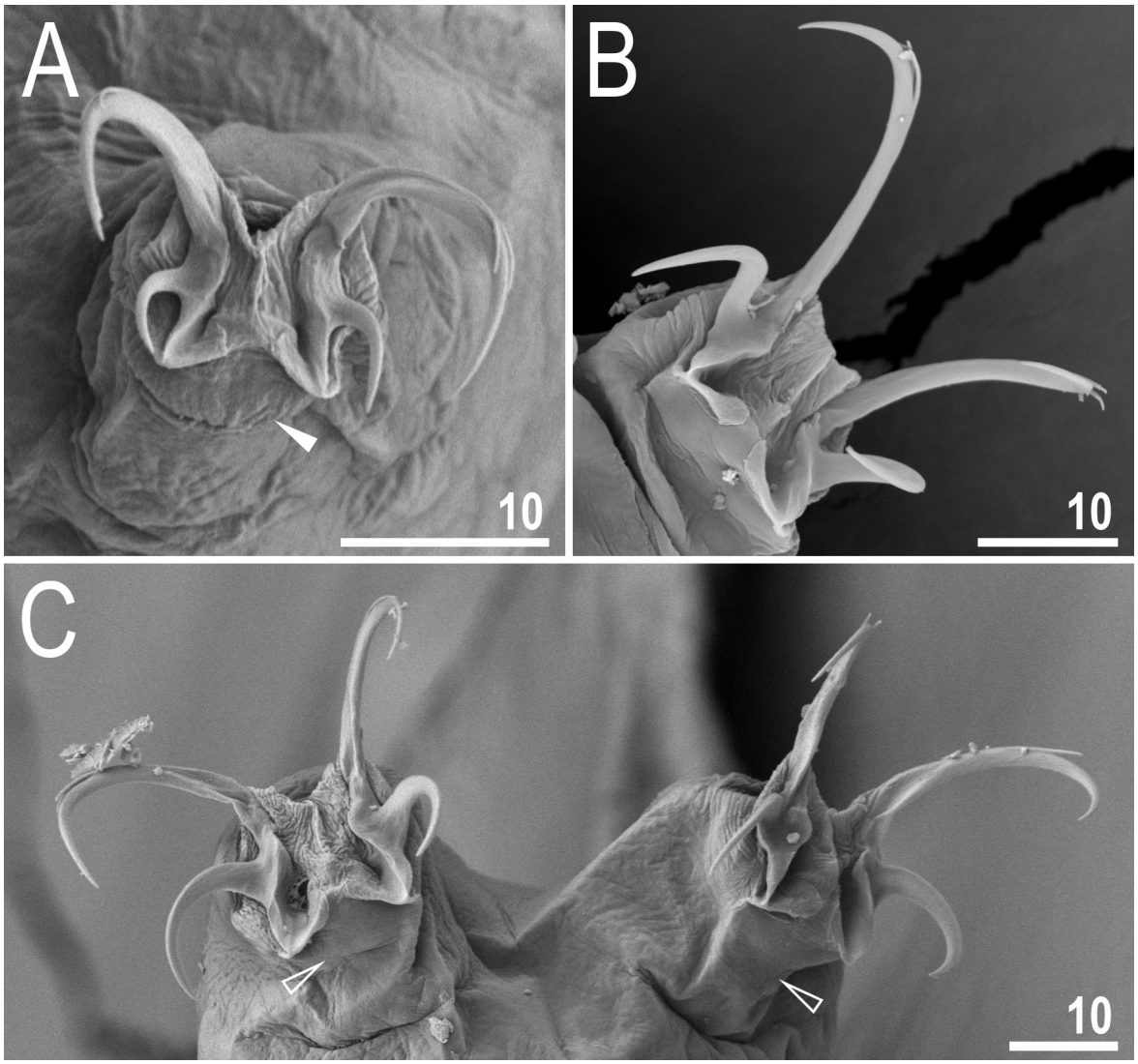

Fig. 5. *Dactylobiotus taiwanensis* sp. nov. – claws (SEM): A, claws II; B–C, claws IV. Filled arrowheads indicate the cuticular bar/thickening, while empty arrowheads indicate faintly marked smoother area above the claws IV. Please note that the claws in figure are oriented upwards. Scale bars in μm.

measurements. Body length and ventral lamina length again contributed more to PC2 than PC1, with PC1 explaining 52.88% of the variance and PC2 explaining 9.78%. In the PCA analysis of egg morphometric data, the two species were clearly separated. The PC1 explained 70.79% of the variance, while PC2 explained 13.12%. The egg morphometric traits contributing most to this separation were the number of processes around the egg circumference (positive), the ratio of process base width to height (positive), and the interprocess distance (negative). Other traits contributed similarly to PC1 and PC2, except for process height, which contributed more to PC2 than PC1.

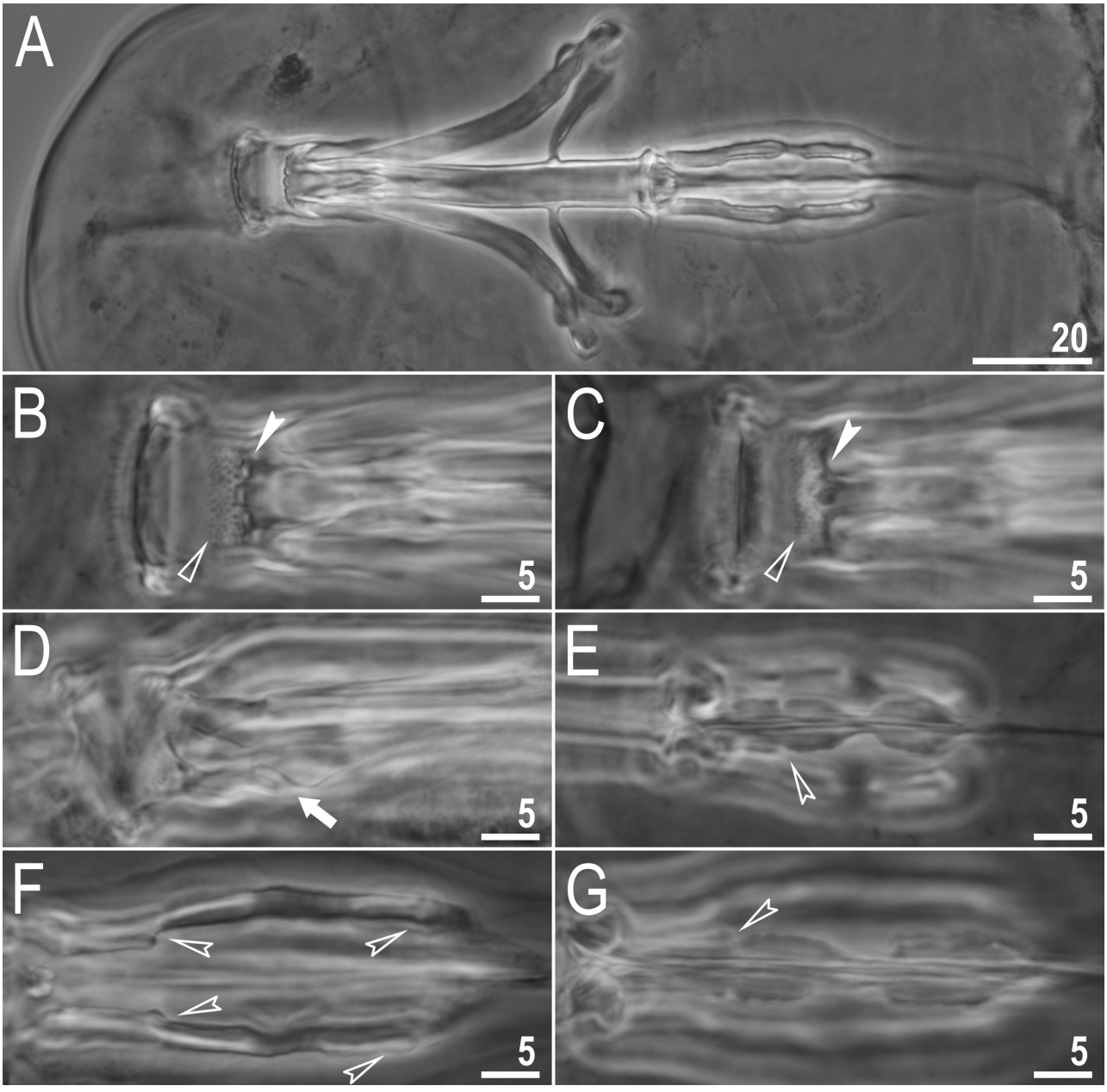

Fig. 6. *Dactylobiotus taiwanensis* sp. nov. – buccal apparatus and oral cavity armature observed in PCM (all paratypes): A, dorso-ventral view of the buccal apparatus; B–C, oral cavity armature in dorsal (B) and ventral (C) views; D, lateral view of the anterior portion of the buccal apparatus, showing the ventral lamina and the incision that indicates the presence of a ventral hook; E–G, placoid morphology in ventral (E, smaller specimen), dorsal (F, larger specimen), and ventral (G, larger specimen) views, respectively. Empty flat arrowheads indicate the second band of teeth; filled indented arrowheads indicate the third band of teeth; arrows point to the ventral hook; and empty indented arrowheads show constrictions in the macroplacoids. Scale bars in μm.

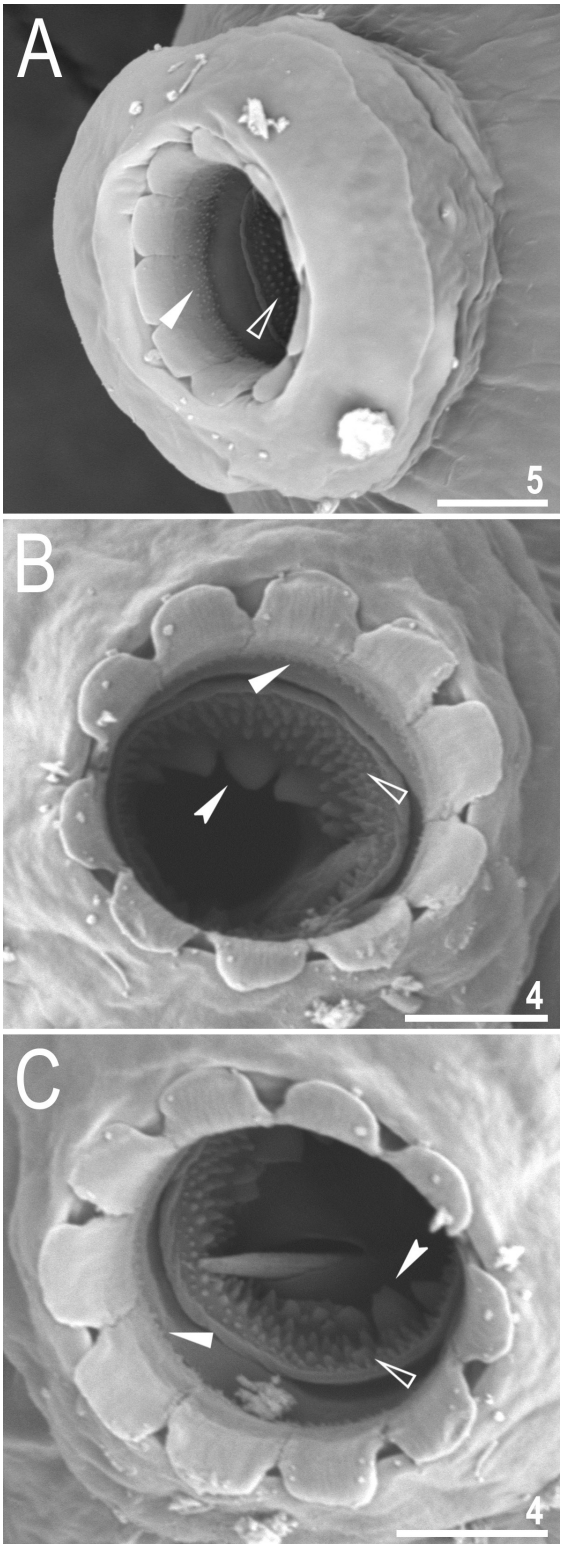

Fig. 7. *Dactylobiotus taiwanensis* sp. nov. – oral cavity armature observed in SEM: A, mouth opening; B–C, oral cavity armature from different angles, dorsal (B) and ventral (C) views, respectively. Filled flat arrowhead indicates the first band of teeth, empty flat arrowheads indicate the second band of teeth, and filled indented arrowheads indicate the third band of teeth. Scale bars in μm.

Genus: *Dactylobiotus* Schuster, 1980 (in Schuster et al. (1980)) *Dactylobiotus* cf. *octavi*

(Figs. 11–12)

Material examined: Four animals and 50 eggs were extracted from the sample collected in Greenland. The DNA sequences were obtained from one embryonated egg (Table 2). Fifteen eggs were prepared and examined under SEM (SEM stub: TAR.19), while the remaining specimens were mounted on microscope slides in Hoyer's medium (Slides: GL.004.01–05).

Material depositories: All specimens are deposited at the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016, Kraków, Poland.

Remarks: The specimens and eggs found in this sample resemble those of *D. ampullaceus* (Thulin, 1911) and *D. octavi*. Although the eggs of *D. ampullaceus* exhibit processes similar to those observed in the newly discovered Greenlandic population, they lack the pores around the process bases (Thulin 1911) that are clearly visible under light microscopy in our population as well as in *D. octavi*. Consequently, the examination of the prepared specimens allowed their identification as *D.* cf. *octavi*, as all observed characteristics matched the original description of animals and eggs, except for some details in egg ornamentation. Guidetti et al. (2006) noted that the egg processes in *D. octavi* are crater-like, often featuring a small elevation at the center. Specifically, most processes are conical with introverted apices, while only a few are fully conical. In contrast, the eggs from the newly discovered population analyzed in this study are distinctly conical in shape (Figs. 11 and 12). Their distal parts form a broad cone, tapering proximally into a short, slender tip (Figs. 11 and 12). Notably, some processes appeared not fully extended, giving the impression of introverted apices (Fig. 12A, C).

Other investigated taxa: The examination of the type material for *D. caldarellai* and *D. lombardoi*, along with their original descriptions, revealed no reliable characters to differentiate these species from their congeners. Furthermore, as eggs are unknown for these species and the type specimens are in very poor condition (SM. 3), we designate them as *nomina dubia* with the following combinations: *Dactylobiotus caldarellai* Pilato and Binda, 1994 nom. dub., and *Dactylobiotus lombardoi* Binda and Pilato, 1999 nom. dub. The challenges surrounding the identification of these three *Dactylobiotus* taxa are further elaborated and discussed in detail in the corresponding discussion section below.

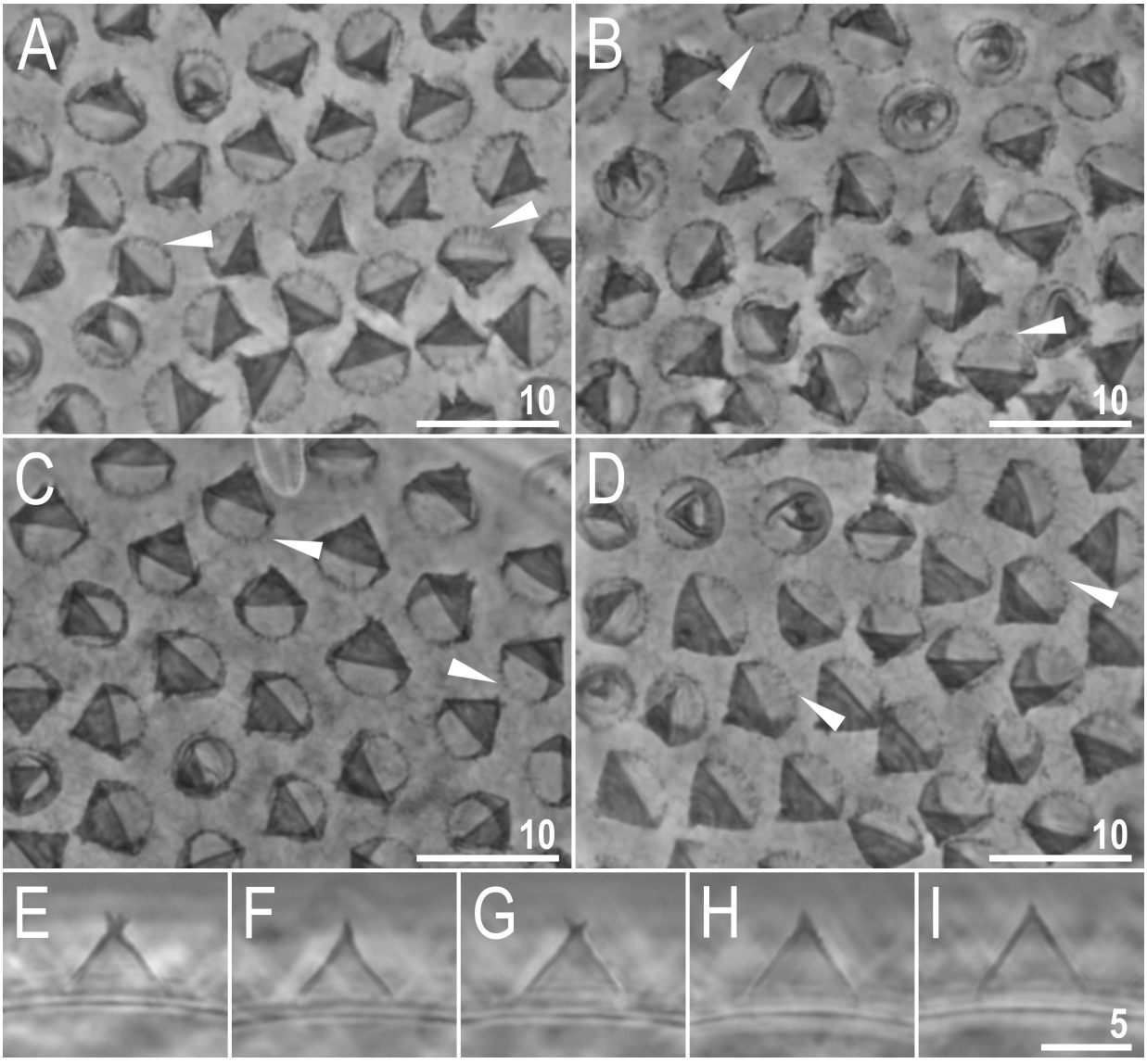

Fig. 8. *Dactylobiotus taiwanensis* sp. nov. – egg chorion morphology viewed in PCM: A–D, overview of the egg surface; E–I, midsections of egg processes. Filled flat arrowheads indicate crowns of faint thickenings/projections Filled flat arrowheads indicate crowns of faint thickenings or projections that extend into wrinkles (though these are only sometimes and faintly visible) as well as pores around the bases of the egg processes. Scale bars in µm.

Table 4. Measurements [in µm] of selected morphological structures of the eggs of *Dactylobiotus taiwanensis* sp. nov. mounted in Hoyer's medium

Character	N	Range			Mean	SD
Egg bare diameter	11	82.5	–	98.2	91.1	5.1
Egg full diameter	11	94.0	–	109.0	101.9	5.1
Process height	33	3.8	–	6.4	5.0	0.6
Process base width	33	5.0	–	7.2	5.9	0.6
Process base/height ratio	33	104%	–	146%	121%	12%
Inter-process distance	33	1.2	–	2.7	1.8	0.4
Number of processes on the egg circumference	11	38	–	42	39.8	1.2

N, number of eggs/structures measured; Range refers to the smallest and the largest structure among all measured specimens; SD, standard deviation.

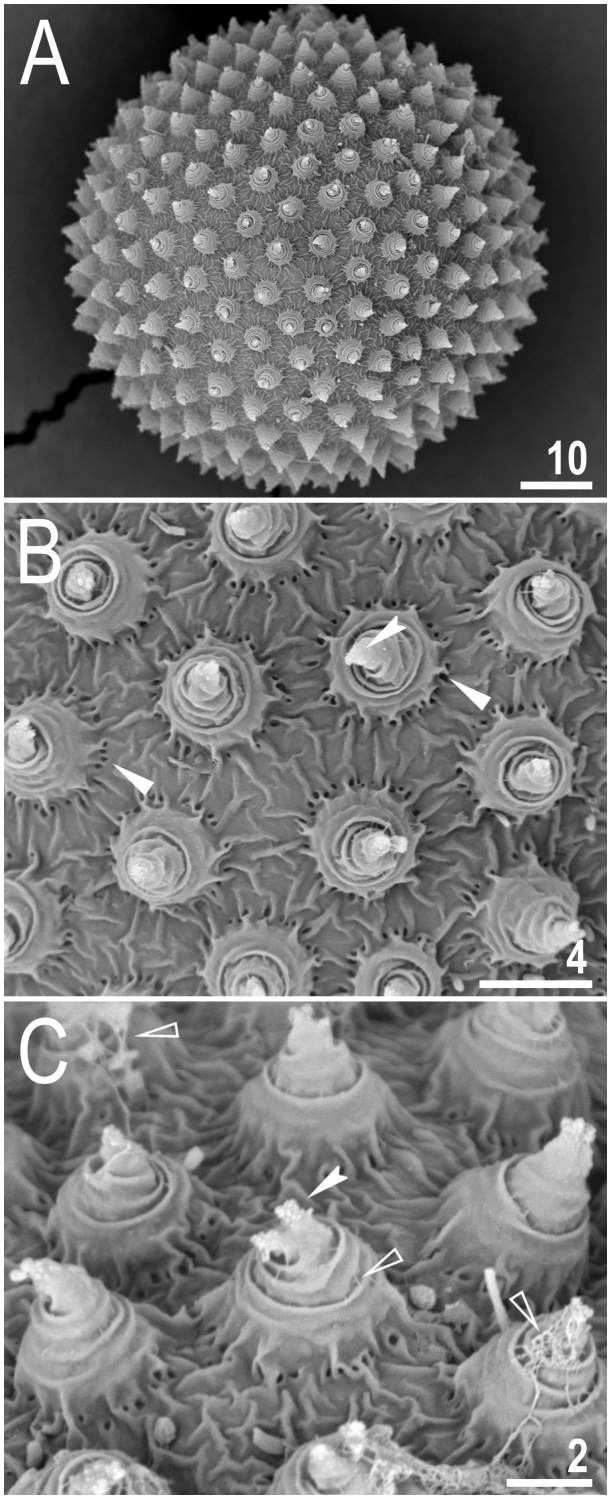

Fig. 9. *Dactylobiotus taiwanensis* sp. nov. – egg chorion morphology observed in SEM: A, entire egg; B–C, details of the egg surface and egg processes. Filled flat arrowheads indicate cuticular thickenings and pores around the bases of the egg processes; filled indented arrowheads indicate microgranulation at the apices of the egg processes; empty flat arrowheads indicate filamentous remains of mucus. Scale bars in μm.

DISCUSSION

Dactylobiotus morphogroups

The species of the genus *Dactylobiotus* can be divided into two morphogroups, distinguished by the presence or absence of papillae on the dorso-caudal portion of the body. Although this division currently lacks clear molecular confirmation, it remains useful for species classification during morphological analyses, as reported for other genera (*e.g.*, Massa et al. 2024; Vecchi et al. 2023; Kaczmarek and Michalczyk 2017; Stec 2022).

The *D. dispar* morphogroup is characterized by the presence of flat or cone-shaped papillae in the dorso-caudal region of the body and includes the following species: *D. dispar*, *D. grandipes*, *D. parthenogeneticus*, *D. selenicus*, and *D. taiwanensis* sp. nov. The second group, the *D. ambiguus* morphogroup, is characterized by the absence of papillae in the dorso-caudal region and comprises the following species: *D. ambiguus*, *D. ampullaceus*, *D. dervizi* Biserov, 1998, *D. haplonyx* Maucci, 1980, *D. luci* Kaczmarek, Michalczyk and Eggermont, 2008, *D. octavi*, *D. ovimutans*, and *D. vulcanus* Kaczmarek, Schabetsberger, Litwin and Michalczyk, 2012. Regarding egg morphology, the species within these morphogroups exhibit variable ornamentation, including eggs with small to large cones, sometimes bearing bi-, tri-, or poly-furcated apices. The surface between egg processes may be smooth, porous, or wrinkled. Despite this variability, the most common eggs in both groups feature small conical processes that are uniform in size and shape. These processes are sometimes furcated but never bear filaments and are always well-spaced from one another. Future phylogenetic analyses with expanded taxonomic and phylogenetic sampling will provide a better understanding of the value of morphological characters, allowing greater phylogenetic significance to be assigned to features such as dorso-caudal papillae or egg ornamentation. However, given the evolutionary convergence observed in another genus (*Murrayon*) regarding egg morphology and the previously documented high rate of morphological evolution in egg ornamentation (Guidetti et al. 2013; Stec et al. 2016 2021), it is anticipated that dorso-caudal papillae (never observed in close relatives of *Dactylobiotus*) will hold greater phylogenetic relevance. Although our phylogenetic analyses were based on a dataset that remains phylogenetically and taxonomically limited, we believe that a congruence with the morphogroup division is already evident in the provided phylogeny. Specifically, all *Dactylobiotus* taxa in our dataset that

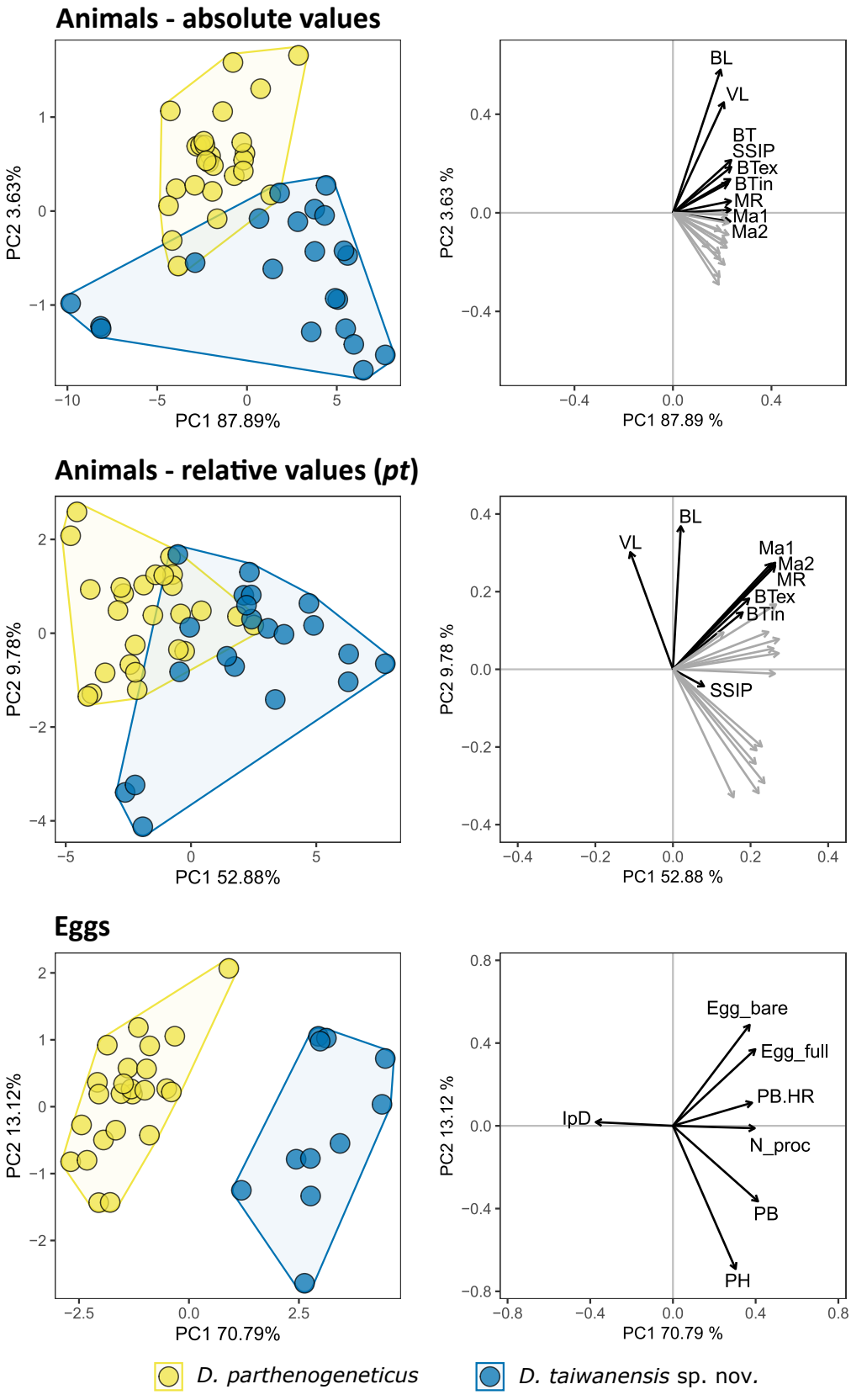

Fig. 10. Results of PCA of animals and eggs measurements of *D. taiwanensis* sp. nov. and *D. parthenogeneticus*. For animals the PCA was done on absolute and relative (*pt*) measurements values. Left quadrants show score scatterplots while right quadrants show loading plots.

exhibit dorsal papillae (*D. grandipes*, *D. selenicus*, *D. parthenogeneticus*, and *D. taiwanensis* sp. nov.) cluster together to form a well-supported clade. However, the relationship of this clade with other *Dactylobiotus* taxa remains poorly supported in our analyses (Fig. 1).

Doubtful *Dactylobiotus* taxa

Several explanations could account for the discrepancies in egg ornamentation morphology observed between the newly discovered Greenlandic

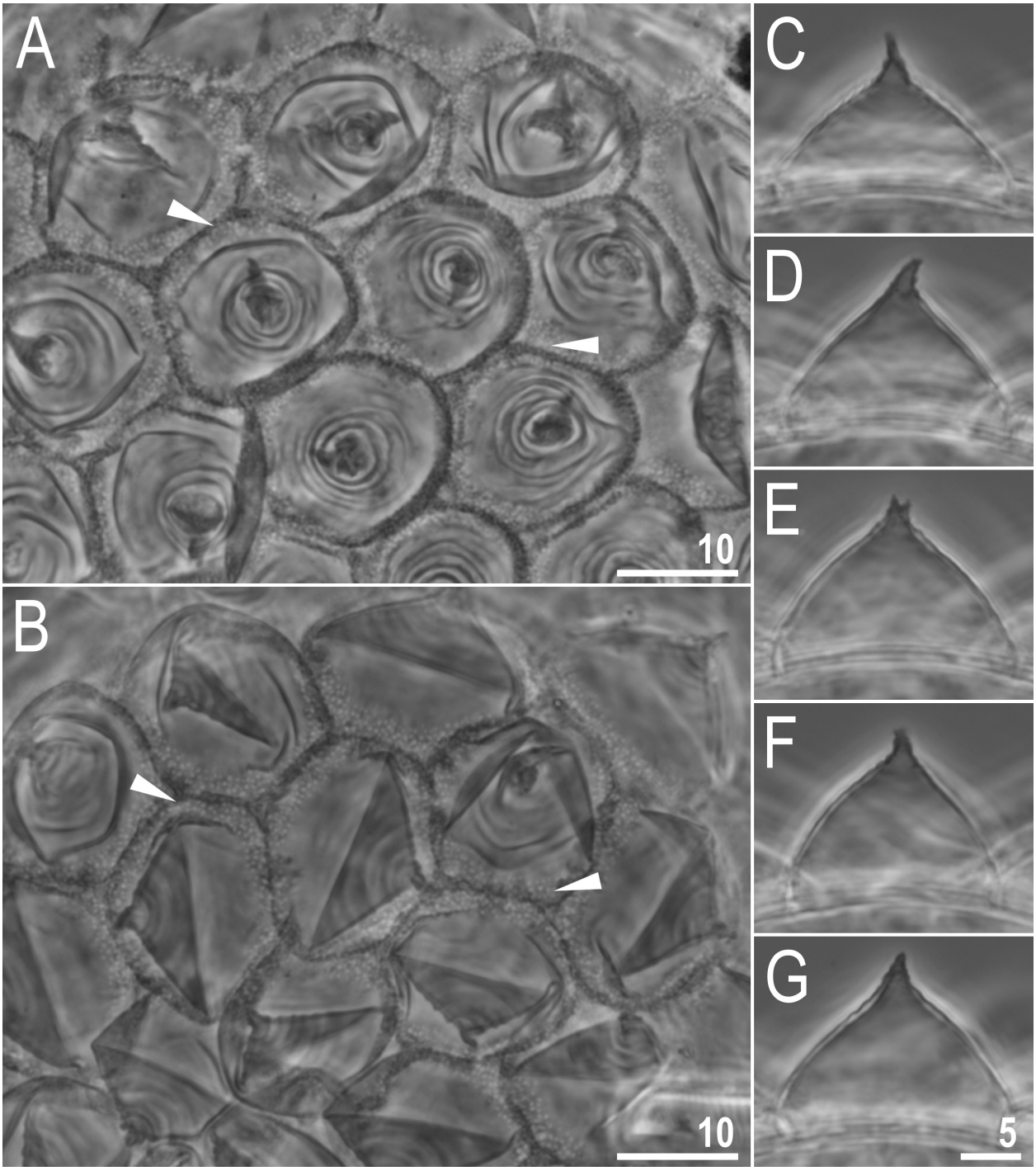

Fig. 11. *Dactylobiotus* cf. *octavi* from Greenland–egg chorion morphology observed in PCM: A–B, egg surface showing conspicuous pores between the processes; C–G, midsections of egg processes. Filled flat arrowheads indicate delicate pores around the bases of the egg processes. Scale bars in μm.

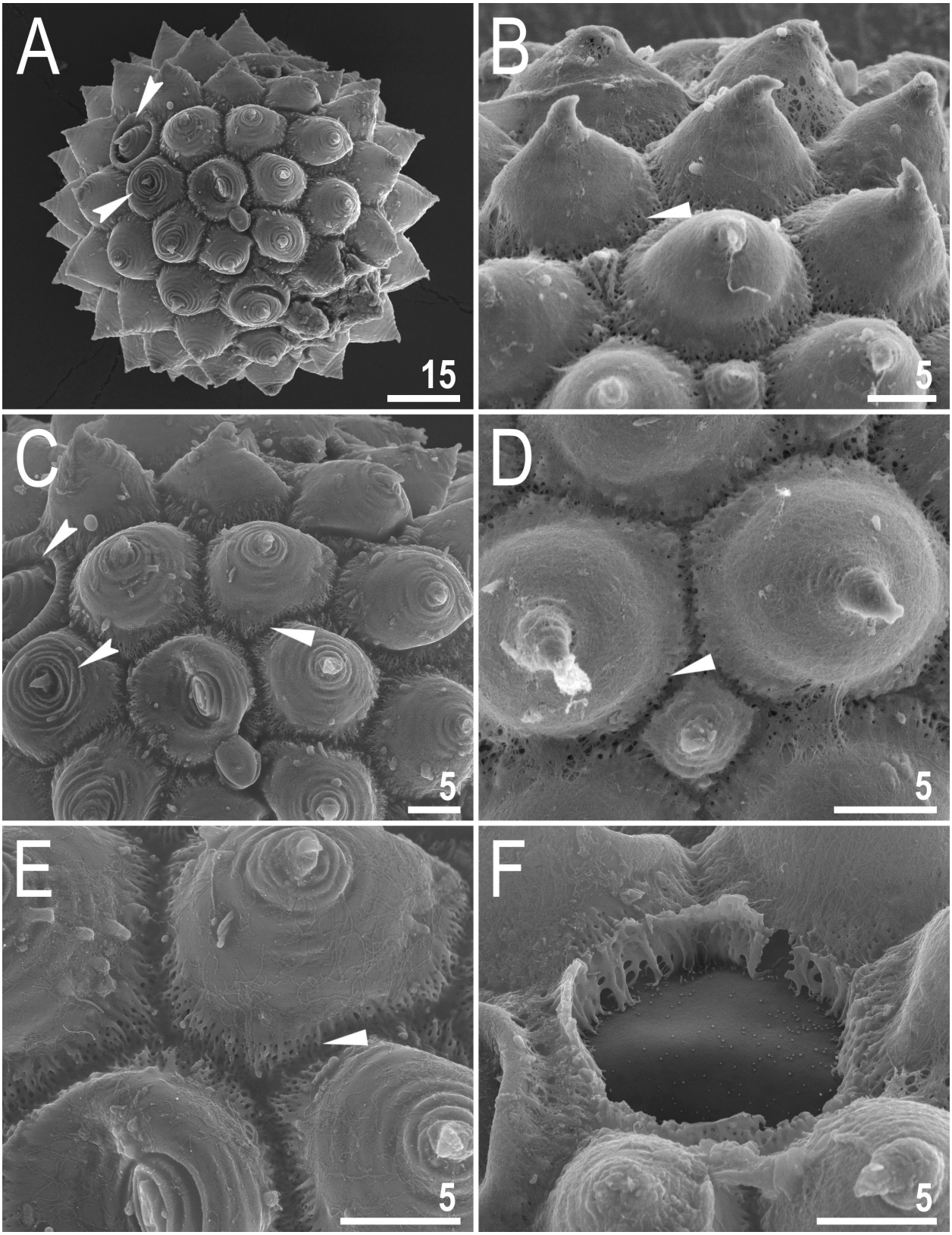

Fig. 12. *Dactylobiotus* cf. *octavi* from Greenland–egg chorion morphology observed in SEM: A, entire egg; B–E, details of the egg surface and egg processes; F, broken egg surface with one egg process detached. Filled flat arrowheads indicate delicate pores around the bases of the egg processes, and filled indented arrowheads indicate egg processes with introverted apices. Scale bars in μm.

population and *D. octavi*. Specifically: (i) the eggs in the type material may have had undeveloped (not fully extended) processes, (ii) the processes may have been distorted by environmental factors or during preparation, or (iii) the varying morphologies could reflect intraspecific variability.

Intraspecific variability in egg morphology has been previously documented in several other tardigrade genera, such as *Bertolanius* Özdikmen, 2008 (primarily variability in the apical portion of the processes; Dastych 1983), *Ramazzottius* Binda and Pilato, 1986 (mainly in process length and shape; Stec et al. 2016 2017; Vecchi and Stec 2024), and *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar and Wolf, 2009 (notably in minor differences in the shape of the processes, sometimes even within the same egg; Guidetti et al. 2019). However, an extreme case was recently reported in the latter genus for *Paramacrobiotus bifrons* Brandoli, Cesari, Massa, Vecchi, Rebecchi and Guidetti, 2024, which exhibits two morphologically distinct egg forms. Regarding the considerable intraspecific variability of egg ornamentation in *Dactylobiotus*, this phenomenon has so far been reported only for *D. ovimutans*. Eggs of this species, thoroughly examined in a culture maintained under stable laboratory conditions by Kihm et al. (2020), exhibited variability in the number, size, and inflation of egg processes. This variability was unlikely to result from seasonality or the production of dormant or active eggs, given the consistent conditions under which the culture was maintained (Kihm et al. 2020). Thus, it cannot be excluded that a similar variability may also occur in *D. octavi*, given the correspondence in all egg characters between the type population and the newly examined population, except for the number and inflation of processes. Specifically, fewer and less inflated processes were observed in the type population from Greenland (Guidetti et al. 2006), whereas eggs with more processes, which were always well extended, were found in the Greenlandic population studied here. Interestingly, differences in the shape of egg processes, attributed to developmental stages, have also been reported for *Paramacrobiotus derkai* (Degma, Michalczyk and Kaczmarek 2008) and *P. bifrons* (Brandoli et al. 2024; Degma et al. 2008). This suggests that similar situations might occur in other genera as well. Given these uncertainties and the inability to compare DNA sequences of variable markers from the population studied here with those of *D. octavi*, we classify our population as *D.* cf. *octavi* until further data become available.

After examining the type material of *D. caldarellai* and reviewing its original description, we concluded that this species is insufficiently diagnosed. Pilato and Binda (1994) described *D. caldarellai* based on two specimens collected from two different locations in Tierra del Fuego, without finding any eggs. The authors considered a morphologically identical population of *D. ambiguus* reported by Dastych (1984) on King George Island as conspecific with *D. caldarellai*. Their rationale was based on Dastych's (1984) observation that the eggs of the newly found population of *D. ambiguus* differed from those of the population from the species' type locality in Europe. Notably, the two locations (Tierra del Fuego (type locality of *D. caldarellai*) and King George Island) are more than 300 km apart. Given the morphological uniformity among animals of different species within the genus and the absence of egg description for *D. caldarellai*, it cannot be confidently determined whether these populations represent the same or different species. Furthermore, the suboptimal condition of the holotype and paratype of *D. caldarellai* hinders a detailed examination (SM. 3). Therefore, until further analyses and a potential integrative redescription based on material from the *locus typicus* are conducted, *D. caldarellai* should be considered a *nomen dubium*, as designated in the results section.

For the second dubious species, *D. lombardoi*, the original description was based on two specimens also collected in Tierra del Fuego. In their study, Binda and Pilato (1999) provided a table of *pt* values derived from the measurements of a single specimen of *D. lombardoi*. These values fall within the *pt* range of the most similar species, *D. parthenogeneticus*, as reported in the same paper, with the exception of buccal tube width and ventral lamina length. However, it is likely that these small differences are the result of an insufficient number of measured specimens or variations in measurement techniques used by different authors. Importantly, when the original measurements from Binda and Pilato (1999) are compared with those provided for *D. parthenogeneticus* by Pogwizd and Stec (2020), the specimen falls perfectly within the newly reported measurement ranges. Although dorsal papillae are not mentioned in the original description of *D. lombardoi*, it has been suggested that this species can be distinguished from *D. parthenogeneticus* by the absence (*D. lombardoi*) or presence (*D. parthenogeneticus*) of these structures. However, despite the poor condition of the type series, it appears that dorsal papillae may indeed be present in specimens of *D. lombardoi* (SM. 3). Given the vague diagnosis of this species, which prevents its clear distinction from other congeners, the lack of a population with a sufficient number of specimens, the unknown egg morphology, and the poor condition of the type material, *D. lombardoi* should be considered a *nomen dubium*. This designation, as outlined in the results section, will remain until further analyses confirm whether it represents a distinct species.

As a result, all species for which eggs have never been found (*i.e.*, *D. aquatilis*, *D. caldarellai*, *D. henanensis*, *D. kansae*, *D. lombardoi*, *D. macronyx*), with the exception of *D. haplonyx* Maucci, 1981, are considered nomina dubia. Regarding *D. haplonyx*, although no specimens from the type series have been examined, it is important to note that the type series comprises individuals collected from multiple locations, with the holotype originating from a different locality than the paratypes (a total of 33 paratypes from five distinct locations, all different from that of the holotype). Furthermore, the species description lacks not only an account of the eggs but also distinctive diagnostic traits that clearly differentiate it from other species. Consequently, further analyses are necessary to assess its validity. Therefore, we consider it appropriate to designate this species as a nomen inquirendum with the following combination: *Dactylobiotus haplonyx* Maucci, 1981 nom. inq.

Dichotomous key

Given that egg ornamentation often comprises fundamental diagnostic characters for distinguishing *Dactylobiotus* taxa, the presented key includes only valid species for which eggs have been described (12 species), excluding from the key all the species designated as *nomina dubia* or *nomina inquirenda*.

1. Two dorso-lateral papillae present 2
- two dorso-lateral papillae absent 6
2. (1). Accessory points not visible with LM, eggs with trunco-conical (crater-like) processes ..... *Dactylobiotus selenicus*
- Eggs with conical processes 3
3. (2). Secondary branch of each claw less than one-third the length of the primary branch; distal portion of egg processes not divided into multiple apices 4
- Secondary branch of each claw more than one-third the length of the primary branch; distal portion of egg processes divided into multiple apices 5
4. (3). *Pt* value of the IV claws < 55, width of the egg processes lower than its height, ca. 40 processes present on the egg circumference ..... *Dactylobiotus dispar*
- *Pt* value of the IV claws > 70, width of the egg processes similar to its height, ≥ 50 processes present on the egg circumference .... *Dactylobiotus grandipes*
5. (3). Egg process bases surrounded by a line of around 25 pores faintly visible with PCM ..... *Dactylobiotus taiwanensis**
- Few pores, randomly distributed around the bases of egg processes, not visible with PCM .... *Dactylobiotus parthenogeneticus**
6. (1). Egg processes clearly spaced from each other 7
- Egg processes in contact with each other, with almost no space left between them 8
7. (6). Delicate reticulation on the egg surface between processes present ..... *Dactylobiotus vulcanus*
- Delicate reticulation on the egg surface between processes absent 9
8. (6). Width of egg processes < 15 µm, more than 20 processes on the egg circumference ..... *Dactylobiotus ambiguus***
- Width of egg processes > 15 µm, less than 20 processes on the egg circumference 10
9. (7). Egg process bases width > 10 µm, pores around egg processes bases regularly distributed and visible under light microscope ..... *Dactylobiotus ovimutans*
- Egg process bases width < 10 µm, egg surface between processes with irregularly distributed pores or pores absent/not visible under light microscope 11
10. (8). Large, conical egg processes, pores around egg processes not visible under light microscope, width of egg processes bases ≤ 23 µm ..... *Dactylobiotus ampullaceus*
- Large, dome-like (or conical) processes, pores around each process well visible under light microscope, width of egg processes bases ≥ 27 µm ..... *Dactylobiotus octavi****
11. (9). Distal portion of egg processes occasionally bi- or tri-furcated with short tips; width of egg process bases < 8 µm, egg surface between processes with irregularly distributed pores, 31–36 processes on the egg circumference .... *Dactylobiotus dervizi*
- Distal portion of egg processes bi-, tri- or multifurcated and often divided into short branches; width of egg processes bases about 9 µm, egg surface between processes without pores or pores not visible under light microscope, 37–41 processes on the egg circumference ..... *Dactylobiotus luci*

*There are also two minor differences in egg morphology between these species, as their ranges slightly overlap. *Dactylobiotus taiwanensis* has 38–42 processes on the egg circumference, with process bases measuring 5–6.5 µm, whereas *Dactylobiotus parthenogeneticus* has 34–38 processes, with process bases ranging from 3 to 5.5 µm. Notably, differences in egg morphometrics clearly distinguish the two species (Fig. 10).

**The measurement was obtained by proportionally scaling a drawing from the species description by Murray (1907), which reported the egg diameter, including processes, as 130 µm. Additionally, data from Thulin (1911) indicated that the process bases had a diameter of 9.5 µm.

***It must be stressed that further investigations are necessary to confirm whether the processes can also have a conical shape. For more details, please refer to the section on *Dactylobiotus* cf. *octavi* in this study, which discusses the shape of egg processes in *Dactylobiotus octavi*.

CONCLUSIONS

The genus *Dactylobiotus* remains understudied in terms of its phylogeny, and further analyses are necessary to better understand interspecies relationships. Egg morphology and morphometry have proven fundamental for species recognition. Due to the importance of these traits and the poor condition of the type material, two additional taxa (*D. caldarellai* and *D. lombardoi*) have been classified as *nomina dubia* in this study; moreover, due to the numerous localities reported for the type series and the incomplete species description, *D. haplonyx* is here designated as a *nomen inquirendum*. Resampling at their respective type localities and the discovery of their eggs are essential to determine whether they truly represent valid species. In

this study, two populations of the genus *Dactylobiotus* were investigated using an integrative approach. One population represents a new tardigrade species, which was formally described as *D. taiwanensis* sp. nov. This discovery contributes to a better understanding of the Taiwanese tardigrade fauna, which is considered poorly recognized (Gąsiorek et al. 2021). The second population resembles *Dactylobiotus octavi*, but discrepancies in egg morphology identified during our analyses led to its provisional identification as *D.* cf. *octavi*. The data obtained in this study have contribute to the understanding of the genus *Dactylobiotus* and the phylogenetic positioning of currently sequenced taxa. Additionally, we constructed a new dichotomous key for all valid species of the genus, which will facilitate future species identification.

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Supplementary materials

SM. 1. Morphometric data for *Dactylobiotus taiwanensis* sp. nov. (download)

SM. 2. The best evolutionary models of sequence evolution selected for phylogenetic analyses together with final raw trees. (download)

SM. 3. Photomicrographs of the types of *Dactylobiotus caldarellai* and *Dactylobiotus lombardoi*. (download)

SM. 4. R code for PCA analysis performed in this study. (download)

SM. 5. Input data for PCA analysis performed in this study. (download)