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Deceptive conservatism of claws: distinct phyletic lineages concealed within Isohypsibioidea (Eutardigrada) revealed by molecular and morphological evidence

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Abstract

Isohypsibioidea are most likely the most basally branching evolutionary lineage of eutardigrades. Despite being second largest eutardigrade order, phylogenetic relationships and systematics within this group remain largely unresolved. Broad taxon sampling, especially within one of the most speciose tardigrade genera, *Isohypsibius* Thulin, 1928, and application of both comparative morphological methods (light contrast and scanning electron microscopy imaging of external morphology and buccal apparatuses) and phylogenetic framework (18S + 28S rRNA sequences) resulted in the most comprehensive study devoted to this order so far. Two new families are erected from the currently recognised family Isohypsibiidae: Doryphoribiidae fam. nov., comprising all aquatic isohypsibioids and some terrestrial isohypsibioid taxa equipped with the ventral lamina; and Halobiotidae fam. nov., secondarily marine eutardigrades with unique adaptations to sea environment. We also split *Isohypsibius* into four genera to accommodate phylogenetic, morphological and ecological variation within the genus: terrestrial *Isohypsibius s.s.* (Isohypsibiidae), with smooth or sculptured cuticle but without gibbosities; terrestrial *Dianea* gen. nov. (Isohypsibiidae), with small and pointy gibbosities; terrestrial *Ursulinius* gen. nov. (Isohypsibiidae), with

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large and rounded gibbosities; and aquatic *Grevenius* gen. nov. (Doryphoribiidae fam. nov.), typically with rough cuticle and claws with branches of very similar heigths. Claw morphology is reviewed and, for the first time, shown to encompass a number of morphotypes that correlate with clades recovered in the molecular analysis. The anatomy of pharynx and cuticle are also shown to be of high value in distinguishing supraspecific taxa in Isohypsibioidea. Taxonomy of all isohypsibioid families and genera is discussed, with special emphasis on the newly erected entities. Finally, a dychotomous diagnostic key to all currently recognised isohypsibioid families and genera is provided.

Keywords

Tardigrada – Isohypsibiidae – taxonomy – buccal apparatus – phylogeny – 18S rRNA – 28S rRNA

Introduction

Tardigrada are a phylum of microinvertebrates inhabiting almost all environments across the globe (Nelson et al., 2015). Despite the undeniable progress in disentangling tardigrade phylogeny, both tardigrade relationships with other metazoans (e.g., Campbell et al., 2011; Gross & Mayer, 2015) and many within-group affinities remain unclear (e.g., Sands et al., 2008; Bertolani et al., 2014a). One of major tardigrade groups with unresolved phylogeny and taxonomy is Isohypsibioidea Guil et al., 2019. This recently established eutardigrade order is considered problematic because it is based on traits that show high levels of morphological stasis (Marley et al., 2011). In fact, this group was erected relatively late mainly because for decades various taxa with *Isohypsibius* and *Hypsibius* type claws were traditionally grouped into a single order Hypsibioidea Guil et al., 2019. Although the erection of Isohypsibioidea clarified the taxonomy of the Eutardigrada, the few efforts to resolve phylogenetic relationships within the order suffered from insufficient sampling and resulted in prevailing polytomies (Bertolani et al., 2014a; Gąsiorek et al., 2019). So far, only a single study (Cesari et al., 2016) identified a monophyletic lineage within Isohypsibioidea. They found morphological and molecular support to erect the family Hexapodibiidae Cesari et al., 2016, the only other isohypsibioid family apart from Isohypsibiidae Sands et al., 2008.

Thus, in order to elucidate the taxonomy and phylogenetic relationships within the order Isohypsibioidea, here we employ comparative analyses of over fifty species representing eleven isohypsibioid genera. Our study embraces a range of analytical methods that included light and scanning electron microscopy observations of external and internal morphology as well as DNA sequencing of two nuclear markers. We uncovered four distinct phylogenetic lineages, corresponding to two previously identified and two new families. The largest tardigrade genus, *Isohypsibius* Thulin, 1928, as indicated earlier (Bertolani et al., 2014a; Cesari et al., 2016; Gąsiorek et al., 2019), is artificial and comprises at least five distinct evolutionary lineages. For three of these, we propose new formal taxonomic ranks and we demonstrate, for the first time, that even slight qualitative differences in claw anatomy, the number of macroplacoids in the pharynx, and the presence of cuticular gibbosities can be of high taxonomic importance in delineating isohypsibioid genera. This work is, therefore, another step towards making all isohypsibioid families and genera monophyletic.

Materials and methods

Sample processing and comparative material

Tardigrades were isolated from moss, lichen, mixed moss and lichen, or water sediment samples, collected by various persons (see table 1), and processed following the protocol described by Stec et al. (2015). In addition to newly collected material, slides with type specimens of taxa described by Ramazzotti, Maucci, Pilato and Biserov, deposited in the Natural History Museum in Verona, were examined using phase contrast microscope (PCM; see table 1 for details), courtesy of Dr. Roberto Guidetti of the University of Modena and Reggio Emilia, Italy.

Microscopy and imaging

Specimens for light microscopy and morphometry were mounted on microscope slides in Hoyer's medium according to Morek et al. (2016) and examined under a Nikon Eclipse 50i phase contrast microscope (PCM) fitted with a Nikon Digital Sight DS-L2 digital camera. Specimens for imaging in the scanning electron microscope (SEM) were prepared according to Stec et al. (2015). Buccal apparatuses were extracted following the protocol provided by Eibye-Jacobsen (2001) with modifications described in Gąsiorek et al. (2016). Both animals and apparatuses were examined under high vacuum in a Versa 3D DualBeam SEM at the ATOMIN facility of Jagiellonian University, Kraków, Poland. For deep structures that could not be fully focused under PCM in a single photograph, a series of 2–6 images were taken every ca. 0.2 µm and then assembled with Corel into a single deep-focus image.

Morphometrics, terminology and classification

All measurements are given in micrometres (µm). Structures were measured only if they were intact and orientated in a flat plane. Terminology for the structures within the buccal apparatus and for the claws follows that of Pilato & Binda (2010) and Gąsiorek et al. (2017). Additionally, in order to avoid misunderstandings and aid comparisons between isohypsibioid taxa, we propose new terminology describing the details of the oral cavity armature (OCA); see also fig. 1. All sclerified elements in the oral cavity are referred to as 'teeth' as their location and shape strongly suggest their function as teeth (see also Hansen & Katholm, 2002, and Michalczyk & Kaczmarek, 2003, for similar proposals in eohypsibiids and macrobiotids, respectively). At the same time, we suggest to abandon the use of unspecific terms such as intrabuccal/infrabuccal 'baffles', 'mucrones' or 'ridges' as they refer to solely shape/appearance rather than to function and therefore may be enigmatic. In all isohypsibioids analysed with SEM, either one or two bands of teeth were observed (Biserov, 1992; Nelson et al., 1999; Jørgensen, 2001, the present study). The first band of teeth was present in all individuals and it was always located on the ring fold (a circular and soft portion of the oral cavity wall; Michalczyk & Kaczmarek, 2003) in the middle of the oral cavity. The second band, if present, was always located behind the first band, at the rear of the oral cavity, just before the buccal tube opening (fig. 1). Therefore, we refer to these bands as 'the first band of teeth' and 'the second band of teeth', respectively. The numbering of bands of teeth is introduced solely for practical reasons, to allow a concise description of the OCA, and are not for the formulation of hypotheses on homologies. Stylet support insertion point is abbreviated as SSIP, whereas apophyses for the insertion of the stylet muscles – as AISM. Claws were measured following Beasley et al. (2008). In order to quantify the relative difference in height between the secondary and the

TABLE 1 List of isohypsibioid taxa used in presented analyses. All species were examined under PCM, and some also under SEM. In most cases type material was analysed

Taxon	Locality	Coordinates	Altitude (m asl)	Sample type	Collector	Type material	SEM analysis
Doryphoribiidae fam. nov.							
Apodibius confusus Dastych, 1983	Welzow-South, Germany	ca. 51°34'N, 14°14'E	?	soil	K. Hohberg	–	+
Apodibius nuntius Binda, 1984	Inhambane, Mozambique	?	?	moss on soil	G. Costa, A. Petralia	+	–
Doryphoribius bindae Lisi, 2011	Udzangwa Mountains, Mwanihana	ca. 7°49'S, 36°50'E	2100	moss	T. Pape, N. Scharff	–	–
Doryphoribius dawkinsi Michalczyk & Kaczmarek, 2010	Heredia, Costa Rica	10°00'10"N, 84°06'44"W	1161	moss from tree	Ł. Kaczmarek	+	+
Doryphoribius korganovae Biserov, 1994	Assumption Island, Seychelles	ca. 9°44'S, 46°38'E	5–10	moss on calcareous slabs	G.A. Korganova	+	–
Doryphoribius monstruosus (Maucci, 1991)	Vegarredonda, Spain	ca. 43°14'N, 4°59'W	?	moss on soil	B. Osella	+	–
Doryphoribius polynettae Biserov, 1988	Naryan-Mara, Russia	?	?	moss	V.I Biserov	+	–
Doryphoribius taiwanus X. Li & H. Li, 2008	Rio de Janeiro, Brasil	22°57'09"S, 43°13'40"W	60	moss	Ł. Skoczylas	–	–
Grevenius baldii (Ramazzotti, 1945) comb. nov.	Lago di Tovel, Italy	ca. 46°15'N, 10°56'E	1180	sediment	M. Balsamo	–	–
Grevenius granulifer (Thulin, 1928) comb. nov.	Kraków, Poland	50°03'27"N, 19°50'19"E	294	liverworts and algae from stump in temporary pond	P. Gąsiorek, W. Morek	–	+
	Fontainebleau, France	48°24'05"N, 2°42'13"E	69	sediment	D. Stec	–	–
Grevenius longiunguis (Pilato, 1974) comb. nov.	Randazzo, Sicily, Italy	ca. 37°52'N, 14°56'E	710–750	sediment	G. Pilato	+	–

TABLE 1 List of isohypsibioid taxa used in presented analyses. All species were examined under PCM, and some also under SEM. In most cases type material was analysed (*cont.*)

Taxon	Locality	Coordinates	Altitude (m asl)	Sample type	Collector	Type material	SEM analysis
Grevenius monoicus (Bertolani, 1981) comb. nov.	Corsonna river, Italy	44°05'06"N, 10°29'36"E	365	sediment	R. Bertolani	+	–
Grevenius pushkini (Tumanov, 2003) comb. nov.	Kraków, Poland	50°03'45"N, 19°57'27"E	205	sediment	A. Oczkowski, B. Surmacz	–	+
Grevenius sismicus (Maucci, 1978) comb. nov.	Van, Turkey	ca. 38°30'N, 43°20'E	1650–1670	moss on soil	W. Maucci	+	–
Pseudobiotus megalonyx (Thulin, 1928)	Kraków, Poland	50°03'27"N, 19°50'19"E	294	sediment and rotting leaves	P. Gąsiorek	–	+
Thulinius ruffoi (Bertolani, 1982)	Zelków, Poland	50°09'52"N, 19°48'26"E	368	sediment	M. Wojtaszek	–	+
Thulinius stephaniae (Pilato, 1974)	Randazzo, Sicily, Italy	ca. 37°52'N, 14°56'E	710–750	sediment	G. Pilato	+	–
Halobiotidae fam. nov.							
Halobiotus arcturulius Crisp & Kristensen, 1983	Advent Bay, Spitsbergen	ca. 78°14'N, 15°35'E*	?	sediment	L. Kotwicki	–	+
Halobiotus crispae Kristensen, 1982	Nipissat Bay, Greenland	ca. 69°26'N, 54°12'W	?	sediment	R.M. Kristensen	+	+
Hexapodibiidae							
Hexapodibius micronyx Pilato, 1969	Gela, Sicily, Italy	ca. 37°04'N, 14°15'E	20–50	moss on sand	G. Pilato	+	–
	Saint-Maur-des-Fossés, France	48°48'23"N, 2°28'44"E	38	moss on tomb	W. Morek	–	+
Hexapodibius pseudomicronyx Robotti, 1972	Avigliana, Italy	ca. 45°05'N, 7°24'E	340–380	moss on wall	C. Robotti	+	–
Parhexapodibius castrii (Ramazzotti, 1964)	Cerro Pajonal, Chile	ca. 22°26'S, 68°54'W	4150	moss	?	+	–
Parhexapodibius lagrecai (Binda & Pilato, 1969)	Ustica, Italy	ca. 38°42'N, 13°10'E	?	moss	M.G. Binda, G. Pilato	+	–

Parhexapodibius ramazzottii Manicardi & Bertolani, 1987	Paradiso Pass, Italy	ca. 46°13’N, 10°34’E	2570	soil	G.C. Manicardi, R. Bertolani	+	–
Isohypsibiidae							
Dianea basalovoi (Durante & Maucci, 1972) comb. nov.	Basalovo, Italy	44°32’22”N, 10°59’55”E	343	moss on soil	M.V. Durante, W. Maucci	+	–
Dianea sattleri (Richters, 1902) comb. nov.	Kamienna Góra, Poland	50°46’39”N, 16°03’22”E	490	moss from tree trunk	G. Przybycień	–	+
	Ringvassøya, Hansnes	ca. 69°57’N, 19°37’E	3	moss from soil	W. Hlebowicz	–	+
Dianea vejdovskyi (Bartoš, 1939) comb. nov.	Carpathians, Czech Republic	?	?	moss on soil	E. Bartoš	+	–
Eremobiotus alicatai (Binda, 1969)	Gela, Sicily, Italy	ca. 37°04’N, 14°15’E	20–50	moss on sand	M.G. Binda	+	–
Eremobiotus ovezovae Biserov, 1992	Kara-Kum Desert, Turkmenistan	?	?	moss on sand	A. Ovezova	+	–
Eremobiotus **sp. nov.**	Gül Baba, Budapest, Hungary	47°31’01”N, 19°02’06”E	136	moss on wall	A. Witwicka	+	+
Fractonotus gilvus (Biserov, 1986)	Mussa-Aci Tara, Russia	ca. 43°16’N, 41°37’E	1835	moss on stump	V.I. Biserov	+	–
Fractonotus verrucosus (Richters, 1900)	Creag Meagaidh, Scotland	56°57’03”N, 4°36’09”W	1100	lichen on rock	B. Blagden	+	+
Isohypsibius arbiter Binda, 1980	Lucania, Italy	?	?	moss on soil	M.G. Binda	+	–
Isohypsibius ceciliae Pilato & Binda, 1987	Magdalena Bay, Spitsbergen	ca. 79°31’N, 10°42’E	?	moss on soil	?	+	–
Isohypsibius chiarae Maucci, 1987	Mount Washburn, Wyoming, USA	ca. 44°47’N, 110°26’W	?	moss on soil	W. Maucci	+	–
Isohypsibius coulsoni Kaczmarek et al., 2012	Hornsund, Spitsbergen	77°00’40”N, 15°22’20”E	437	moss from rock	Ł. Kaczmarek, J. Smykla	+	+
Isohypsibius dastychi Pilato et al., 1982	Modena, Italy	ca. 44°38’N, 10°55’E	30–50	moss on soil	R. Bertolani	+	–
	Paris, France	48°53’19”N, 2°19’44”E	56	moss on tomb	W. Morek	–	–
Isohypsibius prosostomus Thulin, 1928	Tatra Mountains, Poland	49°14’49”N, 20°05’42”E	1037	moss from tree bark	P. Gąsiorek	–	+
Isohypsibius reticulatus Pilato, 1973	Simeto river, Sicily, Italy	ca. 37°36’N, 14°56’E	490–510	?	G. Pilato	+	–

TABLE 1 List of isohypsibioid taxa used in presented analyses. All species were examined under PCM, and some also under SEM. In most cases type material was analysed (*cont.*)

Taxon	Locality	Coordinates	Altitude (m asl)	Sample type	Collector	Type material	SEM analysis
Isohypsibius sculptus (Ramazzotti, 1962)	Cordillera de Nahuelbuta, Chile	?	1100	lichen from tree trunk	F. di C. Liviero	+	–
Isohypsibius wilsoni (Horning et al., 1978)	Taumaka, Open Bay Islands	43°51'44"N, 166°52'56"E	5–20	lichen from rock	D.S. Horning	+	–
Ursulinius dudichi (Iharos, 1964) comb. nov.	Dauphiné Alps, Ailefroide, France	44°53'38"N, 6°19'38"E	2500	moss from rock	D. Frydryszak	–	–
Ursulinius duranteae (Maucci, 1978) comb. nov.	Sakçagözü, Turkey	ca. 37°10'N, 36°56'E	?	moss on soil	W. Maucci	+	–
Ursulinius elegans (Binda & Pilato, 1971) comb. nov.	Catania, Sicily, Italy	ca. 37°30'N, 15°05'E	?	moss on soil	M.G. Binda, G. Pilato	+	–
Ursulinius lunulatus (Iharos, 1966) comb. nov.	Chybie, Poland	49°55'36"N, 18°55'34"E	260	moss from concrete wall	A. Witwicka	–	+
Ursulinius pappi (Iharos, 1966) comb. nov.	Chybie, Poland	49°55'36"N, 18°55'34"E	260	moss from concrete wall	A. Witwicka	–	+
Ursulinius pilatoi (Durante Pasa & Maucci, 1979) comb. nov.	Kvalsund, Norway	ca. 70°30'N, 23°58'E	?	moss from rock	M.V. Durante Pasa, W. Maucci	+	–
Ursulinius ronsisvallei (Binda & Pilato, 1969) comb. nov.	Dilijan, Armenia	ca. 40°44'N, 44°51'E	?	moss on soil	V.I. Biserov	–	–
Ursulinius silvicola (Iharos, 1966) comb. nov.	Lago di Doberdò, Italy	45°49'60"N, 13°33'10"E	3	moss from tree trunk	P. Gąsiorek	–	–
Incertae sedis							
Ramajendas dastychi Kaczmarek et al., 2014	Antarctic Peninsula, Wilhelm Archipelago	65°09'56"S, 64°08'47"W	9	lichen from rock	K. Janko	+	–
Ramajendas renaudi (Ramazzotti, 1972)	Kerguelen Islands	ca. 49°20'S, 70°20'E	?	sediment	J. Renaud-Mornant	+	–

*See details in Wiktor et al. (2016).

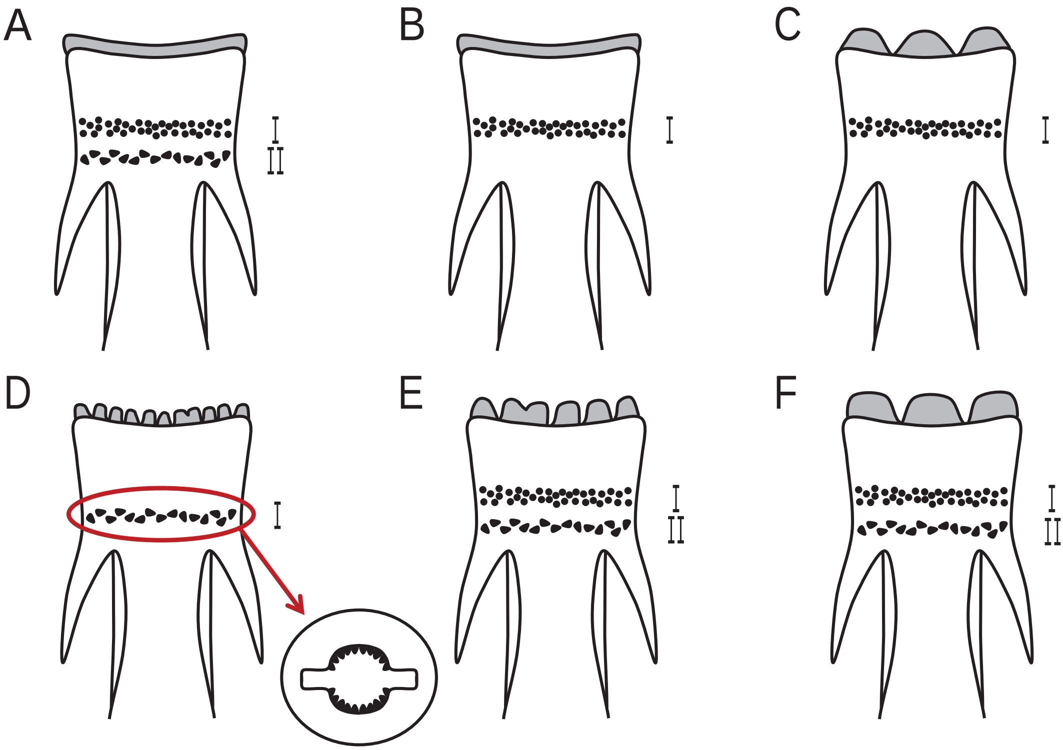

FIGURE 1 Schematic presentation of the oral cavity armature (OCA) in Isohypsibioidea, the first and/or the second band of teeth are marked by Roman numerals: A – a continuous peribuccal lamina, two bands of teeth (*Apodibius*, *Grevenius* gen. nov., *Halobiotus*, *Hexapodibius*); B – a continuous peribuccal lamina, the first band of teeth (*Fractonotus, Isohypsibius*, *Ursulinius* gen. nov.); C – six convex peribuccal papulae, the first band of teeth (*Eremobiotus*); D – rectangular peribuccal lamellae, the first band of teeth with lateral toothless intervals (*Pseudobiotus*); E – rectangular peribuccal lamellae, two bands of teeth (*Thulinius*); F – six large peribuccal lamellae, two bands of teeth (*Haplomacrobiotus*). Both bands of teeth contain variable number of rows, depending on the genus

primary claw branches, we introduce the ***br* ratio**, i.e., ratio of the height of the secondary claw branch to the height of the primary claw branch (the more the branches are similar in height, the closer to 1.0 the *br* ratio is and the shorter the secondary branch relative to the primary branch, the lower the *br* ratio).

During the review process of this manuscript, the tardigrade phylogeny by Guil et al. (2019) was published. Consequently, we adjusted the taxonomy of the high ranks in our work to the system proposed in Guil et al. (2019), however, in our opinion the new taxonomy is controversial and requires further work: possibly rank shift and taxon membership rearrangements, as the majority of high rank taxa (orders, families) remained in polytomies, compared to the relatively well-resolved relationships from the previous phylogeny by Bertolani et al. (2014). Given that superfamily Isohypsibioidea was elevated to the order level by Guil et al. (2019), according to the article 36.1 of International Code of Zoological Nomenclature (1999), the authorship of the superfamily (Sands et al., 2008) is now superseded by the latest authority.

Genotyping

DNA was extracted from individual animals using Chelex® 100 resin (Casquet et al., 2012; Stec et al., 2015). Paragenophores of all sequenced species were mounted on permanent slides and are deposited in the collection of Institute of Zoology and Biomedical Research (Pleijel et al., 2008). We sequenced two DNA fragments: a small ribosome subunit (18S rRNA) and a large ribosome subunit (28S rRNA). All fragments were amplified and sequenced according to the protocols described by Stec et al. (2015), using the primers and specific PCR programmes from: Sands et al. (2008) and Zeller (2010) (18S rRNA) or Mironov et al. (2012) (28S rRNA). Sequencing products were read with the ABI 3130xl sequencer at the Molecular Ecology Laboratory of the Institute of Environmental Sciences at Jagiellonian University. Sequences were processed using version 7.2.5 of BioEdit (Hall 1999).

Phylogenetics

We aligned all available isohypsibioid and hypsibioid (outgroup taxa) 18S + 28S rRNA sequences from GenBank together with our new sequences (see table 2) using the Q-INS-I strategy, which considers the secondary structure of RNA, in MAFFT version 7 (Katoh et al., 2002; Katoh & Toh, 2008). Currently available partial 28S rRNA sequences for *Halobiotus crispae* Kristensen, 1982, *Hexapodibius micronyx* Pilato, 1969 and *Pseudobiotus megalonyx* (Thulin, 1928) represent a different region of this marker than the ones sequenced by us, thus they were not included in the dataset. The aligned fragments were edited and checked manually in BioEdit. The best substitution model and partitioning scheme for posterior phylogenetic analysis was chosen under the Akaike Information Criterion (AIC), using PartitionFinder version 2.1.1 (Lanfear et al., 2016). As best-fit partitioning scheme, PartitionFinder suggested to retain two predefined partitions separately and for each of them the best fit model was GTR+I+G.

Maximum-likelihood (ML) topologies were constructed using RAxML v8.0.19 (Stamatakis, 2014). Strength of support for internal nodes of ML construction was measured using 1000 rapid bootstrap replicates. Bootstrap (BS) support values ≥70% on the final tree were regarded as significant statistical support. Bayesian inference (BI) marginal posterior probabilities were calculated using MrBayes v3.2 (Ronquist & Huelsenbeck, 2003). Random starting trees were used and the analysis was run for ten 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 v1.3 (Rambaut et al., 2014) 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 consensus tree was obtained after summarising the resulting topologies and discarding the 'burn-in'. The BI consensus tree, clades recovered with posterior probability (PP) between 0.95 and 1.00 were considered well supported, those with PP between 0.90 and 0.94 were considered moderately supported and those with lower PP were considered unsupported. All final consensus tree were viewed and visualized by FigTree v.1.4.3 available from http://tree.bio.ed.ac.uk/software/figtree. The sequence HQ604951, representing *E. alicatai* (Binda, 1969), was characterised by highly unstable position in the trees calculated in both methods, and it never clustered with newly sequenced *Eremobiotus* sp. nov., suggesting it could be a misidentification or a mislabelling. Therefore, we excluded this taxon from the final dataset. Additionally, the aligned fragments were trimmed to the size of the shortest available alignment (i.e., 745 bp for

TABLE 2 List of the GenBank accession numbers for isohypsibioid and hypsibioid (outgroup) sequences used for phylogenetic analyses in the present study (new sequences are marked in bold)

Taxon	18S rRNA	28S rRNA	Reference
Isohypsibioidea			
Apodibius confusus Dastych, 1983	KC582830	KC582834	Dabert et al. (2014)
Fractonotus verrucosus (Richters, 1900)	MG800855	MG800856	Gąsiorek et al. (2019)
Dianea papillifera (Murray, 1905)	EU266925	–	Sands et al. (2008)
Dianea sattleri (Richters, 1902)	**MK675926,** **MK675927**	**MK675915,** **MK675916**	**present study**
Doryphoribius flavus (Iharos, 1966)	HQ604940	–	Bertolani et al. (2014a)
Doryphoribius macrodon Binda et al., 1980	HQ604942	–	Bertolani et al. (2014a)
Eremobiotus sp. nov.	**MK675928**	**MK675917**	**present study**
Grevenius granulifer (Thulin, 1928)	EF620403, KT778603	–	Møbjerg et al. (2007), Cesari et al. (2016)
Grevenius pushkini (Tumanov, 2003)	**MK675929**	**MK675918**	**present study**
Halobiotus crispae Kristensen, 1982	AY582121, EF620402	–	Jørgensen & Kristensen (2004), Møbjerg et al. (2007)
Haplomacrobiotus utahensis Pilato & Beasley, 2005	KT778600	–	Cesari et al. (2016)
Hexapodibius micronyx Pilato, 1969	**MK675930,** HQ604915	**MK675919**	**present study,** Bertolani et al. (2014a)
Isohypsibius arbiter Binda, 1980	KT778602	–	Cesari et al. (2016)
Isohypsibius cambrensis (Morgan, 1976)	AM500652	–	Kiehl et al. (2007)
Isohypsibius dastychi Pilato et al., 1982	HQ604954	–	Bertolani et al. (2014a)
Isohypsibius prosostomus Thulin, 1928	EF620404	–	Møbjerg et al. (2007)
Pseudobiotus kathmanae Nelson et al., 1999	HQ604957	–	Bertolani et al. (2014a)
Pseudobiotus megalonyx (Thulin, 1928)	**MK675931,** HQ604959	**MK675920**	**present study,** Bertolani et al. (2014a)
Thulinius augusti (Murray, 1907)	KF360230	–	Bertolani et al. (2014b)
Thulinius ruffoi (Bertolani, 1982)	**MK675932**	**MK675921**	**present study**
Thulinius stephaniae (Pilato, 1974)	GQ925701	–	unpublished
Ursulinius lunulatus (Iharos, 1966)	**MK675933**	**MK675922**	**present study**
Ursulinius pappi (Iharos, 1966)	**MK675934**	**MK675923**	**present study**
Ursulinius silvicola (Iharos, 1966)	**MK675935**	**MK675924**	**present study**
Hypsibioidea (outgroup)			
Acutuncus antarcticus (Richters, 1904)	EU266943	–	Sands et al. (2008)
Adropion belgicae (Richters, 1911)	HQ604925	–	Bertolani et al. (2014a)
Adropion scoticum (Murray, 1905)	HQ604927		Bertolani et al. (2014a)
Astatumen trinacriae (Arcidiacono, 1962)	FJ435733, HQ604922	–	Guil & Giribet (2012), Bertolani et al. (2014a)
Borealibius zetlandicus (Murray, 1907)	HQ604924	–	Bertolani et al. (2014a)

TABLE 2 List of the GenBank accession numbers for isohypsibioid and hypsibioid (outgroup) sequences used for phylogenetic analyses in the present study (new sequences are marked in bold) (*cont.*)

Taxon	18S rRNA	28S rRNA	Reference
Calohypsibius ornatus (Richters, 1900)	MH279652, HQ604914	MK680130	Gąsiorek et al. (2019), Bertolani et al. (2014a)
Diphascon higginsi Binda, 1971	HQ604932	–	Bertolani et al. (2014a)
Diphascon pingue (Marcus, 1936)	FJ435736, HQ604937	FJ435778	Guil & Giribet (2012), Bertolani et al. (2014a)
Diphascon puniceum (Jennings, 1976)	EU266949	–	Bertolani et al. (2014a)
Hebesuncus conjungens (Thulin, 1911)	AM500646	–	Kiehl et al. (2007)
Hebesuncus ryani Dastych & Harris, 1994	EU266956	–	Sands et al. (2008)
Hypsibius convergens (Urbanowicz, 1925)	FJ435726	FJ435771	Guil & Giribet (2012)
Hypsibius exemplaris Gąsiorek et al., 2018	MG800327	MG800337	Gąsiorek et al. (2018)
Hypsibius dujardini (Doyère, 1840)	MG777532	MG777533	Gąsiorek et al. (2018)
Hypsibius klebelsbergi Mihelčič, 1959	KT901827	KC582835	Dabert et al. (2014, 2015)
Hypsibius pallidus Thulin, 1911	HQ604945	–	Bertolani et al. (2014a)
Hypsibius scabropygus Cuénot, 1929	AM500649	–	Dabert et al. (2014)
Mesocrista revelata Gąsiorek et al., 2016	KU528627	KU528628	Gąsiorek et al. (2016)
Mesocrista spitzbergensis (Richters, 1903)	KX347532	KX347533	Gąsiorek et al. (2016)
Microhypsibius bertolanii Kristensen, 1982	HQ604992	–	Bertolani et al. (2014a)
Mixibius saracenus (Pilato, 1973)	HQ604955	–	Bertolani et al. (2014a)
Pilatobius nodulosus (Ramazzotti, 1957)	HQ604934	–	Bertolani et al. (2014a)
Pilatobius patanei (Binda & Pilato, 1971)	HQ604935	–	Bertolani et al. (2014a)
Pilatobius ramazzottii (Robotti, 1970)	HQ604939	–	Bertolani et al. (2014a)
Pilatobius recamieri (Richters, 1911)	KX347526	–	Gąsiorek et al. (2017)
Platicrista angustata (Murray, 1905)	HQ604948	–	Bertolani et al. (2014a)
Ramazzottius oberhaeuseri (Doyère, 1840)	MG573241,	MG573242	Stec et al. (2018),
Ramazzottius aff. *oberhaeuseri*	AY582122		Jørgensen & Kristensen (2004)
Ramazzottius subanomalus (Biserov, 1985)	MF001997	MF001998	Stec et al. (2017)
Ramazzottius varieornatus Bertolani & Kinchin, 1993	HQ604950	MG432818	Bertolani et al. (2014a), Zawierucha et al. (2018)

18S rRNA and 756 bp for 28S rRNA), and uncorrected pairwise distances were calculated using MEGA7 (Kumar et al., 2016).

Results and discussion

Molecular phylogeny of Isohypsibioidea

Isohypsibioidea were highly supported in our phylogenetic analysis (fig. 2). Isohypsibioidea were divided into two major, well-supported clades: clade I (Isohypsibiidae *s.s.*) embraced exclusively terrestrial, bryophilous or lichenophilous taxa, whereas clade II comprised taxa that inhabit various environments, both aquatic (seas, ponds and rivers) and terrestrial (soil and bryophytes in case of some *Doryphoribius* Pilato, 1969 spp.).

Clade I embraced Isohypsibiidae *s.s.* and was further divided into three subclades

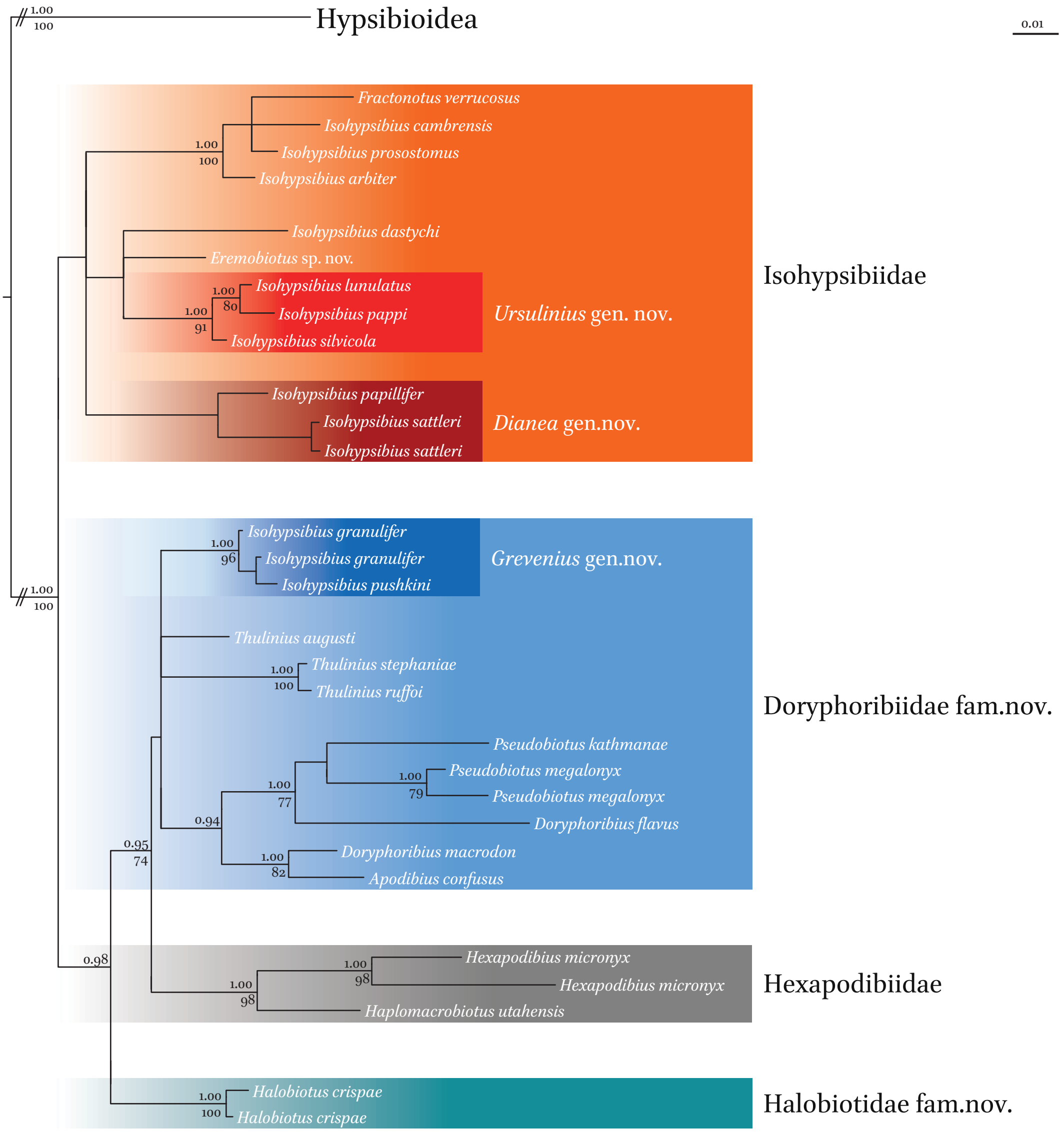

FIGURE 2 The phylogeny of Isohypsibioidea Sands et al., 2008 based on concatenated 18S rRNA and 28S rRNA seqences. New families and genera are marked in bold. Values above branches indicate Bayesian posterior probability values (BI), whereas those under branches show bootstrap values (ML). Branches with support below 0.9 in BI (70% in ML) were collapsed. Scale bar and branch lengths refer to the Bayesian analysis

with unresolved phylogenetic relationships: *Isohypsibius s.s.* + *Fractonotus* Pilato, 1998; *Dianea* gen. nov. (including the species hitherto known as *Isohypsibius sattleri* (Richters, 1902) and related spp.); and *Ursulinius* gen. nov. (including the species hitherto known as *Isohypsibius pappi* (Iharos, 1966) and related spp.) + *Isohypsibius dastychi* Pilato et al., 1982 + *Eremobiotus* Biserov, 1992. Clade II also comprised three distinct phyletic lineages in paraphyletic relationships: Halobiotidae fam. nov. (marine), Hexapodibiidae (soil-dwelling), and Doryphoribiidae fam. nov. (both freshwater and terrestrial/bryophilous). The latter

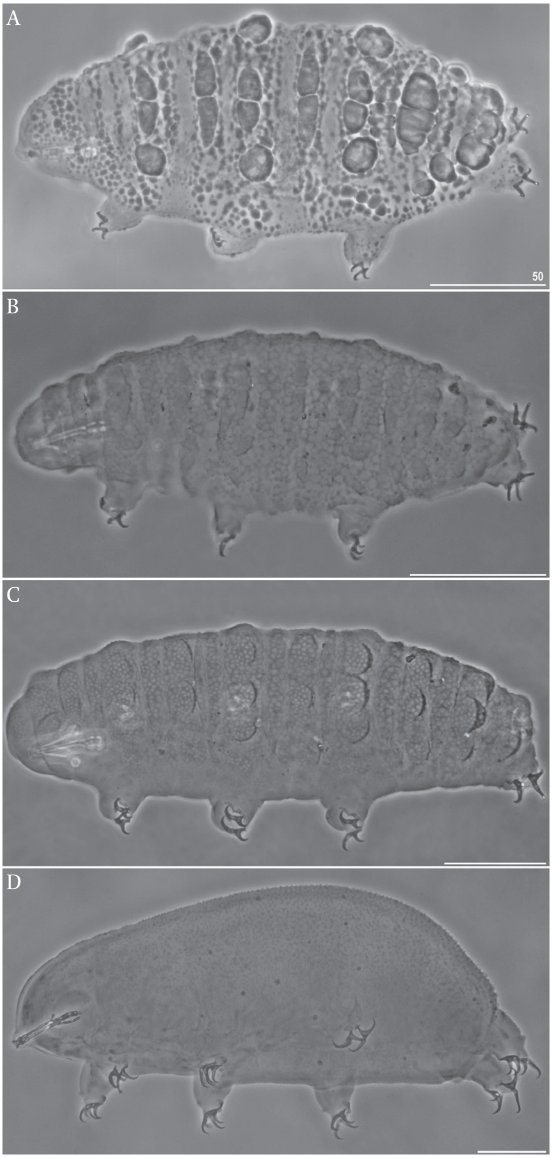

FIGURE 3 Nominal species for the recently transferred or newly erected genera of Isohypsibioidea (PCM): A – *Fractonotus verrucosus* (Richters, 1900) (Isohypsibiidae); B – *Dianea sattleri* (Richters, 1902) comb. nov. (Isohypsibiidae); C – *Ursulinius pappi* (Iharos, 1966) comb. nov. (Isohypsibiidae); D – *Grevenius granulifer* (Thulin, 1928) comb. nov. (Doryphoribiidae fam. nov.). Scale bars = 50 µm

family consisted of four subclades (with not fully resolved affinities) that embraced five genera: polyphyletic *Doryphoribius* and *Thulinius* Bertolani, 2003, as well as monophyletic *Pseudobiotus* Nelson, 1980 (in Schuster et al., 1980), *Apodibius* Dastych, 1983, and *Grevenius* gen. nov. (the species hitherto known as *Isohypsibius granulifer* Thulin, 1928 and kin).

Head morphology and peribuccal structures in Isohypsibioidea

The head in all Isohypsibioidea is terminated bluntly, with anteroventral mouth opening (figs. 3–4). The frontal part of the head is smooth or equipped with either of two types of structures: frontal lobes or cephalic papillae (Pilato & Binda, 2010). Cephalic

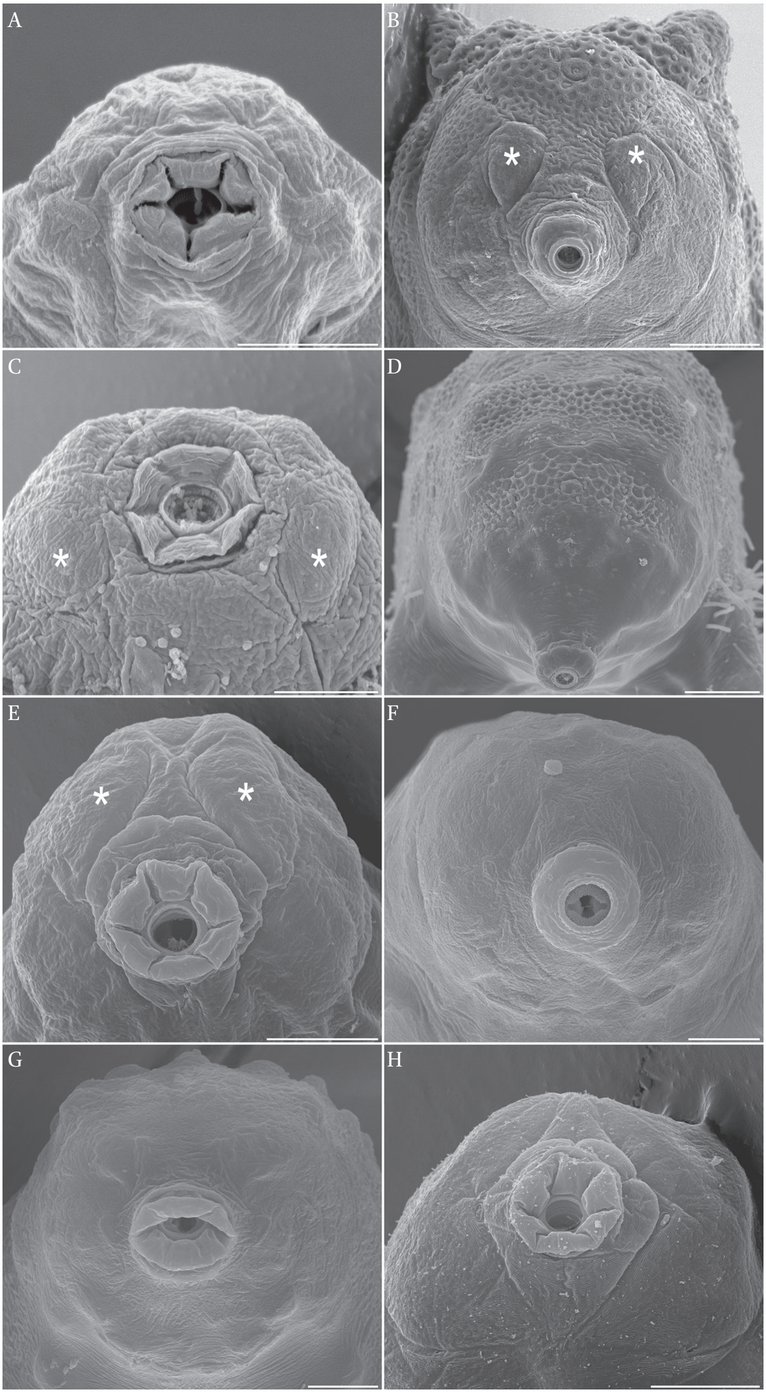

FIGURE 4 Cephalic region of various members of Isohypsibioidea (SEM): A – *Isohypsibius prosostomus* Thulin, 1928 (Isohypsibiidae); B – *Ursulinius pappi* comb. nov. (Isohypsibiidae); C – *Halobiotus crispae* Kristensen, 1982 (Halobiotidae fam. nov.); D – *Doryphoribius dawkinsi* Michalczyk & Kaczmarek, 2010 (Doryphoribiidae fam. nov.); E – *Apodibius confusus* Dastych, 1983 (Doryphoribiidae fam. nov.); F – *Pseudobiotus megalonyx* (Thulin, 1928) (Doryphoribiidae fam. nov.); G – *Grevenius granulifer* comb. nov. (Doryphoribiidae fam. nov.); H – *Hexapodibius micronyx* Pilato, 1969 (Hexapodibiidae). Asterisks indicate frontal lobes or cephalic papillae. Scale bars = 10 μm

papillae, present only in Halobiotidae fam. nov. (fig. 4C; Møbjerg et al., 2007), are less clearly delimited from the surrounding cuticle than lobes. Frontal lobes, on the other hand, are present in some remaining isohypsibioids, although they can vary in size and shape, for example they are smaller and slightly more roundish in *Ursulinius pappi* (fig. 4B) than in *Apodibius confusus* Dastych, 1983 (fig. 4E). *Paradiphascon* Dastych, 1992 has large, dome-shaped frontal lobes (Dastych, 1992). Given that Halobiotidae fam. nov., in contrast to all remaining Isohypsibioidea, secondarily adapted to marine environment, cephalic papillae are most likely a halobiotid autapomorphy. If, as hypothesised by Dastych (1992), frontal lobes are homologous remnants of heterotardigrade cephalic papillae, they should be considered an isohypsibioid plesiomorphy. Moreover, in some genera, additional regular circular cuticular wrinkles can be present around the mouth opening (*Apodibius* and *Hexapodibius* Pilato, 1969; figs. 4E, H, 5F, J). Mouth ring is smooth (figs. 5A–B, H), or with six either weakly developed or clearly separated peribuccal lobes (figs. 5E, G and figs. 5C–D, F, I–J, respectively). Peribuccal chemosensory organs are present exclusively in *Halobiotus crispae* (fig. 5D), but our SEM observations showed delicate lobe depressions in *Apodibius*, *Thulinius*, and *Hexapodibius*, which may indicate chemoperception ability in these genera (figs. 5F–G, J). The mouth opening in the majority of genera (*Apodibius*, *Doryphoribius*, *Fractonotus*, *Grevenius* gen. nov., *Halobiotus*, *Hexapodibius*, *Isohypsibius*, *Ursulinius* gen. nov.) is surrounded by a continuous peribuccal lamina (figs. 1A–B, 5A–F, I–J). However, in three genera peribuccal lamellae, often fused at their bases or on their entire height, are present (figs. 1D–F, 5G–H; see also Schuster et al., 1980 for the complete fusion of lamellae into a continuous lamina in *Thulinius saltursus* (Schuster et al., 1978)). The number of peribuccal lamellae is considered a generic trait (12 in *Thulinius*, 30 in *Pseudobiotus* and an undermined number in *Paradiphascon*; Schuster et al., 1980; Bertolani, 1982; Nelson et al., 1999; Dastych, 1992). Finally, in *Eremobiotus*, and likely in *Dastychius* Pilato, 2013, six peribuccal papulae are present (Biserov, 1992; Pilato, 2013). The continuous peribuccal lamina is definitely the most widespread morphotype, and likely the ancestral one, which independently evolved into divided or semi-divided peribuccal lamellae in two doryphoribiid genera. Nonetheless, our SEM observations question the validity of peribuccal lamellae as the main trait distinguishing *Thulinius* and *Pseudobiotus* (figs. 5G–H), since these structures have variable morphology.

Oral cavity armature in Isohypsibioidea and other Eutardigrada

In the great majority of isohypsibioid species, OCA is visible only under SEM and all our observations are based on this technique. In all analysed taxa, the oral cavity was equipped with at least one band of conical teeth located on the ring fold, in the central part of the oral cavity (fig. 1). However, in the majority of isohypsibioid genera a second band of teeth was also detected (*Apodibius*, *Grevenius* gen. nov., *Halobiotus*, *Hexapodibius*, *Thulinius*; the second band could be present also in *Pseudobiotus*, see below for details). There are no SEM observations of the oral cavity for *Dastychius*. The OCA system in *Paradiphascon* is obscure (Dastych, 1992) and the number or exact shape of peribuccal lamellae are unknown, thus the genus is not included in the present schematic depiction. The first band of teeth, comprising 2–5 rows of teeth, consists either of small and sparse conical teeth in *Fractonotus* and *Halobiotus* (figs. 5C–D), medium-sized and more densely arranged conical teeth in *Isohypsibius*, *Eremobiotus*, *Apodibius*, *Thulinius*, *Grevenius* gen. nov., *Hexapodibius* (figs. 5A, F–G, I–J, 13B–C, 14B), or large conical teeth divided into a dorsal and a ventral

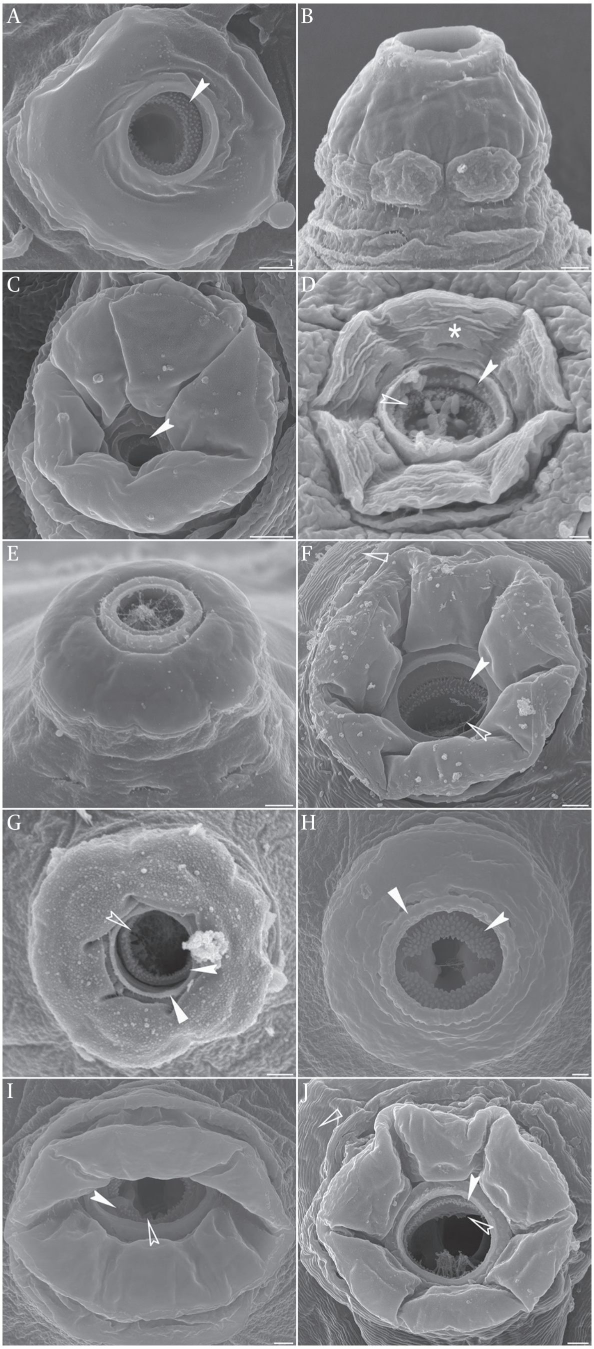

FIGURE 5 Peribuccal structures of various members of Isohypsibioidea (SEM): A – *Isohypsibius coulsoni* Kaczmarek et al., 2012 (Isohypsibiidae); B – *Ursulinius pappi* comb. nov. (Isohypsibiidae); C – *Fractonotus verrucosus* (Isohypsibiidae); D – *Halobiotus crispae* (Halobiotidae fam. nov.); E – *Doryphoribius dawkinsi* (Doryphoribiidae fam. nov.); F – *Apodibius confusus* (Doryphoribiidae fam. nov.); G – *Thulinius ruffoi* (Bertolani, 1981) (Doryphoribiidae fam. nov.); H – *Pseudobiotus megalonyx* (Doryphoribiidae fam. nov.); I – *Grevenius granulifer* comb. nov. (Doryphoribiidae fam. nov.); J – *Hexapodibius micronyx* (Hexapodibiidae). Incised arrowheads indicate the first row of teeth, empty incised arrowheads – the second row of teeth, arrowheads – fused peribuccal lamellae, empty arrowheads – peribuccal wrinkles, and the asterisk points the peribuccal chemosensory organ. Scale bars = 1 μm.

row in *Pseudobiotus* (figs. 1D, 5H; Nelson et al., 1999). The second band of teeth, composed of 1–4 rows of teeth, comprises conical teeth that are typically larger than those in the first band, and are located immediately behind the first band and before the buccal tube opening (figs. 5D, F–G, I, 13B–C, 14B). In all examined species, the second band was continuous, except for *Hexapodibius micronyx*, in which the band was divided into a short dorsal and ventral row of irregular teeth (fig. 5J).

In a wider context, so far, greatest attention was paid to OCA in Macrobiotoidea Guil et al., 2019 (Thulin, 1911; Pilato, 1975; Michalczyk & Kaczmarek, 2003; Guidetti et al., 2012) and Eohypsibioidea Guil et al., 2019 (e.g., Hansen et al., 2017), but very little is known about OCA in Hypsibioidea (Gąsiorek et al., 2016, 2018; Stec et al., 2017, 2018) and the topic has not been addressed systematically in Isohypsibioidea (OCA was only mentioned occasionally in several species, e.g., in Pilato, 1975; Biserov, 1992; Jørgensen, 2001; Lisi, 2011). The lack of data for Hypsibioidea prevents the formulation of sound hypotheses about the evolution of OCA both within isohypsibioids and in all eutardigrades.

Nevertheless, OCA in isohypsibioids seems to have supra-generic significance. Our observations showed that Isohypsibiidae have only one band of teeth, whereas the three other families exhibit two bands (fig. 1). The only exception in Doryphoribiidae fam. nov. – *Pseudobiotus* with only the first band of teeth visible in the oral cavity – has to be treated with caution as the first band of teeth in this genus is very large and it obscures the view of the posterior part of the oral cavity, therefore it is not possible to say whether the second band of teeth is lacking or it is simply not visible when looking through the mouth opening. However, based on phylogeny, we hypothesise that *Pseudobiotus* exhibits two bands of teeth. Given that the two sister clades constituting Isohypsibioidea (i.e., Isohypsibiidae vs Halobiotidae fam. nov. + Hexapodibiidae + Doryphoribiidae fam. nov.) exhibit one vs two bands of teeth in the oral cavity, it is not possible to state whether the last common ancestor for all isohypsibioids had one or two bands of teeth.

The differences in the location of teeth in the OCA between Isohypsibioidea and both Eohypsibioidea and Macrobiotoidea may suggest that bands of teeth in Isohypsibioidea and in the two latter orders are not homologous. OCA in Eohypsibioidea and Macrobiotoidea consists of three bands of teeth: first (minute cones located in the very anterior of the oral cavity), second (larger cones or ridges parallel to the main axis of the buccal apparatus, located in the rear of the oral cavity just behind the ring fold), and third (a system of ventral and dorsal transverse crest/ridge-shaped teeth, located in the rear of the oral cavity just behind the second band of teeth and before the buccal tube opening in Macrobiotidae Thulin, 1928, Murrayidae Guidetti et al., 2000 and some Richtersiidae Guidetti et al., 2016 or a band of conical teeth in some Richtersiidae). Thus, Isohypsibioidea do not exhibit the most anterior band of teeth, termed as the first (or anterior) band, that is present in both Eohypsibioidea and Macrobiotoidea in the very anterior of the oral cavity. Moreover, except for *Richtersius* Pilato & Binda, 1989 (which exhibits a highly modified OCA), neither in Eohypsibioidea nor in Macrobiotoidea were the teeth observed on the ring fold. The only congruence in the location of teeth in the oral cavity concerns the most posterior teeth: in Eohypsibioidea and Macrobiotoidea the third band of teeth is located immediately behind the ring fold, i.e., in the same place as the second band of teeth in Isohypsibioidea. Nevertheless, as already mentioned above, the current state of knowledge on the OCA in eutardigrades does not allow to conclude whether the third band in eohypsibiids and macrobiotids is homologous with the second band in isohypsibioids.

Interestingly, regardless of phylogenetic relationships and location of teeth in the oral cavity, larger teeth (e.g., in the first band in *Pseudobiotus* and in the third band in Eohypsibiidae Bertolani & Kristensen, 1987 and Macrobiotidae) tend to be arranged in two rows, ventral and dorsal. We hypothesise that lateral toothless intervals are necessary to allow stylet extrusion through the oral cavity and mouth opening (stylets are positioned laterally, parallel to the buccal tube, and they are extruded in a scissor-like manner; Guidetti et al., 2013).

Buccal apparatus morphology in the Doryphoribiidae fam. nov. + Hexapodibiidae clade

Two evolutionary pathways can be recognised in the anatomy of the buccal apparatus in Doryphoribiidae fam. nov.: buccal tube without ventral lamina and with unmodified AISM (*Grevenius* gen. nov., *Pseudobiotus*, *Thulinius*), and the other with the buccal tube enforced with ventral lamina, which is associated with modifications of AISM (*Apodibius*, *Doryphoribius*) (Pilato & Binda, 2010). The buccal apparatus of *Grevenius pushkini* (Tumanov, 2003) comb. nov. and other aquatic "*Isohypsibius*" spp. is generally more similar to that in *Thulinius* spp. than to terrestrial *Isohypsibius* spp. (figs. 13, 14), which is not surprising given the close phylogenetic relationship between the two taxa (fig. 2) and same, aquatic, habitat. Specifically, aquatic "*Isohypsibius*" spp. and *Thulinius* spp. have two rows of teeth in the oral cavity (figs. 13B–C, 14B) and narrow apophyses for the insertion of the stylet muscles (AISM) (figs. 13D, 14C) whereas terrestrial *Isohypsibius* spp. exhibit one row of buccal teeth (fig. 5A) and proportionally broader AISM. The anatomy of buccal apparatus in Hexapodibiidae is more conserved, since only the morphotype with ventral lamina exists (Cesari et al., 2016). Buccal apparatus of *Hexapodibius*, similarly to that of *Haplomacrobiotus*, has reduced AISM due to the developed ventral lamina (figs. 15A–C, 16D). Isohypsibioid taxa equipped with ventral lamina, i.e., Hexapodibiidae and some Doryphoribiidae fam. nov.: *Doryphoribius* and *Apodibius*, share extreme resemblance of the buccal apparatus anatomy. For example, they all exhibit unmodified *Hypsibius* type furcae (figs. 15D, 16) and two or three short, often almost granular macroplacoids in the pharynx (figs. 15E, 16; Hohberg & Lang, 2016). This is in contrast to taxa without the ventral lamina, *Thulinius*, *Pseudobiotus*, and *Grevenius* gen. nov., which all have elongated macroplacoids. Interestingly, ventral lamina is present in terrestrial but not in freshwater representatives of the Halobiotidae fam. nov. + Hexapodibiidae + Doryphoribiidae fam. nov. clade (single exceptions can be found in polyphyletic *Doryphoribius*). Ventral lamina in both *Doryphoribius* and Hexapodibiidae has two different morphotypes: a short, delicate lamina reaching no farther than to the half of the buccal tube length in *Apodibius*, *Hexapodibius*, or some *Parhexapodibius* Pilato, 1969, and *Doryphoribius* (figs. 15A–C, 16A, C–D, F); or a long, robust lamina reaching almost the level of the stylet support insertion point in some *Doryphoribius* and *Parhexapodibius* (figs. 16B, E). The presence of ventral lamina in all hexapodibiids but only in some doryphoribiids suggests that either the common ancestor of Hexapodibiidae + Doryphoribiidae fam. nov. exhibited the lamina, which was later independently lost in *Thulinius*, *Pseudobiotus*, and *Grevenius* gen. nov., or lamina evolved independently two or three times: in (1) Hexapodibiidae, (2) ancestor of *Apodibius* and some *Doryphoribius* spp., and (3) in remaining *Doryphoribius* spp. (see fig. 2). The lack of ventral lamina in Heterotardigrada Marcus, 1927, Apotardigrada, Hypsibioidea and many Isohypsibioidea suggests that it is a derived trait that evolved independently in Isohypsibioidea and in the ancestor of Macrobiotoidea. In other words, the presence of the ventral lamina should be treated as an example of parallel evolution within Eutardigrada, being at the same time the autapomorphy of Macrobiotoidea as well as of Hexapodibiidae and some genera of Doryphoribiidae fam. nov. (Marley et al., 2011).

Cuticle morphology in Isohypsibioidea

In contrast to the majority of eutardigrades, isohypsibioids frequently exhibit distinct cuticular sculpturing (figs. 3, 6). Five major kinds of sculpturing can be distinguished within the order: (I) reticulum, (II) circular tubercles of various size, (III) pointy gibbosities, (IV) round gibbosities, and (V) plaques. The most unique type of cuticle morphology characterises *Fractonotus*, which has symmetrically arranged dorsal plaques (figs. 3A, 6B) as well as densely arranged smooth tubercles that cover the entire dorsum and limbs (figs. 3A, 6A; Gąsiorek et al., 2019). Isohypsibioid gibbosities can be generally divided into two types: small, weakly demarcated (almost flat in LM) and pointy gibbosities present in *Dianea* gen. nov. (figs. 3B, 6C), or large, mamillose and round gibbosities with developed reticulum or complex ornamentation in *Ursulinius* gen. nov. and many *Doryphoribius* spp. (figs. 3C, 6D–F; Ramazzotti & Maucci, 1983). Gibbosities of *Dianea* gen. nov. are less regular and clearly narrow towards the apex in contrast to hemispherically convex gibbosities in the two latter genera. The usage of dorsal gibbosities as a generic trait was a subject of criticism (Pilato, 1982), as, according to some descriptions, in one species there could be a considerable variation in gibbosity development (e.g., Binda & Pilato, 1971). The same variability was ascribed to cuticular sculpturing in general (Kristensen & Hallas, 1980). However, recent data show that in a single sample, numerous, potentially closely related or pseudocryptic species can be found (e.g., see Faurby et al., 2011; Morek et al., 2019). Therefore, the reports of such profound variability in the development of gibbosities given without genetic data should be taken with caution. In species devoid of *Fractonotus* type tubercles or gibbosities, e.g., in *Grevenius* gen. nov., *Thulinius* or *Pseudobiotus* (figs. 3D, 6G), quite often the entire dorsal cuticle is covered with homogenous, rough sculpturing that forms wrinkly epicuticular reticulum or processes (fig. 6H; Bertolani, 1982; Chang et al., 2007; Pilato et al., 2010; Bertolani et al., 2014b). The richness and variability of cuticular sculpturing within Isohypsibioidea indicate independent, autapomorphic origin and prevent hypothesising whether the ancestral cuticle state was smooth or sculptured.

Claw morphology in Isohypsibioidea

Isohypsibioid claws can be divided into six general morphotypes: (I) *Isohypsibius* type, as defined by Ramazzotti & Maucci (1983), the most widespread morphotype, with external and internal claws on the same limb of similar size and with branches forking at a ca. right (90°) angle, (figs. 7A–D, H, K, 8, 9B, 10); (II) *Eremobiotus* type, with all claws with branches forming an obtuse, approaching a straight (ca. 180°) angle, and external and internal claws on the same limb of similar size, but dissimilar branch heights (*br* < 70%), which is a highly modified *Isohypsibius* type (figs. 7E–F, 9C–D; Biserov, 1992); (III) *Fractonotus* type, with all claws with V-shaped branches and with secondary branches forming a continuous curve with the basal tract and significantly shorter than the primary branches (*br* < 70%), which could be seen as an intermediate morphotype between the *Isohypsibius* and the *Hypsibius* type claw (figs. 7G, 9A; Gąsiorek et al., 2019); (IV) strongly reduced hexapodibiid claws, with indistinct basal tracts and partly or completely reduced secondary branches (fig. 7Q; Cesari et al., 2016); (V) *Paradiphascon* type, with internal and anterior claws of the *Isohypsibius* type and external and posterior claws of the *Hypsibius* type with very broad bases (Dastych, 1992); (VI) *Pseudobiotus* type

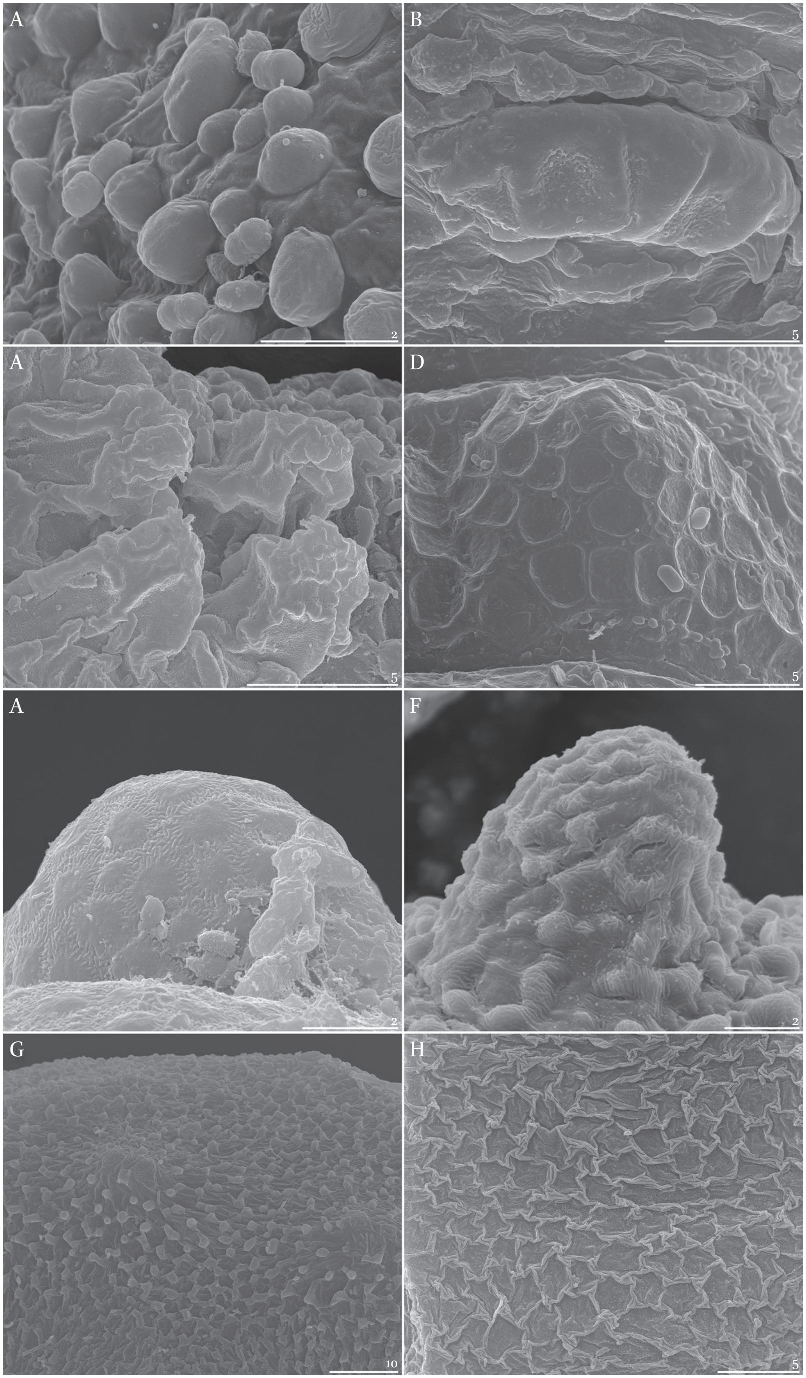

FIGURE 6 Cuticular surface of various members of Isohypsibioidea (SEM): A–B – *Fractonotus verrucosus* (Isohypsibiidae), obtuse tubercles and plaques; C – *Dianea sattleri* comb. nov. (Isohypsibiidae), small wrinkled gibbosities; D – *Ursulinius pappi* comb. nov. (Isohypsibiidae), large reticulated gibbosities; E – *Ursulinius elegans* (Binda & Pilato, 1971) comb. nov. (Isohypsibiidae), large ornamented gibbosities; F – *Doryphoribius dawkinsi* (Doryphoribiidae fam. nov.), large sculptured gibbosities; G – *Grevenius granulifer* comb. nov. (Doryphoribiidae fam. nov.), irregular small tubercles; H –*Grevenius pushkini* (Tumanov, 2003) comb. nov. (Doryphoribiidae fam. nov.), cuticular wrinkles. Scale bars in micrometres

with secondary and primary branches similar in heigth (*br* typically >70%, see table 2), elongated basal tracts, and typically prominent humps on primary branches of internal and anterior claws (figs. 7I–J, L–P, 11). The *Pseudobiotus* type claws are common for the genera *Pseudobiotus*, *Halobiotus*, *Thulinius* and *Grevenius* gen. nov. The peculiar morphology of OCA (see above), together with anatomical modifications related to copulation and parental care in *Pseudobiotus* (hook-like claws on the first pair of legs in males (fig. 7N) and reduced hind claws in females who carry shed exuviae with eggs), seem to be more suitable taxonomic criteria to differentiate *Pseudobiotus* and *Thulinius* rather than the number of peribuccal lamellae, as *Thulinius* is parthenogenetic (or at least does not exhibit sexual dimorphism, however thelytoky was confirmed in *T. augusti* (Murray, 1907) – see Bertolani, 1976, and *T. ruffoi* (Bertolani, 1981) – see Kosztyła et al., 2016) and lacks parental care and associated morphological modifications (Rebecchi & Nelson, 1998)).

Morphotypes II–VI are internally homogenous, however *Isohypsibius* morphotype can be further divided into three distinct subgroups: (Ia) *I. prosostomus* type, with secondary branches clearly shorter than primary branches (*br* ranges from around 40% to 70%, see table 3), claw bases without pseudolunulae, and with single bars under claws (figs. 7A–B, 8A–D); (Ib); *I. dastychi* type, with branches forking at an obtuse, approaching a straight (ca. 180°) angle, with developed pseudolunulae, *br* ≈ 70% and double bars under claws (fig. 8E; according to Tumanov (2005), bars are absent only in *I. panovi* Tumanov, 2005); (Ic) *U. pappi* type, with evident pseudolunulae, and double bars under claws (*br* ≈ 50%–70%; figs. 7C–D, 10). The ancestral state of claw morphology remains unknown, as relationships within Isohypsibiidae *s.s.* are unclear (fig. 2). Types II–VI have been already used in erections of supra-specific entities, and we hypothesise that all subtypes of type I could also be suitable for differentiating higher taxonomic levels.

Taxonomy of Isohypsibiidae *sensu stricto*

Isohypsibius *Thulin, 1928 and related genera*

For a considerable time, *Isohypsibius* was the second largest tardigrade genus (Degma & Guidetti, 2007; Degma et al., 2009–18). Despite the erections of new genera from *Isohypsibius*, including those erected in the present study, the genus still remains relatively speciose (42 spp. vs 16, 36 and 35 spp. in the newly erected *Dianea*, *Ursulinius* and *Grevenius* gen. nov., respectively; see Appendix). However, as recently suggested by Gąsiorek et al. (2019), some *Isohypsibius* spp. appear more closely related to *Fractonotus* than to *Isohypsibius s.s.*, which could explain the current paraphyletic character of *Isohypsibius* with respect to *Fractonotus* (fig. 2). Moreover, there are at least two more claw morphotypes that are divergent from the *I. prosostomus* (i.e., *Isohypsibius s.s.*) type defined in this work (figs. 8A–D). The distinctiveness of the first group, *I. dastychi* group, has been already noticed by Tumanov (2005). The *I. dastychi* group exhibits claws with branches forking at a very wide, approaching a 180° angle, present also in *Eremobiotus* (fig. 8E). Interestingly, the topology of the tree indicates the affinity of these two groups as *I. dastychi* and *Eremobiotus* sp. nov. are in a single polytomous clade (that includes also *Ursulinius* gen. nov.). The second morphotype is currently represented only by a single species, *Isohypsibius chiarae* Maucci, 1987. Secondary branches in this species are reduced, being short and acute (fig. 8F). Taking into consideration that in the present study, morphological peculiarities of a similar magnitude induced the erections of three new genera, including one representing a different family (*Grevenius* gen. nov., in Doryphoribiidae fam. nov.), it should be noted that *I. chiarae* does not belong to *Isohypsibius s.s.*

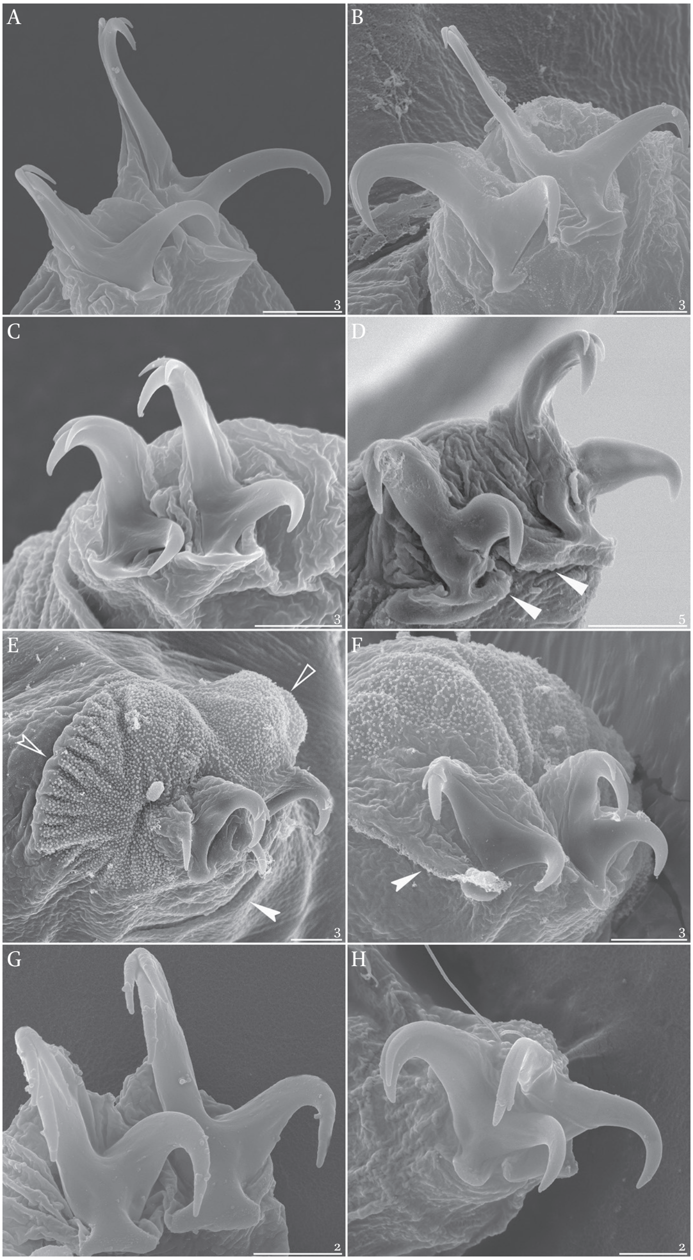

FIGURE 7 Claw types of various members of Isohypsibioidea (SEM): A – *Isohypsibius prosostomus* (Isohypsibiidae); B – *Isohypsibius coulsoni* (Isohypsibiidae); C – *Ursulinius pappi* comb. nov. (Isohypsibiidae), claws I–III; D – *Ursulinius pappi* comb. nov. (Isohypsibiidae), modified claws IV (arrowheads indicate evident pseudolunulae); E – *Eremobiotus* sp. nov. (Isohypsibiidae), external side of claws I–III (incised arrowheads indicate longitudinal internal bar, empty incised arrowheads – the furbelow structure covered with minute granulation, the empty arrowhead – pedal gibbosity); F – *Eremobiotus* sp. nov. (Isohypsibiidae), internal side of claws I–III; G – *Fractonotus verrucosus* (Isohypsibiidae); H – *Dianea sattleri* comb. nov. (Isohypsibiidae); (*Cont. on next page*)

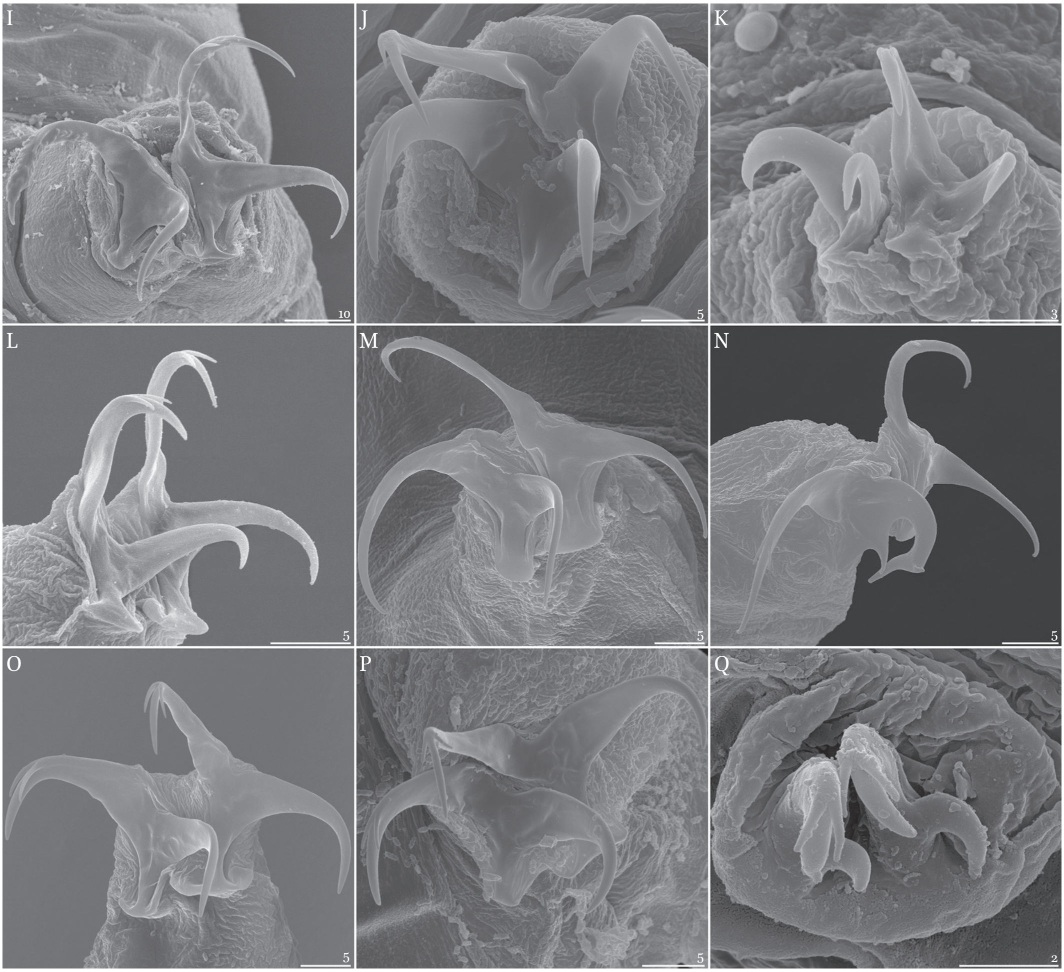

FIGURE 7 (*Cont.*) I – *Halobiotus arcturulius* Crisp & Kristensen, 1983 (Halobiotidae fam. nov.); J – *Halobiotus crispae* (Halobiotidae fam. nov.); K – *Doryphoribius dawkinsi* (Doryphoribiidae fam. nov.); L – *Thulinius ruffoi* (Doryphoribiidae fam. nov.); M – *Pseudobiotus megalonyx* (Doryphoribiidae fam. nov.); N – *Pseudobiotus megalonyx* (Doryphoribiidae fam. nov.), modified male claws I; O – *Grevenius granulifer* comb. nov. (Doryphoribiidae fam. nov.); P – *Grevenius pushkini* comb. nov. (Doryphoribiidae fam. nov.); Q – *Hexapodibius micronyx* (Hexapodibiidae), reduced claws with undeveloped bases. Scale bars in micrometres

Systematic position of **Eremobiotus** *Biserov, 1992*

Both morphological and genetic data undoubtedly show that *Eremobiotus* is a member of Isohypsibiidae (fig. 2). The morphological uniformity of the clade *Eremobiotus* + *Ursulinius* gen. nov. + the *I. dastychi* group (uniting *Isohypsibius* spp. with two macroplacoids and smooth cuticle) is expressed by several traits: two macroplacoids in the pharynx, typically well-developed pseudolunulae (figs. 8E, 10), and evident double bars in *Eremobiotus* and *Ursulinius* gen. nov. (fig. 10) (see also Lisi et al., 2016). However, the monophyly of the genus should be treated with caution since claws in *E. ovezovae* Biserov, 1992, unlike claws of the remaining two described *Eremobiotus* spp., are significantly reduced (compare

TABLE 3 Secondary/primary claw branch ratios (br) for various *Isohypsibius*-like taxa expressed in percentages. Claws of the fourth pair of legs were measured. Data source: O – original species description, M – measurements performed in the present study

Species	Habitat	RANGE			MEAN	SOURCE
Dianea sattleri (Richters, 1902)	terrestrial	58	–	72	64	M
Eremobiotus sp. nov.	terrestrial	50	–	66	58	M
Fractonotus verrucosus (Richters, 1900)	terrestrial	54	–	59	56	M
Isohypsibius altai Kaczmarek & Michalczyk, 2006	terrestrial	38	–	67	44	O
Isohypsibius archangajensis Kaczmarek & Michalczyk, 2004	terrestrial	50	–	71	62	O
Isohypsibius condorcanquii Kaczmarek et al., 2014	terrestrial	50	–	55	53	O
Isohypsibius coulsoni Kaczmarek et al., 2012	terrestrial	56	–	68	63	M
Isohypsibius dastychi Pilato et al., 1982	terrestrial	70	–	70	?	M
Isohypsibius prosostomus Thulin, 1928	terrestrial	59	–	70	63	M
Ursulinius austriacus (Iharos, 1966)	terrestrial	61	–	61	?	M
Ursulinius dudichi (Iharos, 1964)	terrestrial	62	–	70	66	M
Ursulinius elegans (Binda & Pilato, 1971)	terrestrial	64	–	64	?	M
Ursulinius lunulatus (Iharos, 1966)	terrestrial	54	–	69	62	M
Ursulinius pappi (Iharos, 1966)	terrestrial	62	–	70	65	M
Ursulinius silvicola (Iharos, 1966)	terrestrial	60	–	66	63	M
Grevenius baldiioides (Tumanov, 2003)	aquatic	72	–	75	?	O
Grevenius granulifer (Thulin, 1928)	aquatic	83	–	99	91	M
Grevenius karenae (Zawierucha, 2013)	aquatic	70	–	81	76	O
Grevenius kotovae (Tumanov, 2003)	aquatic	68	–	76	71	O
Grevenius ladogensis (Tumanov, 2003)	aquatic	74	–	79	76	O
Grevenius laevis (McInnes, 1995)	aquatic	78	–	78	?	O
Grevenius pushkini (Tumanov, 2003)	aquatic	71	–	72	72	M

figs. 9C–D). Thus, the possibility that *E. ovezovae* represents an independent evolutionary line that has convergently evolved claws with widely angled branches must be considered. In fact, it would not be surprising if this claw morphotype evolved more than once in Isohypsibiidae, especially that, for example, claw reduction has been shown to evolve independently in several eutardigrade lineages (Bertolani & Biserov, 1996).

Taxonomy of Doryphoribiidae fam. nov.

Polyphyly of Doryphoribius *Pilato, 1969*

In the redesigned systematics of Isohypsibioidea presented herein, the position and composition of *Doryphoribius* appeared as the most problematic. The genus *Doryphoribius* is polyphyletic and this is clearly visible both from earlier studies (Bertolani et al., 2014a) and the current molecular phylogeny

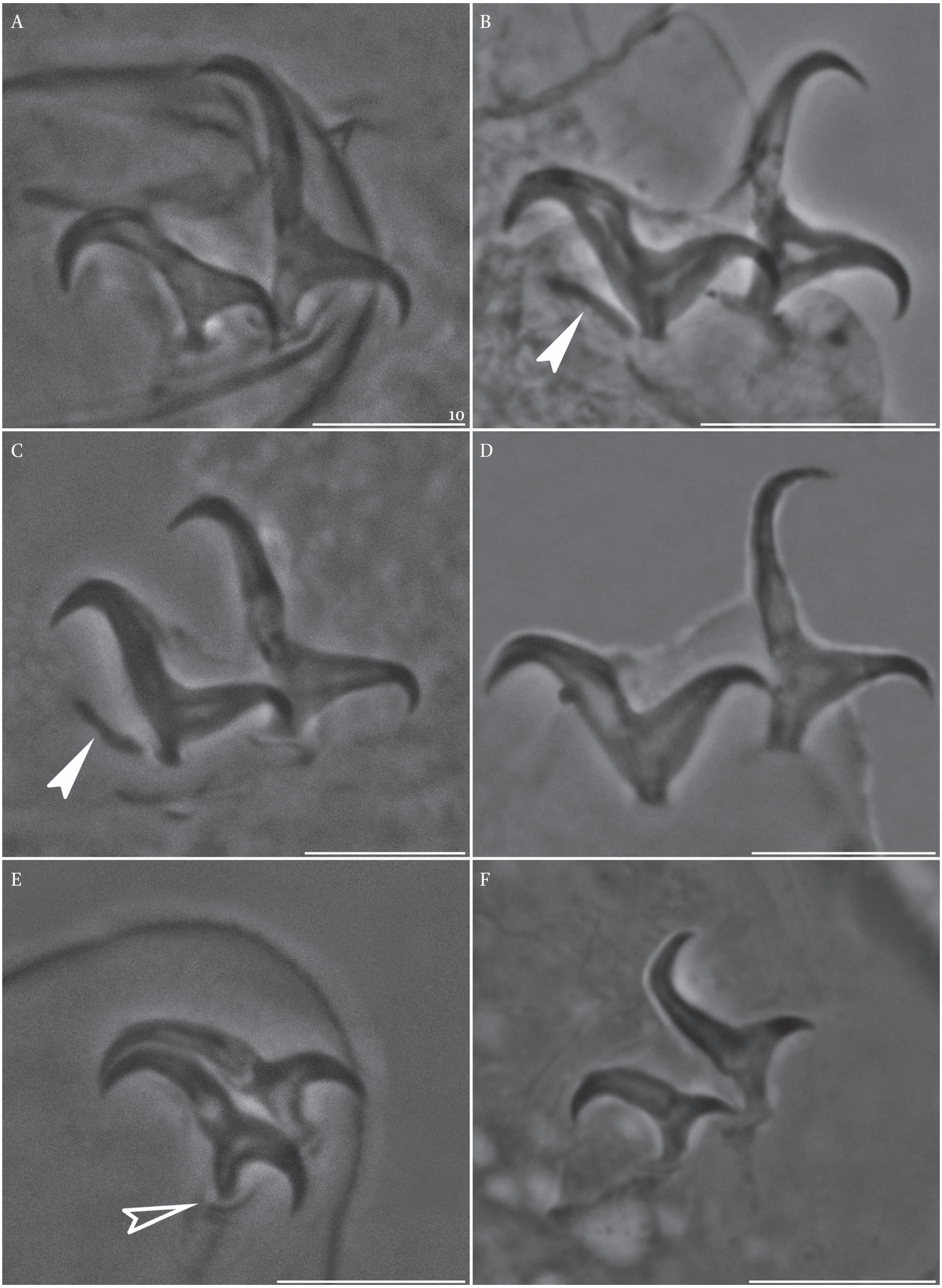

FIGURE 8 *Isohypsibius* type claws (Isohypsibiidae, PCM): A – *Isohypsibius prosostomus*; B – *Isohypsibius arbiter* Binda, 1980; C – *Isohypsibius coulsoni*; D – *Isohypsibius wilsoni* (Horning et al., 1978); E – *Isohypsibius dastychi* Pilato et al., 1982; F – *Isohypsibius chiarae* Maucci, 1987. Note singular bars (incised arrowheads) and weakly developed or lacking pseudolunulae (empty incised arrowhead). Scale bars = 10 μm

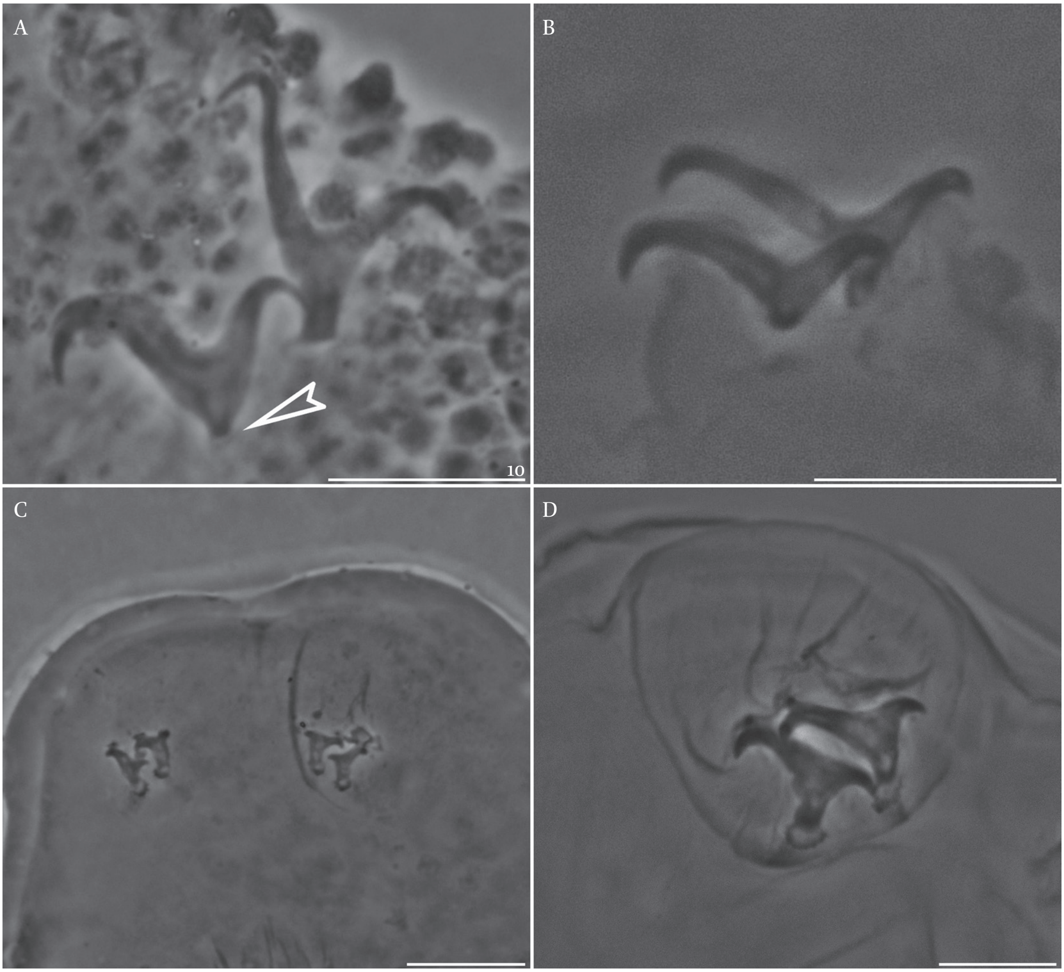

FIGURE 9 Modified *Isohypsibius* type claws (Isohypsibiidae, PCM): A – *Fractonotus gilvus* (Biserov, 1986), note weakly developed pseudolunulae (empty incised arrowheads); B – *Dianea sattleri* comb. nov.; C – *Eremobiotus ovezovae* Biserov, 1992; D – *Eremobiotus* sp. nov. Scale bars = 10 μm

(fig. 2). Specifically, *D. macrodon* Binda et al., 1980 clustered with *A. confusus*, which is not very surprising since both species have the ventral lamina and two short macroplacoids in the pharynx (fig. 2). However, at the same time, another *Doryphoribius*, *D. flavus* (Iharos, 1966), grouped with *Pseudobiotus*, which is unexpected as the two taxa exhibit dissimilar buccal apparatuses and claw morphologies as well as different habitat preferences (terrestrial vs freshwater). This discrepancy between morphology and genetics is not easy to interpret. On one hand, the ventral lamina has most likely evolved independently at least several times within Doryphoribiidae fam. nov., which makes the phylogenetic inference based on buccal apparatus anatomy questionable. On the other hand, it cannot be ruled out that the ribosomal markers are too conservative to resolve phyletic affinities within Doryphoribiidae fam. nov. and real relationships within Doryphoribiidae fam. nov. may

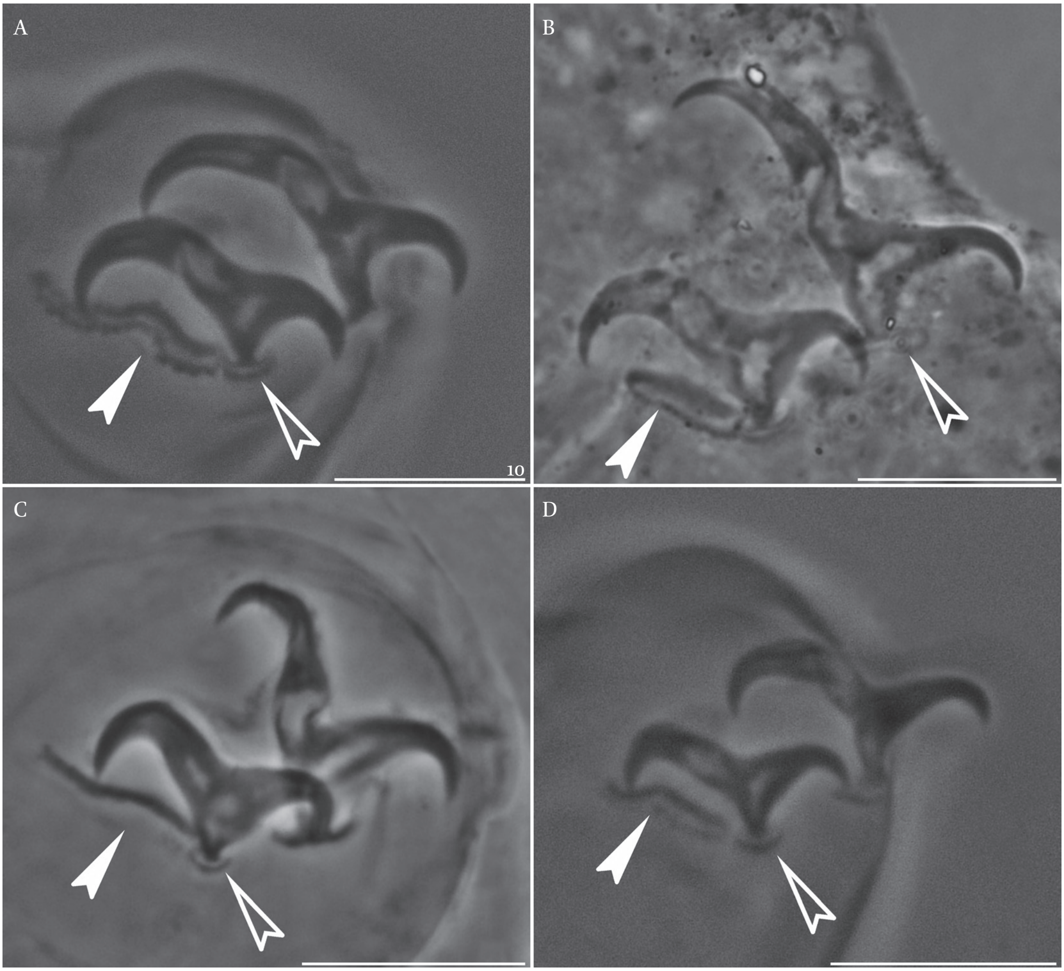

FIGURE 10 Modified *Isohypsibius* type claws (Isohypsibiidae, PCM): *Ursulinius* type claws: A – *Ursulinius pappi* comb. nov.; B – *Ursulinius duranteae* (Maucci, 1978) comb. nov.; C – *Ursulinius ronsisvallei* (Binda & Pilato, 1969) comb. nov.; D – *Ursulinius dudichi* (Iharos, 1964) comb. nov. Note double bars (incised arrowheads) and well-developed pseudolunulae (empty incised arrowheads). Scale bars = 10 μm

differ from the inferred. Finally, the possibility of misidentification or mislabelling of samples with *D. flavus* with a species representing another genus cannot be excluded. Thus, more *Doryphoribius* spp. and additional genetic markers need to be sequenced to verify phyletic relationships within the family. Nevertheless, the presence of a monophyletic clade characterised by the presence of the ventral lamina (i.e., *Doryphoribius s.l.* + *Apodibius*) within Doryphoribiidae fam. nov. seems unlikely since this structure may have evolved independently during the natural history of this group (as it has evolved convergently also in other eutardigrades).

Our phylogenetic analysis confirmed that also *Isohypsibius* was polyphyletic (Sands et al., 2009; Guil & Giribet, 2011; Bertolani et al., 2014a and Cesari et al., 2016) and, in order to accommodate different evolutionary lineages, we divided the genus into three isohypsibiid terrestrial genera (*Isohypsibius*,

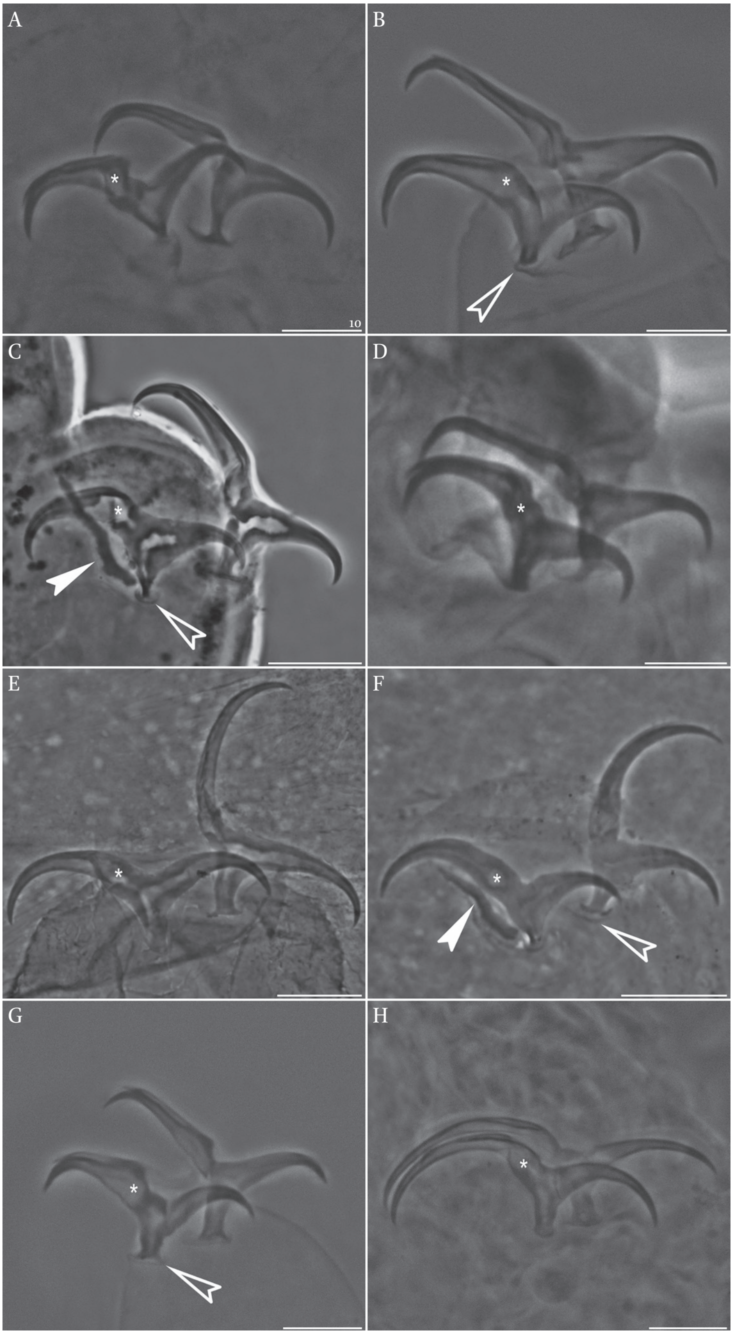

FIGURE 11 Claws of aquatic isohypsibioids, *i.e.* Doryphoribiidae fam. nov. (PCM): A – *Grevenius granulifer* comb. nov.; B – *Grevenius pushkini* comb. nov.; C – *Grevenius sismicus* (Maucci, 1978) comb. nov.; D – *Grevenius karenae* (Zawierucha, 2013) comb. nov.; E – *Grevenius monoicus* (Bertolani, 1981) comb. nov.; F – *Grevenius longiunguis* (Pilato, 1974) comb. nov.; G – *Thulinius ruffoi*; H – *Pseudobiotus megalonyx*. Note singular bars (incised arrowheads) and pseudolunulae (empty incised arrowheads). Asterisks indicate evident internal and anterior claw primary branch widening, the claw curvature forms an obtuse angle (A–C, E) or the expansion is knob-like (D, F). Scale bars = 10 µm

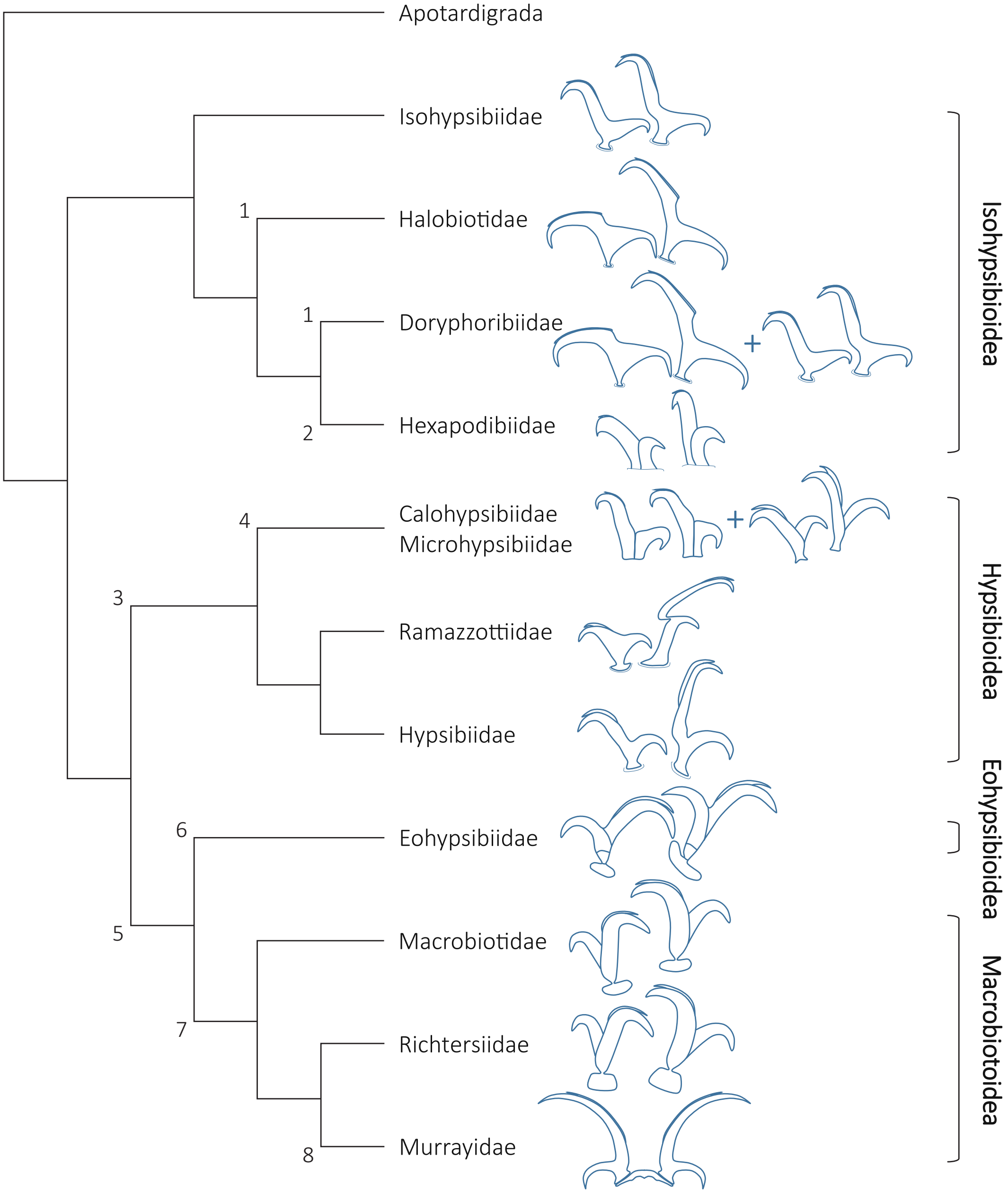

FIGURE 12 Hypothesised claw evolution scheme within the class Eutardigrada. Common Eutardigrade Ancestor (CEA) exhibited asymmetric (anisonych/heteronych) claws. Most significant changes in the overall morphology of claws are marked with numerals: (1) – secondary branch elongation; (2) – claw reduction, basal portion indistinctly merged with cuticle; (3) – branch curving; (4) – miniaturisation; (5) – evolution of true lunulae; (6) – claws tripartite; (7) – claw symmetry, claws bipartite; (8) – primary branch elongation, secondary branch reduction, lunulae transformed into longitudinal bars, exclusively aquatic. Drawings are based on SEM and/or PCM microphotographs. Phylogenetic relationships are based on the consensus results from recent published works (Bertolani et al., 2014a; Cesari et al., 2016; Guidetti et al., 2016) and the present study

FIGURE 13 *Grevenius pushkini* comb. nov., the buccal apparatus: A – habitus; B – mouth opening (the incised arrowhead indicates the first band of teeth, whereas the empty incised arrowhead – the second band of teeth); C – oral cavity armature; D – buccal crown (dorsal view); E – buccal crown (lateral view); F – pharynx (dorsal view); G – pharynx (lateral view). Scale bars in micrometres

Dianea gen. nov., *Ursulinius* gen. nov.) and one doryphoribiid aquatic genus (*Grevenius* gen. nov.). Moreover, our study demonstrated that the presence and morphology of gibbosities bear taxonomic importance at the genus level. Thus, it would not be surprising if cuticular gibbosities could be used as diagnostic traits to differentiate new genera in other tardigrade groups. In addition to the presence/absence of cuticular gibbosities, Michalczyk & Kaczmarek (2010) used, in parallel, the number of placoids (2 vs 3) to divide

FIGURE 14 *Thulinius ruffoi*, the buccal apparatus: A – habitus; B – mouth opening (the incised arrowhead indicates the first band of teeth, whereas the empty incised arrowhead – the second band of teeth); C – buccal crown (dorsal view); D – furca. Scale bars in micrometres

Doryphoribius into four groups (*doryphorus*, *evelinae*, *vietnamensis*, and *zappalai* group) to aid the taxonomy of the genus. Recent analyses support the hypothesis that the number of placoids in the pharynx is likely to be stable at the genus level (Gąsiorek et al., 2016, 2018; Guidetti et al., 2016; Vecchi et al., 2016), meaning that all the genera with no single pattern of number of placoids are polyphyletic and artificial, e.g., *Adropion*, *Doryphoribius*, *Microhypsibius*, *Mixibius*, and, still, *Isohypsibius*. Finally, our study suggests that some lineages may dwell in a specific habitat (e.g., terrestrial or aquatic). *Doryphoribius* can be divided into taxa with two and three macroplacoids in the pharynx, into taxa with and without gibbosities (Michalczyk & Kaczmarek, 2010), and into terrestrial/xerophilous and strictly aquatic taxa (the present study; see Appendix). Moreover, within the genus, several claw morphotypes can be distinguished (e.g., Bertolani, 1983; Pilato & Lisi, 2004; Beasley et al., 2008; Lisi, 2011; figs. 17B–C) and at least two ventral lamina morphotypes are present (figs. 16B–C, see also Pilato, 2013). This high intrageneric variability clearly suggests that the genus *Doryphoribius* is polyphyletic. By adding the criterion of habitat to the two morphological criteria proposed by Michalczyk & Kaczmarek (2010), we obtained seven distinct groups of species:

terrestrial with gibbosities and two placoids (the *Doryphoribius flavus* group), aquatic with gibbosities and two placoids (the *Doryphoribius evelinae* group), terrestrial with two placoids but with no gibbosities (the *Doryphoribius doryphorus* group = *Doryphoribius s.s.*), aquatic with two placoids but with no gibbosities (the *Doryphoribius koreanus* group), terrestrial with gibbosities and three placoids (the *Doryphoribius vietnamensis* group), terrestrial with three placoids but with no gibbosities (the *Doryphoribius bertolanii* group), and, finally, aquatic with three placoids but with no gibbosities (the *Doryphoribius zappalai* group) (see Appendix for species compositions of groups within *Doryphoribius s.l.*). However, some of these groups are heterogenous in regards to claw and ventral lamina morphology. Thus, the number of potential genera concealed within this polyphyletic genus may be even higher than seven.

Systematic position of **Grevenius** *gen. nov.*

Genetic distinctiveness of *Grevenius* gen. nov. became first apparent in Sands et al. (2008), where close affinities between *I. asper* (Murray, 1906), *I. granulifer*, and *Thulinius stephaniae* (Pilato, 1974) were demonstrated. This clade, although weakly supported in the Maximum Parsimony analysis (MP = 50), was clearly distinct from the well-supported *Isohypsibius s.s.* clade (represented at the time only by *I. prosostomus* Thulin, 1928 and *I. cambrensis* (Morgan, 1976); MP = 99, BI = 1.0; Sands et al., 2008). New sequences for *Isohypsibius s.s.* and *Grevenius* gen. nov. confirmed the remote affinity between aquatic *Isohypsibius s.l.* spp. and *Isohypsibius s.s.* In addition to distinct genetic divergence, *Grevenius* gen. nov. also clearly differs from *Isohypsibius s.s.* morphologically and ecologically. The new genus exhibits distinct differences in the armature of the oral cavity (two rows of teeth vs only one row in *Isohypsibius s.s.*); see Jørgensen, 2001, where the second row of teeth in *G. malawiensis* (Jørgensen, 2001) comb. nov. is described as 'intrabuccal baffles') and in claw morphology (claws elongated, with a clear hump on the primary branch and with relatively elongated secondary branches (*br* > 70%) in *Grevenius* gen. nov., figs. 11A–F vs claws of the *Isohypsibius* type, without the hump on the primary branch and with a considerable difference in primary and secondary branch height (*br* ≤ 70%) in *Isohypsibius*, figs. 8A–D). Moreover, *Grevenius* gen. nov. inhabits a different ecological niche than in *Isohypsibius s.s.* (freshwater vs terrestrial).

Claws in *Grevenius* gen. nov., similarly to those in *Pseudobiotus* and *Thulinius* (e.g., see Nelson et al., 1999; Bertolani, 2003), are clearly elongated, which is most likely an adaptation to the aquatic habitat (fig. 12). Moreover, internal claws in the new genus have a clear hump (as in *Thulinius*) and the cuticle is typically rough (as in *Pseudobiotus*; e.g., see Bertolani, 1982; Chang et al., 2007 or Pilato et al., 2010). All these similarities suggest a close affinity of the new genus with both *Pseudobiotus* and *Thulinius*, which is in agreement with the molecular phylogeny (fig. 2). However, the exact phyletic relationships between the three genera and relationships within Doryphoribiidae fam. nov. are not fully solved. Thus, more DNA sequences, in particular for intertidal *Grevenius* gen. nov. spp., are needed to better understand its relationships with other doryphoribiid genera.

Morphology of Hexapodibiidae

The problematic systematic position of calohypsibiid genera and species (order Hypsibioidea) has been a subject of long debate (Pilato, 1989; Guil et al., 2013; Bertolani et al., 2014a; Gąsiorek et al., 2019). Recently, Cesari et al. (2016) demonstrated the monophyly

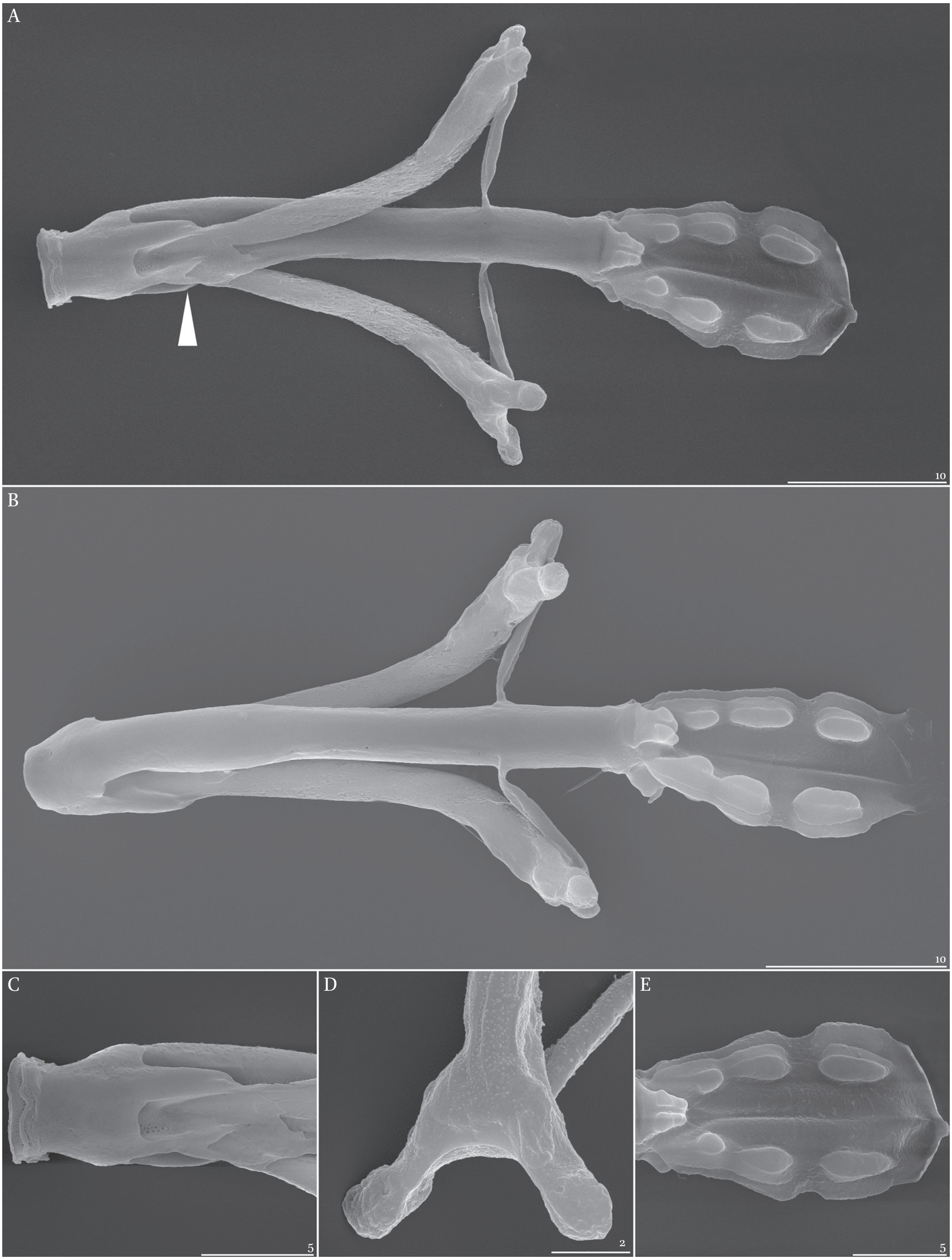

FIGURE 15 *Hexapodibius micronyx*, the buccal apparatus: A – habitus (ventral view, the arrowhead points the ventral lamina); B – habitus (dorsal view); C – buccal crown and ventral lamina (ventral view); D – furca; E – pharynx. Scale bars in micrometres

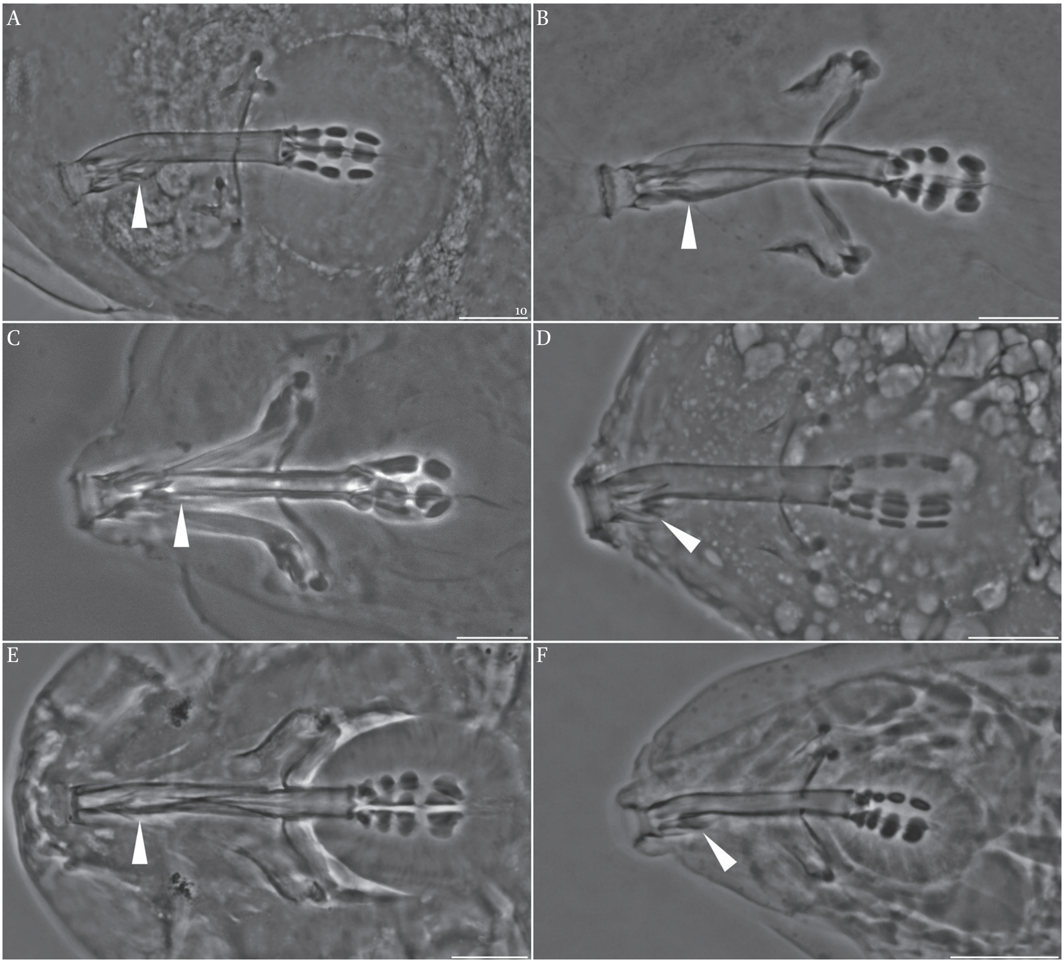

FIGURE 16 Buccal apparatus morphology of Doryphoribiidae fam. nov. and Hexapodibiidae members equipped with ventral lamina (arrowheads): A – *Apodibius nuntius* Binda, 1984; B – *Doryphoribius korganovae* Biserov, 1994; C – *Doryphoribius bindae* Lisi, 2011; D – *Hexapodibius micronyx*; E – *Parhexapodibius castrii* (Ramazzotti, 1964); F – *Parhexapodibius ramazzottii* Manicardi & Bertolani, 1987. Scale bars = 10 µm

of a clade comprising four of the former five members of Calohypsibiidae (*Haplohexapodibius* Pilato & Beasley, 1987, *Haplomacrobiotus* May, 1948, *Hexapodibius*, *Parhexapodibius*) and instituted a new family rank for eutardigrades equipped with the ventral lamina and exhibiting various degrees of claw reduction. All four hexapodibiid genera share the same general morphology of the buccal apparatus, i.e., reduced dorsal AISM, ventral lamina and three granular macroplacoids (compare figs. 15, 16D–F herein and the buccal apparatus of *Haplomacrobiotus* in Cesari et al., 2016). Interestingly, a similar buccal apparatus morphotype is also present in two doryphoribiid genera: *Apodibius* and *Doryphoribius* (although with two macroplacoids in some species), but absent in the remaining doryphoribiid genera (*Pseudobiotus*, *Thulinius* and *Grevenius* gen. nov.). Thus, at the moment, it is not possible to establish whether a similar buccal apparatus morphotype evolved independently in Hexapodibiidae as well as in *Apodibius* (Hohberg & Lang, 2016) and *Doryphoribius* (Guidetti et al., 2013) or whether the similarity indicates the ancestral state of

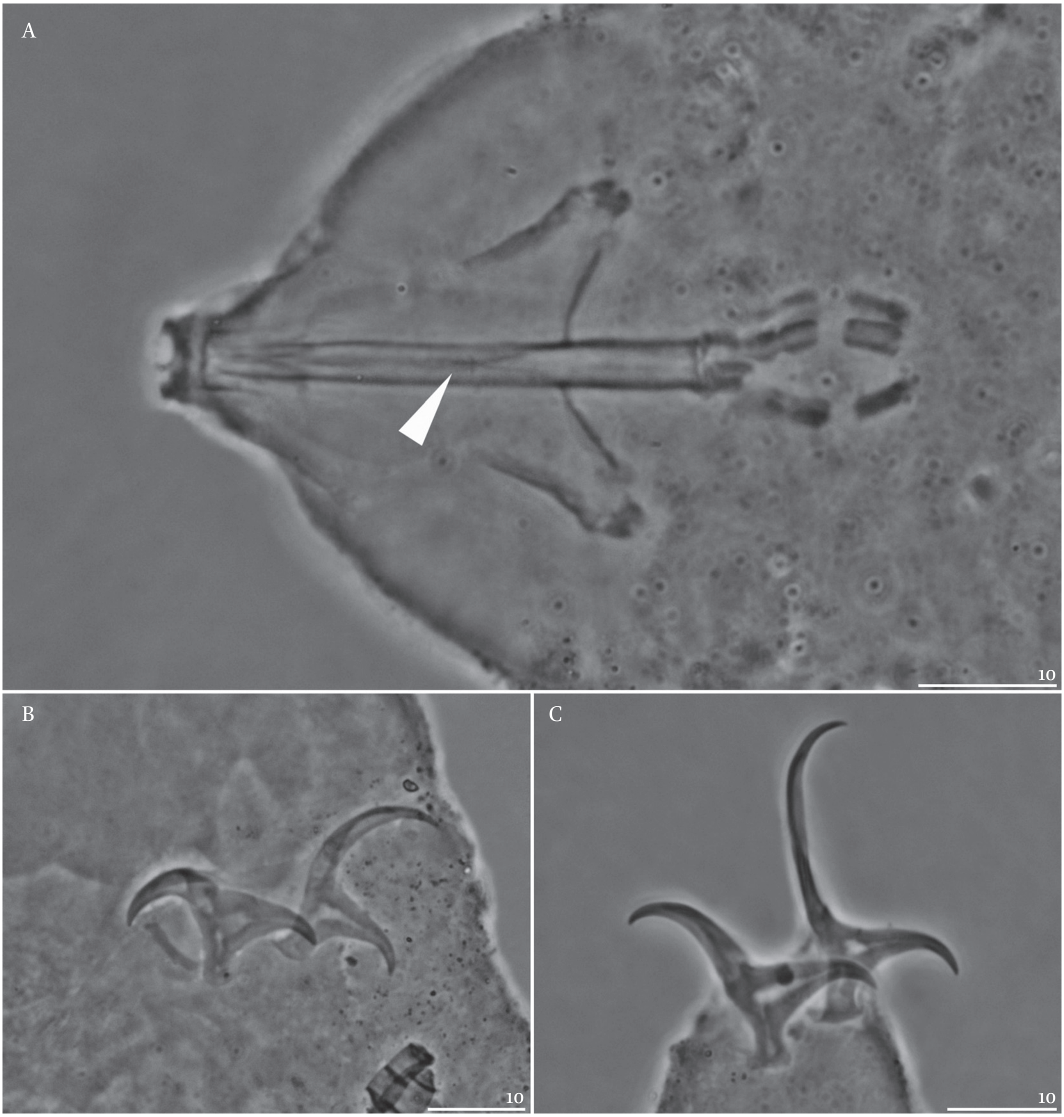

FIGURE 17 *Doryphoribius monstruosus* (Maucci, 1991) comb. nov. (PCM, holotype): A – the buccal apparatus (arrowhead indicates ventral lamina); B – claws I; C – claws IV. Scale bars in micrometres

Hexapodibiidae + Doryphoribiidae fam. nov. Nevertheless, an independent (convergent) origin of the ventral lamina within this clade seems more likely as it is a more parsimonious explanation: given that the evolution of the ventral lamina is tightly linked with the reduction of dorsal AISM (same pattern was observed also in other eutardigrades), hypothesising that the ancestor of Hexapodibiidae + Doryphoribiidae fam. nov. had ventral lamina, which was secondarily lost, and ridge-like AISM evolved again, appears less probable. In other words, a plesiomorphic ventral lamina would require a subsequent atrophy of this structure, re-establishing of the dorsal apophysis and the restoration of the overall symmetry of AISM in *Grevenius* gen. nov., *Pseudobiotus* and *Thulinius*.

Despite representing different families, both *Hexapodibius* and *Apodibius* exhibit peculiar peribuccal circular wrinkles (figs. 5F, J). Interestingly, it must be noted that these structures are found exclusively in soil isohypsibioids (*Haplomacrobiotus* being an exception (Cesari et al., 2016)). Similarly, frontal lobes are present mainly in soil genera (*Apodibius*, *Haplomacrobiotus*, *Paradiphascon*) and they occur also in *Ursulinius* gen. nov. Thus, these organs could be another adaptation to the terrestrial habitat.

In accordance with analyses of Guil et al. (2013), claw morphology in Hexapodibiidae represents three levels of reduction: (I) shortened secondary branches and the basal tract being continuous with cuticle surface (reduced pseudolunulae in *Parhexapodibius*; see Manicardi & Bertolani, 1987), (II) shortened primary branches and lack of claws IV in *Hexapodibius* (see fig. 7Q), and (III) complete reduction of secondary branches in *Haplohexapodibius* and *Haplomacrobiotus* (see Cesari et al., 2016). The reduction is commonly viewed as an adaptation to soil habitat, preferred by hexapodibiids (Bertolani & Biserov, 1996; Hohberg et al., 2011).

Autapomorphies of Halobiotidae fam. nov.

The erection of Halobiotidae fam. nov. is firmly supported both by DNA sequences as well as unique morphology and anatomy, which are most likely the result of secondary adaptation to marine habitat. Traits exclusive to *Halobiotus*, such as cephalic papillae, peribuccal chemosensory organs, and gigantic Malpighian tubules, most probably serve in perception of external stimuli and osmoregulation, respectively (Kristensen, 1982; Møbjerg & Dahl, 1996; Møbjerg et al., 2007; Halberg et al., 2013). Claws of *Halobiotus* (figs. 7I–J) are similar to the most common morphotype of doryphoribiid claws, i.e., with elongated stalks and branches of similar heigths, present also in *Grevenius* gen. nov., *Paradiphascon*, *Pseudobiotus*, *Thulinius*, and some *Doryphoribius* spp. Therefore, presumably the ancestral claw type of the clade [Halobiotidae fam. nov. (Doryphoribiidae fam. nov. + Hexapodibiidae)] was close to this morphotype.

Incertae sedis: Ramajendas Pilato & Binda, 1991 *and Thalerius* Dastych*, 2009*

Exhibiting a mixture of hypsibioid and isohypsibioid morphological traits, two enigmatic genera, *Ramajendas* and *Thalerius*, are a subject of an ongoing debate on their taxonomic affinity. Originally placed in Isohypsibiidae (Marley et al., 2011; Guil et al., 2013), they were later tentatively transferred to Ramazzottiidae Sands et al., 2008 (Bertolani et al., 2014a) and most recently, moved back, also provisionally, to Isohypsibiidae (Zawierucha et al., 2018). On one hand external and posterior claws, by having elongated and flexible primary branch, seem to resemble those in the family Ramazzottiidae (Hypsibioidea). On the other hand, however, the shape of internal and anterior claws is similar to that found in some species representing both Hypsibioidea and Isohypsibioidea. Moreover, the two genera lack body pigmentation and paired cephalic elliptical organs (present in Ramazzottidae), which speak against the close affinity with ramazzottiids (Zawierucha et al., 2018). The body shape and the bucco-pharyngeal apparatus morphology (including AISM shape) in *Ramajendas* are indeed near those in aquatic doryphoribiid genera. However, this genus comprises both terrestrial taxa and a marine species (*R. renaudi* (Ramazzotti, 1972)) which strongly indicates that *Ramajendas* may be polyphyletic, as it was shown above that distinct evolutionary lineages often correspond with the type of environment. Furthermore, *Thalerius* exhibits the bucco-pharyngeal apparatus similar to many isohypsibioid genera (three granular macroplacoids, widespread in Isohypsibioidea but rare in Hypsibioidea, except for the polyphyletic *Mixibius* Pilato, 1992 and *Diphascon* Plate, 1889) and claws with concave bases present in some Itaquasconinae (Hypsibioidea).

In fact, this perplexing mix may indicate a need to create a new higher taxon for *Thalerius*. We are of the opinion that neither buccopharyngeal apparatus nor claw morphology should be given priority (see Schuster et al., 1980, and Pilato, 1982, for opposing views on the relevance of these structures used in the formulation of eutardigrade classification on higher taxonomic levels), making clarification of the status of the two genera impossible without molecular data. To conclude, the mixture of traits exhibited by both *Ramajendas* and *Thalerius* make it difficult to ascribe them to any of the isohypsibioid families distinguished in this work. Therefore, we designate the two genera as *incertae sedis* within Isohypsibioidea, pending molecular verification of their taxonomic positions within this or a different eutardigrade order.

Taxonomic account of the families and genera of Isohypsibioidea

Type genera are underlined with a double line.

Phylum: Tardigrada Doyère, 1840
Class: Eutardigrada Richters, 1926
Order: Isohypsibioidea Guil et al., 2019
Amended diagnosis (modified from Bertolani et al., 2014a): Double claws asymmetrical with respect to the median plane of the leg (2121), normally with a similar shape and size on each leg; double claws with the external secondary branches inserted perpendicularly on the claw basal tract, or partly reduced (very short, without the common basal tract, with a base as large as the sum of the primary and secondary branch widths, and with an evident suture between the primary and the secondary branch), or elsewhere absent. Buccal tube rigid (apart *Paradiphascon*) and often relatively large, without the ventral lamina (*Dastychius*, *Dianea* gen. nov., *Eremobiotus*, *Grevenius* gen. nov., *Halobiotus*, *Isohypsibius*, *Ursulinius* gen. nov., *Paradiphascon*, *Pseudobiotus*, *Ramajendas*, *Thalerius*, *Thulinius*) or with the ventral lamina (*Apodibius*, *Doryphoribius*, *Haplomacrobiotus*, *Haplohexapodibius*, *Hexapodibius*, *Parhexapodibius*). Pharyngeal apophyses and placoids present. Smooth eggs laid in exuviae.

Family: Isohypsibiidae Sands et al., 2008

Amended diagnosis: Terrestrial eutardigrades with six peribuccal lobes or with a continuous peribuccal ring, and peribuccal lamina. Lacking peribuccal lamellae and ventral lamina on the buccal tube. AISM ridge-like and asymmetrical with respect to the frontal plane (only in *Fractonotus*) or symmetrical (remaining five genera). Stylet furcae of the *Hypsibius* type. Claws with secondary branches clearly shorter than primary branches (*br* ≤ 0.70).

Composition: *Dastychius* Pilato, 2013, *Dianea* gen. nov., *Eremobiotus* Biserov, 1992, *Fractonotus* Pilato, 1998, *Isohypsibius* Thulin, 1928, *Ursulinius* gen. nov.

Remarks: Molecular data are not available for representatives of some genera of former Isohypsibiidae *s.l.*, thus their taxonomic assignment may change when the data are obtained. *Dastychius improvisus* (Dastych, 1984) is kept in the family since *Dastychius* type AISM are modified *Isohypsibius* type AISM (ridged AISM, exceptionally elongated towards the SSIP). Together with peculiar cuticular cavities and typical *Isohypsibius* type claws, they currently prevent any taxonomic re-shuffling of this genus.

Differential diagnosis: Isohypsibiidae *s.s.* have no ventral lamina and they retained the plesiomorphic claw morphotype with claw branches of dissimilar heigths (*br* ≤ 0.70). Doryphoribiidae fam. nov., on the other hand, either exhibit the ventral lamina or their claw branches are similar in height (*br* > 0.70) or both (i.e., at least one of these two criteria distinguishes any given member of Doryphoribiidae fam. nov. from Isohypsibiidae). Hexapodibiidae are always equipped with the ventral lamina and their claws are strongly reduced. Halobiotidae fam. nov. have dome-shaped cephalic papillae (absent in other isohypsibioids). Moreover, Isohypsibiidae *s.s.* inhabit terrestrial habitats (mostly mosses and lichens) whereas many Doryphoribiidae fam. nov. inhabit freshwaters, Hexapodibiidae dwell exclusively in soil, and all Halobiotidae fam. nov. are marine.

Genus: *Isohypsibius* Thulin, 1928

Amended diagnosis: Cuticle smooth or sculptured (i.e., reticulated or rugose), but without gibbosities or plaques. Claw bases typically without pseudolunulae. No cuticular bars or only single bars under claws. Only first band of teeth in the oral cavity.

Remarks: The genus *Isohypsibius*, even after the exclusion of species assigned to the three genera erected herein, still remains polyphyletic. For example, *I. dastychi* (the *I. dastychi* group), represents a different evolutionary lineage within Isohypsibiidae. Since the clade *I. dastychi* + *Ursulinius* gen. nov. + *Eremobiotus* lack statistical support (fig. 2), and at least one more representative of this group ought to be sequenced to confirm the monophyly of the *I. dastychi* group, the *dastychi* complex is not erected as a separate genus. Moreover, as recently indicated by Gąsiorek et al. (2019), relationships between *Isohypsibius* and *Fractonotus* need clarification as the only sequenced species, *F. verrucosus* (Richters, 1900), is embedded within the *Isohypsibius* clade (fig. 2).

Type species: *Isohypsibius prosostomus* Thulin, 1928.

Differential diagnosis: *Isohypsibius* is distinguished from *Dastychius* and *Fractonotus* by the AISM shape (symmetric and restricted to the buccal crown vs elongated and extending towards the SSIP and asymmetric, respectively). Moreover, *Dianea* gen. nov. and *Ursulinius* gen. nov. exhibit gibbosities, which are absent in *Isohypsibius*. Finally, *Eremobiotus* has six peribuccal papulae (absent in *Isohypsibius*) and widely forked claw branches (branches forking at a ca. right angle in *Isohypsibius*).

Genus: *Dianea* gen. nov.

Diagnosis: Cuticle with numerous small but distinct, pointy gibbosities covered with reticulum. Claw branches forking at a wide angle; claw bases without pseudolunulae. No cuticular bars under claws.

Etymology: In honour of Diane Nelson, Professor Emerita of the East Tennessee State University, USA, an exquisite American tardigradologist and a great friend.

Type species: *Macrobiotus sattleri* Richters, 1902.

Differential diagnosis: *Dianea* gen. nov. has gibbosities, absent in all isohypsibiids but *Ursulinius* gen. nov. However, gibbosities of *Dianea* gen. nov. are small and pointed, not convex or mamillose as in *Ursulinius* gen. nov. Additionally, *Dianea* gen. nov. has no cuticular bars under claws whereas *Ursulinius* gen. nov. exhibits evident double bars.

Genus: *Ursulinius* gen. nov.

Diagnosis: Cuticle covered with large, mamillose, and sculptured gibbosities. Claw bases with well-developed pseudolunulae. Double cuticular bars under claws.

Etymology: When observed in SEM en face, the first row of dorsal gibbosities look like pointy ears on the head, which results in a teddy bear-like appearance of animals of the new genus (e.g., see fig. 4B). Therefore, the name of the new genus is derived from the Latin word "*ursus*" (bear), being a diminutive to mean "a small bear".

Type species: *Hypsibius pappi* Iharos, 1966.

Differential diagnosis: *Ursulinius* gen. nov. has gibbosities, absent in all isohypsibiids but *Dianea* gen. nov. (see above for the distinction with this genus).

Family: Doryphoribiidae fam. nov.

Diagnosis: Freshwater (limnic) or terrestrial eutardigrades with six peribuccal lobes, or with continuous peribuccal ring. Mouth opening surrounded by peribuccal lamellae, often partially or almost completely fused (*Paradiphascon*, *Pseudobiotus*, *Thulinius*) or by a peribuccal lamina (*Apodibius*, *Doryphoribius*, *Grevenius* gen. nov.). Ventral lamina on the buccal tube present (*Apodibius*, *Doryphoribius*) or absent (*Grevenius* gen. nov., *Paradiphascon*, *Pseudobiotus*, *Thulinius*). AISM ridge-like, well-developed and symmetrical in genera with no ventral lamina or greatly reduced and asymmetrical in genera exhibiting the ventral lamina. Flexible pharyngeal tube present (*Paradiphascon*) or absent (all remaining genera). Two claw types: the dominant type, with secondary branches being similar in heigth to the primary branches (all genera with the exception of some *Doryphoribius* spp.); and the second, with secondary branches being clearly shorter than the primary branches (only in some *Doryphoribius* spp.).

Composition: *Apodibius* Dastych, 1983, *Doryphoribius* Pilato, 1969, *Grevenius* gen. nov., *Paradiphascon* Dastych, 1992, *Pseudobiotus* Nelson, 1980, *Thulinius* Bertolani, 2003.

Remarks: *Paradiphascon manningi* Dastych, 1992 is transferred from the family Isohypsibiidae primarily on the basis of large peribuccal lamellae. Pilato & Binda (1996) considered lamellae in this taxon as papulae, but the term "papulae" refers to rounded peribuccal structures, present e.g., in *Calohypsibius* (Gąsiorek et al., 2019). However, extremely peculiar morphological autapomorphies of the genus (highly modified AISM, annulated pharyngeal tube, dorsoposterior apodeme on the border between the buccal and pharyngeal tube, external and posterior claws with wide bases) require molecular and new morphological evidence to verify the tentative affiliation within Doryphoribiidae fam. nov.

Differential diagnosis: For the differences with Halobiotidae fam. nov. and Hexapodibiidae, see the differential diagnoses for these families. There are two clear ecomorphological groups within Doryphoribiidae fam. nov.: (I) aquatic genera with elongated claws (*Grevenius* gen. nov., *Pseudobiotus*, *Thulinius*) ($br > 0.70$), and (II) terrestrial genera with typical ($br \leq 0.70$) or reduced claws, either with the ventral lamina (*Apodibius*, *Doryphoribius*) or without the lamina but with the flexible pharyngeal tube (*Paradiphascon*). In contrast, Isohypsibiidae *s.s.* do not inhabit limnic habitats (if found in water, they are accidental, ecdemic elements) and they have no peribuccal lamellae around the mouth opening or ventral lamina on the buccal tube.

Genus: *Grevenius* gen. nov.

Diagnosis: Aquatic (limnic or intertidal) or hygrophilic. Cuticle smooth or rough, covered with evident protuberances or wrinkles, sometimes forming reticulum. Claw bases with pseudolunulae. Single bars or no cuticular bars under claws. Two rows of teeth in the oral cavity. Stylet furcae of the *Hypsibius* type.

Etymology: Named after Hartmut Greven, Professor Emeritus of the Department of Zoology, Heinrich Heine University Düsseldorf, and an eminent German zoologist, who studied the biology of the type species of the new genus (Greven & Blom, 1977; Wolburg-Buchholz & Greven, 1979).

Type species: *Isohypsibius granulifer* Thulin, 1928.

Differential diagnosis: The presence of peribuccal lamellae distinguishes *Paradiphascon*, *Pseudobiotus* and *Thulinius* from *Grevenius* gen. nov. (continuous peribuccal lamina). Similarly, the presence of ventral lamina separates *Apodibius* and *Doryphoribius* from *Grevenius* gen. nov. (lack of ventral lamina). *Grevenius* gen. nov. is separated from the former *Isohypsibius s.l.* (= *Isohypsibius* and two other genera established in this work) by the absence of dorsal gibbosities (gibbosities present in *Dianea* gen. nov. and *Ursulinius* gen. nov.), the presence of two bands of teeth in the OCA, and by elongated claws (one band of teeth and, comparatively, markedly shorter claws in *Isohypsibius s.s.*, *Dianea* gen. nov. and *Ursulinius* gen. nov.).

Family: Hexapodibiidae Cesari et al., 2016

Amended diagnosis: Soil eutardigrades with six peribuccal lobes. Peribuccal lamellae (*Haplohexapodibius*, *Haplomacrobiotus*) or peribuccal lamina (*Hexapodibius*, *Parhexapodibius*) around the mouth opening. Ventral lamina on the buccal tube present. AISM reduced. Claws reduced, with indistinct claw bases and no pseudolunulae. Clear sutures between branches. Secondary branches shortened or absent.

Composition: *Haplohexapodibius* Pilato & Beasley, 1987, *Haplomacrobiotus* May, 1948, *Hexapodibius* Pilato, 1969, *Parhexapodibius* Pilato, 1969.

Differential diagnosis: Claws in the family Hexapodibiidae are remarkably reduced (Pilato, 1989; Cesari et al., 2016). The only other example of convergent claw atrophy within Isohypsibioidea is known in *Apodibius*. However, *Apodibius* is completely clawless whereas in Hexapodibiidae entire claws vanished only on the fourth pair of legs (only in *Haplohexapodibius* and *Hexapodibius*).

Family: Halobiotidae fam. nov.

Diagnosis: Marine eutardigrades with six peribuccal lobes equipped with chemosensory organs. Two large, dome-shaped cephalic papillae present. Mouth opening surrounded by the peribuccal lamina. No ventral lamina on the buccal tube. AISM symmetrical, divided into the anterior semilunar hook and the posterior slight thickening. Claws with pseudolunulae.

Composition: *Halobiotus* Kristensen, 1982.

Differential diagnosis: In contrast to exclusively marine Halobiotidae fam. nov., vast majority of isohypsibioids dwell in limnoterrestrial habitats. Moreover, none of them exhibit peribuccal chemosensory organs and giant Malpighian tubules, both being distinctive features of the new family.

Incertae sedis: *Ramajendas* Pilato & Binda, 1991, *Thalerius* Dastych, 2009.

Remarks: The current data do not allow for assigning these two genera to any of the families. Moreover, it is not certain whether they represent Isohypsibioidea (see above for details).

Evolution of traits within the order in relation to other lineages of Eutardigrada

Isohypsibioidea are most likely the most basal lineage in the order Eutardigrada (Sands et al., 2008; Bertolani et al., 2014a). Therefore, unravelling phyletic affinities within this group is of special importance for understanding the evolution of Eutardigrada (Kiehl et al., 2007; Sands et al., 2008). Of the four currently recognised eutardigrade orders, Isohypsibioidea, alongside Hypsibioidea and Eohypsibioidea, exhibit asymmetrical (heteronych) claws and only Macrobiotoidea are characterised by symmetrical (isonych) claws (fig. 12). Given the phylogenetic relationships between the orders (Bertolani et al., 2014a), asymmetrical claws are most likely a plesiomorphy of the Eutardigrada whereas claw symmetry should be considered as a macrobiotid autapomorphy. Nevertheless, in comparison to Hypsibioidea, in which a number of claw morphotypes were recognised (e.g., *Hypsibius*, *Ramazzottius* or *Calohypsibius* type), isohypsibioid claws have always been defined as of a single, general "*Isohypsibius* type", which suggests prevalent conservatism in their morphology (Pilato & Binda, 2010; Marley et al., 2011). However, our study implies that details of claw shape together with the presence or absence of other pedal structures such as pseudolunulae and cuticular bars, which were often considered as of minor taxonomic significance (e.g., they were omitted in the only comprehensive morphological phylogeny of eutardigrades by Guil et al., 2013), may hold sound phylogenetic signal. Some isohypsibioid taxa seem to have claws intermediate between the *Isohypsibius* and the *Hypsibius* types. For example, claws in *Fractonotus* (*Hypsibius*-like claw curvature) or *Paradiphascon* (*Hypsibius*-like difference in the size of external and internal claws), may signalise a closer affinity between Isohypsibioidea and Hypsibioidea than with the two remaining orders. As the relationships between the basal families of Hypsibioidea, Calohypsibiidae Pilato, 1969 and Microhypsibiidae Pilato, 1998, are not resolved (fig. 12), the plesiomorphic condition for this order remains unknown. However, in the recent phylogenies, the polytomy embraced also *Mixibius* and *Acutuncus* Pilato & Binda, 1997, having either hypsibiid-isohypsibiid claws or typical hypsibiid claws, respectively (Bertolani et al., 2014a; Cesari et al., 2016). This suggests that hypsibiid ancestor had claws nearing to the present Hypsibiidae Pilato, 1969, and that the claws of Calohypsibiidae and Microhypsibiidae are considerably modified. Similarly to the hypothesised closer affinity between Isohypsibioidea and Hypsibioidea, the relationship between Eohypsibioidea and Macrobiotoidea is well-supported in the development of true, strongly sclerotised lunulae (in contrast to pseudolunulae present in the former), and narrowing of the basal portion of the claw, which became the peduncle (fig. 12).

Aquatic isohypsibioid species are scattered between more numerous terrestrial taxa, and the basal family, Isohypsibiidae *s.s.*, comprises entirely land taxa (fig. 2). Concerning the entire class Eutardigrada, limnic forms occur only in some Doryphoribiidae fam. nov., Microhypsibiidae, some Hypsibiidae and Eohypsibiidae, and Murrayidae, whereas marine – in Halobiotidae fam. nov. and in some Doryphoribiidae fam. nov. (Nelson & Marley, 2000). The current phylogeny indicates all these are examples of independent invasion of aquatic habitats (figs. 2, 12). Maucci (1973–1974) first formulated the hypothesis on the evolution of claw morphotypes in relation to the inhabited ecological niche for Hypsibiidae and Ramazzottiidae: he noted that aquatic species exhibit longer claws compared to terrestrial taxa. The correlation between secondarily aquatic environment and claw morphology is also expressly visible in Isohypsibioidea, in which aquatic taxa have elongated claws with branches of almost similar heigths, whereas terrestrial species exhibit robust claws with markedly shorter secondary branches (table 3; figs. 7–12; see also Bertolani, 1982 and compare limnic taxa depicted therein: the majority of *Isohypsibius* spp. [now assigned to *Grevenius* gen. nov.], *Pseudobiotus* spp., *Thulinia* Bertolani, 1981 spp. [now *Thulinius*] with terrestrial taxa: *Dianea sattleri* comb. nov. [*Isohypsibius bakonyiensis* (Iharos, 1964) then], *Eremobiotus alicatai* [*Isohypsibius alicatai* then], *Isohypsibius marcellinoi* Binda & Pilato, 1971, *I. prosostomus*, *U. pappi* comb. nov., *U. ronsisvallei* Binda & Pilato, 1969 comb. nov.).

Concerning buccal apparatus morphology, all isohypsibioid AISM shapes could be seen as derived states of the *Isohypsibius* type, i.e., ridged AISM (Marley et al., 2011), suggesting this shape as plesiomorphic for the Eutardigrada. Pilato (2013) also hypothesised about the ancestral state of eutardigrade (parachelan then) AISM shape within Isohypsibioidea, suggesting however the *Dastychius* rather than *Isohypsibius* type (long ridges reaching to the level of SSIP vs short ridges limited to the buccal crown) as a potential candidate. He hypothesised that in the course of evolution, the *Dastychius* AISM became shorter, which resulted in the *Isohypsibius* type AISM. Nonetheless, the current state of knowledge does not allow to determine confidently which of these types is plesiomorphic. The highly modified AISM types in *Fractonotus* and *Halobiotus* probably evolved by the division of both ventral and dorsal apophyses, and subsequent reduction of lateral AISM portions or by forming hook-like portions. On the other hand, the most modified AISM type, with reduced dorsal apophysis, is present in Hexapodibiidae and some groups within Doryphoribiidae fam. nov. (figs. 15–16). The magnitude of these changes is most likely associated with the parallel evolution of ventral lamina, which constitutes an important stylet muscle attachment and therefore changes the distribution of forces in the buccal apparatus, rendering dorsal apophyses less important for the functioning of the stylet musculature. The pattern of reduction of the dorsal AISM is consistently found in all eutardigrades exhibiting the ventral lamina (i.e., Macrobiotoidea; Pilato & Binda, 2010)

Another instance of parallel evolution, next to the independent origin of the ventral lamina, is the development of dorsolateral gibbosities. Among Eutardigrada, cuticular gibbosities evolved most likely independently in two orders, Isohypsibioidea and Hypsibioidea. Mamillose, sculptured gibbosities of a very similar appearance are present in four genera representing four families: *Ursulinius* gen. nov. (Isohypsibiidae), some *Doryphoribius* spp. (Doryphoribiidae fam. nov.), the majority of *Pilatobius* Bertolani et al., 2014 spp. (Hypsibiidae), and in *Ramazzottius szeptyckii* (Dastych, 1979) (Ramazzottiidae). On the other hand, small, terminated at point and wrinkly gibbosities of *Dianea* gen. nov. (Isohypsibiidae) are a unique feature of this genus, therefore they should be recognised as its autapomorphy.

Key to families and genera of Isohypsibioidea

1. Concave claw bases......... *Thalerius* (*inc. sed.*)
–. Straight claw bases or lacking claws............ 2
2(1). Extremely elongated, flexible external claws .... *Ramajendas* (*inc. sed.*)
–. External claws non-flexible or lacking claws 3
3(2). Exclusively marine, with dome-shaped cephalic papillae... ....Halobiotidae fam. nov. (*Halobiotus*)
–. Limno-terrestrial, rarely marine, without cephalic papillae 4
4(3). Reduced claws with indistinct basal tract .... (Hexapodibiidae) 6
–. Claws with distinct basal tract or claws absent.. 5

5(4). Claws always present, with branches of dissimilar heights ($br \leq 70\%$), mouth opening surrounded with peribuccal lamina, no ventral lamina on the buccal tube .... (Isohypsibiidae) 9
–. Claws absent or present, with branches of similar heigths ($br > 70\%$), mouth opening surrounded by peribuccal lamellae or lamina, ventral lamina on the buccal tube may be present ....(Doryphoribiidae fam. nov.) 14
6(3). Minute claws present on all legs............. 7
–. Claws on legs IV absent.................................. 8
7(6). Secondary branches absent or in the form of acute spines.............*Haplomacrobiotus*
–. Secondary branches short..*Parhexapodibius*
8(6). Double claws present on legs I–III ....*Hexapodibius*
–. Single claws present on legs I–III ....*Haplohexapodibius*
9(5). AISM asymmetrical with respect to the frontal plane.......................................*Fractonotus*
–. AISM symmetrical with respect to the frontal plane...10
10(9). Dorsal gibbosities present.......................11
–. Dorsal gibbosities absent...............................12
11(10). – Gibbosities narrow towards apex and with sharp apices, appear flat under PCM .... *Dianea* gen. nov.
–. Gibbosities mamillose/hemispherical, convex (dome-shaped).............*Ursulinius* gen. nov.
12(10). Claw branches on legs IV forking at nearly a 180° angle...........................*Eremobiotus*
–. Claw branches on legs IV forking at an acute angle (<90°)..13
13(12). AISM short, typically not exceeding 1/3 of the buccal tube length................*Isohypsibius*
–. AISM long, reaching almost to the SSIP ....*Dastychius*
14(5). Claws absent on all legs ....*Apodibius*
–. Claws present on all legs 15
15(14). Ventral lamina on the buccal tube present ....*Doryphoribius*
–. Ventral lamina on the buccal tube absent..16
16(15). Flexible pharyngeal tube present ....*Paradiphascon*
–. No pharyngeal tube 17
17(16). Lacking peribuccal lamellae .... *Grevenius* gen. nov.
–. Peribuccal lamellae present 18
18(17). Twelve peribuccal lamellae, primary claw branches with a clear hump......*Thulinius*
–. Thirty peribuccal lamellae, elongated primary claw branches without the hump ....*Pseudobiotus*

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References

Beasley, C.W., Kaczmarek, Ł. & Michalczyk, Ł. (2008) *Doryphoribius mexicanus*, a new species of Tardigrada (Eutardigrada: Hypsibiidae) from Mexico (North America). *Proc. Biol. Soc. Wash.*, 121, 34–40. doi:10.2988/07-30.1.

Bertolani, R. (1976) Osservazioni cariologiche su *Isohypsibius augusti* (Murray, 1907) e *I. megalonyx* Thulin, 1928 (Tardigrada) e ridescrizione delle due specie. *Boll. Zool.*, 43, 221–234.

Bertolani, R. (1981) A new genus and five new species of Italian fresh-water tardigrades. *Boll. Mus. Civ. Stor. Nat. Verona*, 8, 249–254.

Bertolani, R. (1982) Tardigradi (Tardigrada). Guide per il riconoscimento delle specie animale delle acque interne italiane. *Consiglio Nazionale delle Richerche*, 15, 1–104.

Bertolani, R. (1983) Tardigradi muscicoli delle dune costiere Italiane, con descrizione di una nuova specie. *Atti Soc. Tosc. Sci. Nat. Mem. Serie B*, 90, 139–148.

Bertolani, R. (2003) *Thulinius*, new generic name substituting for *Thulinia* Bertolani, 1981 (Tardigrada, Eutardigrada). *Zootaxa*, 195, 1–8. doi:10.11646/zootraxa.314.1.1.

Bertolani, R., Bartels, P.J., Guidetti, R., Cesari, M. & Nelson, D.R. (2014b) Aquatic tardigrades in the Great Smoky Mountains National Park, North Carolina and Tennessee, U.S.A., with the description of a new species of *Thulinius* (Tardigrada, Isohypsibiidae). *Zootaxa*, 3764, 524–536. doi:10.11646/zootaxa.3764.5.2.

Bertolani, R. & Biserov, V.I. (1996) Leg and claw adaptations in soil tardigrades, with erection of two new genera of Eutardigrada, Macrobiotidae: *Pseudohexapodibius* and *Xerobiotus*. *Invert. Biol.*, 115, 299–304. doi:10.2307/3227019.

Bertolani, R., Guidetti, R., Marchioro, T., Altiero, T., Rebecchi, L. & Cesari, M. (2014a) Phylogeny of Eutardigrada: new molecular data and their morphological support lead to the identification of new evolutionary lineages. *Mol. Phyl. Evol.*, 76, 110–126. doi:10.1016/j.ympev.2014.03.006.

Bertolani, R. & Kristensen, R.M. (1987) New records of *Eohypsibius nadjae* Kristensen, 1982, and revision of the taxonomic position of two genera of Eutardigrada (Tardigrada). In: R. Bertolani (Ed.) *Biology of Tardigrades*. Selected Symposia and Monographs U.Z.I., 1, pp. 359–372.

Binda, M.G. & Pilato, G. (1969) Tardigradi muscicoli dell'Isola di Ustica (Sicilia), con descrizione di due specie nuove. *Boll. Accad. Gioenia Sci. Nat. Catania, Serie IV*, 10, 171–180.

Binda, M.G. & Pilato, G. (1969) Ricerche sulla fauna e sulla zoogeografia della Sicilia. XLV. Ulteriore contributo alla conoscenza dei Tardigradi di Sicilia con descrizione di due nuove specie. *Boll. Accad. Gioenia Sci. Nat. Catania, Serie IV*, 10, 205–214.

Binda, M.G. & Pilato, G. (1971) Nuovo contributo alla conoscenza dei Tardigradi di Sicilia. *Boll. Accad. Gioenia Sci. Nat. Catania, Serie IV*, 10, 896–909.

Binda, M.G., Pilato, G. & Dastych, H. (1980) Descrizione di una nuova specie di Eutardigrado: *Doryphoribius macrodon*. *Animalia*, 7, 23–27.

Biserov, V.I. (1992) A new genus and three new species of tardigrades (Tardigrada: Eutardigrada) from the USSR. *Boll. Zool.*, 59, 95–103.

Campbell, L.I., Rota-Stabelli, O., Edgecombe, G.D., Marchioro, T., Longhorn, S.J., Telford, M.J., Philippe, H., Rebecchi, L., Peterson, K.J. & Pisani, D. (2011) MicroRNAs and phylogenomics resolve the relationships of Tardigrada and suggest that velvet worms are the sister group of Arthropoda. *PNAS*, 108, 15920–15924. doi:10.1073/pnas.1105499108.

Casquet, J., Thebaud, C. & Gillespie, R.G. (2012) Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. *Mol. Ecol. Res.*, 12, 136–141. doi:10.1111/j.1755-0998.2011.03073.x.

Cesari, M., Vecchi, M., Palmer, A., Bertolani, R., Pilato, G., Rebecchi, L. & Guidetti, R. (2016) What if the claws are reduced? Morphological and molecular phylogenetic relationships of the genus *Haplomacrobiotus* May, 1948 (Eutardigrada, Parachela). *Zool. J. Linn. Soc.*, 178: 819–827. doi:10.1111/zoj.12424.

Chang, C.Y., Kaczmarek, Ł., Lee, J.M. & Michalczyk, Ł. (2007) *Pseudobiotus spinifer*, a new tardigrade species (Eutardigrada: Hypsibiidae) from Nakdong river, south Korea, with a redescription of *P. vladimiri* Biserov, Dudichev & Biserova. *Zool. Sci.*, 24, 623–629. doi:10.2108/zsj.24.623.

Dabert, M., Dastych, H. & Dabert, J. (2015) Molecular data support the dispersal ability of the glacier tardigrade *Hypsibius klebelsbergi* Mihelčič, 1959 across the environmental barrier (Tardigrada). *Entomol. Mitt. Zool. Mus. Hamburg*, 17, 233–240.

Dabert, M., Dastych, H., Hohberg, K. & Dabert, J. (2014) Phylogenetic position of the enigmatic clawless eutardigrade genus *Apodibius* Dastych, 1983 (Tardigrada), based on 18S and 28S rRNA sequence data from its type species *A. confusus*. *Mol. Phyl. Evol.*, 70, 70–75. doi:10.1016/j.ympev.2013.09.012.

Dastych, H. (1979) *Hypsibius szeptycki* sp. nov., a new species of Tardigrada from South Africa. *Bull. Acad. Pol., Sér. Sci. Biol.*, 27, 505–508.

Dastych, H. (1983) *Apodibius confusus* gen. n., sp. n., a new water-bear from Poland (Tardigrada). *Bull. Pol. Acad. Sci., Biol.*, 31, 41–46.

Dastych, H. (1984) The Tardigrada from Antarctica with descriptions of several new species. *Acta Zool. Cracov.*, 27, 377–436.

Dastych, H. (1992) *Paradiphascon manningi* gen. n. sp. n., a new water-bear from South Africa, with the erecting of a new subfamily Diphasconinae (Tardigrada)., *Mitt. Hamb. Zool. Mus. Inst Hamburg*, 89, 125–139.

Dastych, H. (2009) *Thalerius konradi* gen. nov., sp. nov., a new tardigrade from the periglacial area of the Ötztal Alps, Austria (Tardigrada). *Contr. Nat. Hist.*, 12, 391–402.

Degma, P., Bertolani, R. & Guidetti, R. (2009–18) Actual checklist of Tardigrada species. Online: www.tardigrada.modena.unimo.it/miscellanea/Actual%20checklist%20of%20Tardigrada.pdf, pp. 48. Accessed 21 July 2018.

Degma, P. & Guidetti, R. (2007) Notes to the current checklist of Tardigrada. *Zootaxa*, 1579, 41–53. doi:10.11646/zootaxa.1579.1.2.

Doyère, M.L. (1840) Mémorie sur les Tardigrades. *Ann. Sci. Nat., Paris, Series 2*, 14, 269–362.

Eibye-Jacobsen, J. (2001) A new method for making SEM preparations of the tardigrade buccopharyngeal apparatus. *Zool. Anz.*, 240, 309–319. doi:10.1078/0044-5231-00038.

Faurby, S., Jørgensen, A., Kristensen, R.M. & Funch, P. (2011) Phylogeography of North Atlantic intertidal tardigrades: refugia, cryptic speciation and the history of the Mid-Atlantic Islands. *J. Biogeo.*, 38, 1613–1624. doi:10.1111/j.1365-2699.2011.02533.x.

Gąsiorek, P., Morek, W., Stec, D., Blagden, B. & Michalczyk, Ł. (2019) Revisiting Calohypsibiidae and Microhypsibiidae: *Fractonotus* and its phylogenetic position within Isohypsibiidae (Eutardigrada: Parachela). *Zoosystema*, 41, 71–89. doi:10.5252/zoosystema2019v41a6.

Gąsiorek, P., Stec, D., Morek, W., Zawierucha, K., Kaczmarek, Ł., Lachowska-Cierlik, D. & Michalczyk, Ł. (2016) An integrative revision of *Mesocrista* Pilato, 1987 (Tardigrada: Eutardigrada: Hypsibiidae). *J. Nat. Hist.*, 50, 2803–2828. doi:10.1080/00222933.2016.1234654.

Gąsiorek, P., Stec, D., Morek, W. & Michalczyk, Ł. (2018) An integrative redescription of *Hypsibius dujardini* (Doyère, 1840), the nominal taxon for Hypsibioidea (Tardigrada: Eutardigrada). *Zootaxa*, 4415, 45–75. doi:10.11646/zootaxa.4415.1.2.

Gąsiorek, P., Zawierucha, K., Stec, D. & Michalczyk, Ł. (2017) Integrative redescription of a common Arctic water bear *Pilatobius recamieri* (Richters, 1911). *Pol. Biol.*, 40, 2239–2252. doi:10.1007/s00300-017-2137-9.

Greven, H. & Blom, H.-J. (1977) *Isohypsibius granulifer* Thulin 1928 – ein neuer Tardigrade für Deutschland. *Decheniana* (*Bonn*), 130, 128–130.

Gross, V. & Mayer, G. (2015) Neural development in the tardigrade *Hypsibius dujardini* based on anti-acetylated α-tubulin immunolabeling. *EvoDevo*, 6, 12. doi:10.1186/s13227-015-0008-4.

Guidetti, R., Altiero, T., Marchioro, T., Sarzi Amade, L., Avdonina, A.M., Bertolani, R. & Rebecchi, L. (2012) Form and function of the feeding apparatus in Eutardigrada (Tardigrada). *Zoomorphology*, 131, 127–148. doi:10.1007/s00435-012-0149-0.

Guidetti, R., Bertolani, R. & Rebecchi, L. (2013) Comparative analysis of the tardigrade feeding apparatus: adaptive convergence and evolutionary pattern of the piercing stylet system. *J. Limnol.*, 72, 24–35. doi:10.4081/jlimnol.2013.s1.e4.

Guidetti, R., Rebecchi, L. & Bertolani, R. (2000) Cuticle structure and systematics of the Macrobiotidae (Tardigrada, Eutardigrada). *Acta Zool.*, 81, 27–36. doi:10.1046/j.1463-6395.2000.00034.x.

Guidetti, R., Rebecchi, L., Bertolani, R., Jönsson, K.I., Kristensen, R.M. & Cesari, M. (2016) Morphological and molecular analyses on *Richtersius* (Eutardigrada) diversity reveal its new systematic position and lead to the establishment of a new genus and a new family within Macrobiotoidea. *Zool. J. Linn. Soc.*, 178, 834–845. doi:10.1111/zoj.12428.

Guil, N. & Giribet, G. (2012) A comprehensive molecular phylogeny of tardigrades – adding genes and taxa to a poorly resolved phylum-level phylogeny. *Cladistics*, 28, 21–49. doi:10.1111/j.1096-0031.2011.00364.x.

Guil, N., Jørgensen, A. & Kristensen, R. (2019) An upgraded comprehensive multilocus phylogeny of the Tardigrada tree of life. *Zool. Scr.*, 48, 120–137. doi:10.1111/zsc.12321.

Guil, N., Machordom, A. & Guidetti, R. (2013) High level of phenotypic homoplasy amongst eutardigrades (Tardigrada) based on morphological and total evidence phylogenetic analyses. *Zool. J. Linn. Soc.*, 169, 1–26. doi:10.1111/zoj.12046.

Halberg, K.A., Persson, D.K., Jørgensen, A., Kristensen, R.M. & Møbjerg, N. (2013) Ecology and thermal tolerance of the marine tardigrade *Halobiotus crispae* (Eutardigrada: Isohypsibiidae). *Mar. Biol. Res.*, 9, 716–724. doi:10.1080/17451000.2013.765577.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.*, 41, 95–98. doi:10.1021/bk-1999-0734.ch008.

Hallas, T.E. (1971) Notes on the marine *Hypsibius stenostomus*-complex, with a description of a new species (Tardigrada, Macrobiotidae). *Steenstrupia*, 1, 201–206.

Hansen, J.G. & Katholm, A.K. (2002) A study of the genus *Amphibolus* from Disko Island with special attention on the life cycle of *Amphibolus nebulosus* (Eutardigrada: Eohypsibiidae). Arctic Biology Field Course, Qeqertarsuaq 2002. H.C.Ø. TRYK, Copenhagen, Denmark, pp. 129–163.

Hansen, J.G., Kristensen, R.M., Bertolani, R. & Guidetti, R. (2017) Comparative analyses of *Bertolanius* species (Eohypsibiidae; Eutardigrada) with the description of *Bertolanius birnae* sp. nov. from northern polar regions. *Pol. Biol.*, 40, 123–140. doi:10.1007/s00300-016-1931-0.

Hohberg, K. & Lang, B. (2016) The feeding apparatus and taxonomic position of clawless *Apodibius confusus* Dastych, 1983 (Tardigrada: Isohypsibiidae). *Zool. J. Linn. Soc.*, 178, 828–833. doi:10.1111/zoj.12408.

Hohberg, K., Russell, D.J. & Elmer, M. (2011) Mass occurrence of algal-feeding tardigrade *Apodibius confusus*, in the young soils of a post-mining site. *J. Zool. Syst. Evol. Res.*, 49, 62–65. doi:10.1111/j.1439-0469.2010.00600.x.

Iharos, G. (1964) Neuere Beiträge zur Kenntnis der Tardigraden-Fauna Ungarns, V. *Opuscula Zool., Budapest*, 5, 57–67.

Iharos, G. (1966) Neuere Beiträge zur Kenntnis der Tardigraden-Fauna Ungarns, VI. *Acta Zool. Acad. Sci. Hung.*, 12, 111–122.

ICZN (1999) *International Code of Zoological Nomenclature*. Fourth Edition. The International Trust for Zoological Nomenclature, London, UK.

Jørgensen, A. (2001) Graphical presentation of the African tardigrade fauna using GIS with the description of *Isohypsibius malawiensis* sp. n. (Eutardigrada: Hypsibiidae) from Lake Malawi. *Zool. Anz.*, 240, 441–449. doi:10.1078/0044-5231-00052.

Jørgensen, A. & Kristensen, R.M. (2004) Molecular phylogeny of Tardigrada – investigation of the monophyly of Heterotardigrada. *Mol. Phyl. Evol.*, 32, 666–670. doi:10.1016/j.ympev.2004.04.017.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.*, 30, 3059–3066. doi:10.1093/nar/gkf436.

Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinf.*, 9, 286–298. doi:10.1093/bib/bbn013.

Kiehl, E., Dastych, H., D'Haese, J. & Greven, H. (2007) The 18S rDNA sequences support polyphyly of Hypsibiidae (Eutardigrada). *J. Limnol.*, 66, 21–25. doi:10.4081/jlimnol.2007.s1.21.

Kosztyła, P., Stec, D., Morek, W., Gąsiorek, P., Zawierucha, K., Michno, K., Ufir, K., Małek, D., Hlebowicz, K., Laska, A., Dudziak, M., Frohme, M., Prokop, Z.M., Kaczmarek, Ł. & Michalczyk, Ł. (2016) Experimental taxonomy confirms the environmental stability of morphometric traits in a taxonomically challenging group of microinvertebrates. *Zool. J. Linn. Soc.*, 178, 765–775. doi:10.1111/zoj.12409.

Kristensen, R.M. (1982) The first record of cyclomorphosis in Tardigrada based on a new genus and species from Arctic meiobenthos. *Zeitschr. Zool. Syst. Evol.-Forsch.*, 20, 249–270. doi:10.1111/j.1439-0469.1983.tb00552.x.

Kristensen, R.M. & Hallas, T.E. (1980) The tidal genus *Echiniscoides* and its variability, with erection of Echiniscoididae fam. n. (Tardigrada). *Zool. Scr.*, 9, 113–127. doi:10.1111/j.1463-6409.1980.tb00657.x.

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.*, 33, 1870–1874. doi:10.1093/molbev/msw054.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. & Calcott, B. (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution formolecular and morphological phylogenetic analyses. *Mol. Biol. Evol.*, 34, 772–773. doi:10.1093/molbev/msw260.

Lisi, O. (2011) Remarks on *Doryphoribius flavus* (Iharos, 1966), and description of three new species (Tardigrada, Hypsibiidae). *Zootaxa*, 2834, 17–32. doi:10.11646/zootaxa.2834.1.2.

Lisi, O., Binda, M.G. & Pilato, G. (2016) *Eremobiotus ginevrae* sp. nov. and *Paramacrobiotus pius* sp. nov., two new species of Eutardigrada. *Zootaxa*, 4103, 344–360. doi:10.11646/zootaxa.4103.4.3.

Manicardi, G.C. & Bertolani, R. (1987) First contribution to the knowledge of alpine grassland tardigrades. In: R. Bertolani (Ed.) *Biology of Tardigrades*. Selected Symposia and Monographs U.Z.I., 1, pp. 177–185.

Marcus, E. (1927) Zur Anatomie und Ökologie mariner Tardigraden. *Zool. Jahrb. Abt. Syst. Okol. Geogr. Tiere*, 53, 487–558.

Marley, N.J., McInnes, S.J. & Sands, C.J. (2011) Phylum Tardigrada: A re-evaluation of the Parachela. *Zootaxa*, 2819, 51–64. doi:10.11646/zootaxa.2819.1.2.

Maucci, W. (1973–4) Tardigradi muscicoli del Carso Triestino. *Boll. Soc. Adr. Sci. Trieste*, 59, 107–150.

Maucci, W. (1987) A contribution to the knowledge of the North American Tardigrada with emphasis on the fauna of Yellowstone National Park (Wyoming). In: R. Bertolani (Ed.) *Biology of Tardigrades*. Selected Symposia and Monographs U.Z.I., 1, pp. 187–210.

May, R.-M. (1948) Nouveau genre et espèce de tardigrade du Mexique: *Haplomacrobiotus hermosillensis*. *Bull. Soc. Zool. France*, 73, 95–97.

Michalczyk, Ł. & Kaczmarek, Ł. (2003) A description of the new tardigrade *Macrobiotus reinhardti* (Eutardigrada: Macrobiotidae, *harmsworthi* group) with some remarks on the oral cavity armature within the genus *Macrobiotus* Schultze. *Zootaxa*, 331, 1–24. doi:10.11646/zootaxa.331.1.1.

Michalczyk, Ł. & Kaczmarek, Ł. (2010) Description of *Doryphoribius dawkinsi*, a new species of Tardigrada (Eutardigrada: Hypsibiidae) from the Costa Rican highlands, with the key to the genus *Doryphoribius*. *Zootaxa*, 2393, 46–58. doi:10.11646/zootaxa.2393.1.4.

Mironov, S.V., Dabert, J. & Dabert, M. (2012) A new feather mite species of the genus *Proctophyllodes* Robin, 1877 (Astigmata: Proctophyllodidae) from the Long-tailed Tit *Aegithalos caudatus* (Passeriformes: Aegithalidae): morphological description with DNA barcode data. *Zootaxa*, 3253, 54–61.

Møbjerg, N. & Dahl, C. (1996) Studies on the morphology and ultrastructure of the Malpighian tubules of *Halobiotus crispae* Kristensen, 1982 (Eutardigrada). *Zool. J. Linn. Soc.*, 116, 85–99. doi:10.1111/j.1096-3642.1996.tb02335.x.

Møbjerg, N., Jørgensen, A., Eibye-Jacobsen, J., Halberg, K.A., Persson, D. & Kristensen, R.M. (2007) New records on cyclomorphosis in the marine eutardigrade *Halobiotus crispae* (Eutardigrada: Hypsibiidae). *J. Limnol.*, 66, 132–140. doi:10.4081/jlimnol.2007.s1.132.

Morek, W., Stec, D., Gąsiorek, P., Schill, R.O., Kaczmarek, Ł. & Michalczyk, Ł. (2016) An experimental test of tardigrade preparation methods for light microscopy. *Zool. J. Linn. Soc.*, 178, 785–793. doi:10.1111/zoj.12457.

Morek, W., Stec, D., Gąsiorek, P., Surmacz, B. & Michalczyk, Ł. (2019) *Milnesium tardigradum* Doyère, 1840: The first integrative study of interpopulation variability in a tardigrade species. *J. Zool. Syst. Evol. Res.*, 57, 1–23. doi:10.1111/jzs.12233.

Morgan, C. (1976) Studies on the British tardigrade fauna: some zoogeographical and ecological notes. *J. Nat. Hist.*, 10, 607–632. doi:10.1080/00222937600770491.

Murray, J. (1906) XII. Scottish National Antarctic Expedition: Tardigrada of the South Orkneys. *Trans. R. Soc. Edinburgh, Part II*, 45, 323–334. doi:10.1017/S0080456800022754.

Murray, J. (1907) Scottish Tardigrada, collected by the lake survey. *Trans. R. Soc. Edinburgh, Part III*, 45, 641–668. doi:10.1017/S0080456800011777.

Nelson, D.R., Guidetti, R. & Rebecchi, L. (2015) Phylum Tardigrada. In: J. Thorp & D.C. Rogers (Eds.) *Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates*, Academic Press, 1, pp. 347–380.

Nelson, D.R. & Marley, N.J. (2000) The biology and ecology of lotic Tardigrada. *Fresh. Biol.*, 44, 93–108. doi:10.1046/j.1365-2427.2000.00586.x.

Nelson, D.R., Marley, N.J. & Bertolani, R. (1999) Redescription of the genus *Pseudobiotus* (Eutardigrada, Hypsibiidae) and of the new type species *Pseudobiotus kathmanae* sp. n. *Zool. Anz.*, 238, 311–317.

Pilato, G. (1969) Evoluzione e nuova sistemazione degli Eutardigrada. *Boll. Zool.*, 36, 327–345.

Pilato, G. (1969) Su un interessante Tardigrado esapodo delle dune costiere siciliane: *Hexapodibius micronyx* n. gen. n. sp. *Boll. Accad. Gioenia Sci. Nat. Catania, Serie IV*, 9, 619–622.

Pilato, G. (1974) Tardigradi delle acque dolci siciliane. Terza nota. *Animalia*, 1, 235–244.

Pilato, G. (1975) On the taxonomic criteria of the Eutardigrada. *Mem. Ist. Ital. Idrobiol.*, 32 (Suppl.), 277–303.

Pilato, G. (1982) The systematics of Eutardigrada: a comment. *Sond. aus Z.f. zool. Sys. u. Evol.*, 20, 271–284.

Pilato, G. (1989) Phylogenesis and systematic arrangement of the family Calohypsibiidae Pilato, 1969 (Eutardigrada). *Zeitschr. Zool. Syst. Evol.-Forsch.*, 27, 8–13. doi:10.1111/j.1439-0469.1989.tb00433.x.

Pilato, G. (1992) *Mixibius*, nuovo genere di Hypsibiidae (Eutardigrada). *Animalia*, 19, 121–125.

Pilato, G. (1998) Microhypsibiidae, new family of Eutardigrades, and description of the new genus *Fractonotus*. *Spixiana*, 21, 129–134.

Pilato, G. (2013) The taxonomic value of the structures for the insertion of the stylet muscles in the Eutardigrada, and description of a new genus. *Zootaxa*, 3721, 365–378. doi:10.11646/zootaxa.3721.4.4.

Pilato, G. & Beasley, C.W. (1987) *Haplohexapodibius seductor* n. gen. n. sp. (Eutardigrada, Calohypsibiidae) with remarks on the systematic position of the new genus. *Animalia*, 14, 65–71.

Pilato, G. & Beasley, C.W. (2005) *Haplomacrobiotus utahensis* new species of Calohypsibiidae (Eutardigrada) from North America. *Zootaxa*, 879, 1–7. doi:10.11646/zootaxa.879.1.1.

Pilato, G., Bertolani, R. & Binda, M.G. (1982) Studio degli *Isohypsibius* del gruppo *elegans* (Eutardigrada, Hypsibiidae) con descrizione di due nuove specie. *Animalia*, 9, 185–198.

Pilato, G. & Binda, M.G. (1989) *Richtersius*, nuovo nome generico in sostituzione di *Richtersia* Pilato and Binda, 1987 (Eutardigrada). *Animalia*, 16, 147–148.

Pilato, G. & Binda, M.G. (1991) Tardigradi dell'Antartide. I. *Ramajendas*, nuovo genere di Eutardigrado. Nuova posizione sistematica di *Hypsibius renaudi* Ramazzotti, 1972, e descrizione di *Ramajendas frigidus* n. sp. *Animalia*, 17, 61–71.

Pilato, G. & Binda, M.G. (1996) Additional remarks to the description of some genera of eutardigrades. *Boll. Accad. Gioenia Sci. Nat. Catania*, 29, 33–40.

Pilato, G. & Binda, M.G. (1997) *Acutuncus*, a new genus of Hypsibiidae (Eutardigrada). *Entomol. Mitt. Zool. Mus. Hamburg*, 12, 159–162.

Pilato, G. & Binda, M.G. (2010) Definition of families, subfamilies, genera and subgenera of the Eutardigrada, and keys to their identification. *Zootaxa*, 2404, 1–54. doi:10.5281/zenodo.194138.

Pilato, G. & Lisi, O. (2004) *Doryphoribius neglectus* sp. n. and *Parascon nichollsae* sp. n., new species of eutardigrades from Australia. *Zootaxa*, 545, 1–7. doi:10.11646/zootaxa.545.1.1.

Pilato, G., Lisi, O. & Binda, M.G. (2010) Tardigrades of Israel with description of four new species. *Zootaxa*, 2665, 1–28. doi:10.11646/zootaxa.2665.1.1.

Plate, L.H. (1889) Beiträge zur Naturgeschichte der Tardigraden. *Zool. Jahr.*, 3, 487–550.

Pleijel, F., Jondelius, U., Norlinder, E., Nygren, A., Oxelman, B., Schander, C., Sundberg, P. & Thollesson, M. (2008) Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Mol. Phyl. Evol.*, 48, 369–371. doi:10.1016/j.ympev.2008.03.024.

Ramazzotti, G. (1972) Tardigradi delle Isole Kerguelen e descrizione dell nuova specie *Hypsibius* (*I.*) *renaudi*. *Mem. Ist. Ital. Idrobiol.*, 29, 141–144.

Ramazzotti, G. & Maucci, W. (1983) Il Phylum Tardigrada. III edizione riveduta e aggiornata. *Mem. Ist. Ital. Idrobiol.*, 41, 1–1012.

Rambaut, A., Suchard, M.A., Xie, D. & Drummond, A.J. (2014) Tracer v1.6. Online: https://beast.bio.ed.ac.uk/Tracer.

Rebecchi, L. & Nelson, D. (1998) Evaluation of a secondary sex character in eutardigrades. *Invert. Biol.*, 117, 194–198. doi:10.2307/3226985.

Richters, F. (1900) Beiträge zur Kenntnis der Fauna der Umgegend von Frankfurt a M.. *Bericht der Senckenbergischen Naturforschenden Gesell. Frankfurt am Main*, 21–44.

Richters, F. (1902) Beiträge zur Kenntnis der Fauna der Umgebung von Frankfurt a M.. *Bericht der Senckenbergische Naturforschende Gesselschaft in Frankfurt am Main*, 23–26.

Richters, F. (1926) Tardigrada. In: W. Kükenthal (Ed.) *Handbuch der Zoologie*, pp. 58–61. De Gruyter, Berlin and Leipzig.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574. doi:10.1093/bioinformatics/btg180.

Sands, C.J., McInnes, S.J., Marley, N.J., Goodall-Copestake, W., Convey, P. & Linse, K. (2008) Phylum Tardigarda: an "individual" approach. *Cladistics*, 24, 1–18. doi:10.1111/j.1096-0031.2008.00219.x.

Schuster, R.O., Toftner, E.C. & Grigarick, A.A. (1978) Tardigrada of Pope Beach, Lake Tahoe, California. *Wasmann J. Biol.*, 35, 115–136.

Schuster, R.O., Nelson, D.R., Grigarick, A.A. & Christenberry, D. (1980) Systematic criteria of the Eutardigrada. *Trans. Am. Microsc. Soc.*, 99, 284–303. doi:10.2307/3226004.

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313. doi:10.1093/bioinformatics/btu033.

Stec, D., Morek, W., Gąsiorek, P. & Michalczyk, Ł. (2018) Unmasking hidden species diversity within the *Ramazzottius oberhaeuseri* complex, with an integrative redescription of the nominal species for the family Ramazzottiidae (Tardigrada: Eutardigrada: Parachela). *Syst. Biodivers.*, 16, 357–376. doi:10.1080/14772000.2018.1424267.

Stec, D., Smolak, R., Kaczmarek, Ł. & Michalczyk, Ł. (2015) An integrative description of *Macrobiotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya. *Zootaxa*, 4052, 501–526. doi:10.11646/zootaxa.4052.5.1.

Stec, D., Zawierucha, K. & Michalczyk, Ł. (2017) An integrative description of *Ramazzottius subanomalus* (Biserov, 1985) (Tardigrada) from Poland. *Zootaxa*, 4300, 403–420. doi:10.11646/zootaxa.4300.3.4.

Thulin, G. (1911) Beiträge zur Kenntnis der Tardigradenfauna Schwedens. *Arkiv för Zool.*, 7, 1–60.

Thulin, G. (1928) Über die Phylogenie und das System der Tardigraden. *Hereditas*, 11, 207–266.

Tumanov, D.V. (2003) Four new *Isohypsibius* species from Russian fresh waters. *Bull. Inst. R. Sci. Nat. Belg. Biol.*, 73, 183–189.

Tumanov, D.V. (2005) *Isohypsibius panovi*, a new species of Tardigrada from Ireland (Eutardigrada, Hypsibiidae). *Zootaxa*, 812, 1–4. doi:10.11646/zootaxa.812.1.1.

Vecchi, M., Cesari, M., Bertolani, R., Jönsson, K.J., Rebecchi, L. & Guidetti, R. (2016) Integrative systematic studies on tardigrades from Antarctica identify new genera and new species within Macrobiotoidea and Echiniscoidea. *Invert. Syst.*, 30, 303–322. doi:10.1071/IS15033.

Wiktor, J., Tatarek, A., Węsławski, J.M., Kotwicki, L. & Poulin, M. (2016) Colonies of *Gyrosigma eximium*: a new phenomenon in Arctic tidal flats. *Oceanologia*, 58, 336–340. doi:10.1016/j.oceano.2016.04.007.

Wolburg-Buchholz, K. & Greven, H. (1979) On the fine structure of the spermatozoon of *Isohypsibius granulifer* Thulin 1928 (Eutardigrada) with reference to its differentiation. *Zesz. Nauk. Uniw. Jagiell.*, 529, 191–197.

Zawierucha, K., Stec, D., Lachowska-Cierlik, D., Takeuchi, N., Li, Z. & Michalczyk, Ł. (2018) High mitochondrial diversity in a new of water bear species (Tardigrada: Eutardigrada) from mountain glaciers in central Asia, with the erection of a new genus *Cryoconicus*. *Ann. Zool.*, 68, 179–201. doi:10.3161/00034541ANZ2018.68.1.007.

Zeller, C. (2010) Untersuchung der Phylogenie von Tardigraden anhand der Genabschnitte 18S rDNA und Cytochrom c Oxidase Untereinheit 1 (COX I). MSc Thesis, Technische Hochschule Wildau, Wildau, 105 pp.

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APPENDIX

Taxonomic composition of isohypsibioid families

Type genera are underlined by a double line, and type species by a single line. Taxa described as *species dubiae* are either synonyms of other species or their descriptions are too general and do not allow confident identifications; whereas *nomina inquirenda* embrace most likely valid species, but insufficiently described. This distinction and assessment was done after a careful analysis of the original species descriptions.

Composition of Isohypsibiidae

Genus: *Dastychius* Pilato, 2013

Species: *D. improvisus* (Dastych, 1984).

Genus: *Dianea* gen. nov.

Species: *D. acuminata* nom. nov. et comb. nov. [*Isohypsibius papillifer indicus* (Iharos, 1969)], *D. basalovoi* (Durante & Maucci, 1973) comb. nov., *D. belliforma* (Mihelčič, 1971) comb. nov. et sp. dub., *D. bella* (Mihelčič, 1971) comb. nov. et sp. dub., *D. brevispinosa* (Iharos, 1966) comb. nov. et sp. dub., *D. costata* (Mihelčič, 1971) comb. nov. et sp. dub., *D. effusa* (Mihelčič, 1971) comb. nov. et sp. dub., *D. franzi* (Mihelčič, 1951) comb. nov. et sp. dub., *D. helenae* (Iharos, 1964) comb. nov. et nom. inq., *D. indica* (Murray, 1907) comb. nov. et nom. inq., *D. mammillosa* (Iharos, 1964) comb. nov. et sp. dub., *D. papillifera* (Murray, 1905) comb. nov., *D. sattleri* (Richters, 1902) comb. nov., *D. rahmi* (Li & Wang, 2006) comb. nov., *D. tuberculoides* (Mihelčič, 1951) comb. nov. et sp. dub., *D. vejdovskyi* (Bartoš, 1939) comb. nov. et nom. inq.

Remarks: As stressed by Ramazzotti & Maucci (1983) for *Isohypsibius s.l.* spp. described by Mihelčič & Iharos, the genus consists mainly of dubious and improperly described species, which very likely are synonyms of the nominal species, *D. sattleri* comb. nov. Here, *I. papillifer bulbosus* (Marcus, 1928) is supressed due to lack of significant differences between the putative subspecies and the nominal subspecies. On the other hand, the subspecies *Isohypsibius papillifer indicus* (Iharos, 1969) is elevated to the species rank because of significant morphological differences with the nominal subspecies such as two macroplacoids instead of three, larger and more triangular gibbosities, and no cephalic gibbosities. Given that within *Dianea* gen. nov., the specific name '*indica*' is already occupied by *D. indica* (Murray, 1907), a *nomen novum D. acuminata* comb. nov. is established for *I. papillifer indicus* (Iharos, 1969) (from Latin *acuminatus* = acute, sharp, which refers to the elongated gibbosity apices).

Genus: *Eremobiotus* Biserov, 1992

Species: *E. alicatai* (Binda, 1969), *E. ginevrae* Lisi *et al.*, 2016, *E. ovezovae* Biserov, 1992.

Genus: *Fractonotus* Pilato, 1998

Species: *F. caelatus* (Marcus, 1928), *F. verrucosus* (Richters, 1900), *F. gilvus* (Biserov, 1986).

Genus: *Isohypsibius* Thulin, 1928

Species: *I. altai* Kaczmarek & Michalczyk, 2006, *I. arbiter* Binda, 1980, *I. archangajensis* Kaczmarek & Michalczyk, 2004, *I. arcuatus* (Bartoš, 1934), *I. barbarae* Pilato & Binda, 2002, *I. borkini* Tumanov, 2003, *I. brulloi* Pilato & Pennisi, 1976, *I. cambrensis* (Morgan, 1976) comb. nov., *I. campbellensis* Pilato, 1996, *I. canadensis* (Murray, 1910) sp. dub., *I. ceciliae* Pilato & Binda, 1987, *I. changbaiensis* Yang, 1999 sp. dub., *I. chiarae* Maucci, 1987, *I. condorcanquii* Kaczmarek et al., 2014, *I. coulsoni* Kaczmarek et al., 2012, *I. damxungensis* Yang, 2007 sp. dub., *I. dastychi* Pilato et al., 1982, *I. glazovi* Biserov, 1999, *I. hadzii* (Mihelčič, 1938) sp. dub., *I. jakieli* Dastych, 1984, *I. jingshanensis* Yang, 2003 sp. dub., *I. jinhouensis* Yang, 2007 sp. dub., *I. liae* Li & Wang, 2006 nom. inq., *I. macrodactylus* (Maucci, 1978), *I. marcellinoi* Binda & Pilato, 1971, *I. palmai* Pilato, 1996, *I. panovi* Tumanov, 2005, *I. pauper* (Mihelčič, 1971) nom. inq., *I. prosostomus* Thulin, 1928, *I. pseudundulatus* (da Cunha & do Nascimento Ribeiro, 1964), *I. reticulatus* Pilato, 1973, *I. sabellai* Pilato et al., 2004, *I. schaudinni* (Richters, 1909) nom. inq., *I. sculptus* (Ramazzotti, 1962), *I. sellnicki* (Mihelčič, 1962), *I. solidus* (Mihelčič, 1971), *I. taibaiensis* Li & Wang, 2005, *I. tuberculatus* (Plate, 1889) nom. inq., *I. undulatus* Thulin, 1928, *I. verrucosus* (Della Valle, 1915) nom. inq., *I. wilsoni* (Horning et al., 1978), *I. yunnanensis* Yang, 2002 sp. dub.

Remarks: *I. cambrensis* is raised to a species rank because of the significant distinctiveness from *I. prosostomus* both in terms of external morphology (finely granulated vs completely smooth cuticle) and genetics (fig. 2). On our phylogenetic tree, the clade *I. dastychi* + *Eremobiotus* + *Ursulinius* gen. nov. can be observed (fig. 2). In our opinion, widely forked claws in the *dastychi* complex (*I. borkini*, *I. dastychi*, *I. jakieli*, *I. marcellinoi*, and *I. panovi*) bear resemblance to *Eremobiotus* type claws. This characteristic, together with only two granular macroplacoids in the pharynx and smooth or weakly reticulated cuticle, which make the *dastychi* complex stand out from *Isohypsibius s.s.* could warrant erecting a new genus for the *dastychi* complex if new data support the hypothetical monophyly of the complex. As noted by Gąsiorek et al. (2019), species with very pronounced cuticular sculpture (*I. barbarae*, *I. sabellai*) are potentially more closely related to *Fractonotus* than to *Isohypsibius s.s.*, and their systematic position may change when more ample molecular data are available.

Genus: *Ursulinius* gen. nov.

Species: *U. austriacus* (Iharos, 1966) comb. nov., *U. bartosi* (Iharos, 1966) comb. nov. et sp. dub., *U. bulbifer* (Mihelčič, 1957) comb. nov. et sp. dub., *U. cameruni* (Iharos, 1969) comb. nov., *U. cyrilli* (Mihelčič, 1951) comb. nov., *U. dudichi* (Iharos, 1964) comb. nov., *U. duranteae* (Maucci, 1978) comb. nov., *U. elegans* (Binda & Pilato, 1971) comb. nov., *U. eplenyiensis* (Iharos, 1970) comb. nov., *U. glaber* (Durante Pasa & Maucci, 1979) comb. nov., *U. gracilis* (Iharos, 1966) comb. nov. et nom. inq., *U. gyulai* (Mihelčič, 1971) comb. nov. et sp. dub., *U. hypostomoides* (Mihelčič, 1971) comb. nov., *U. josephi* (Iharos, 1964) comb. nov., *U. latiunguis* (Iharos, 1964) comb. nov. et nom. inq., *U. leithaicus* (Iharos, 1966) comb. nov., *U. lunulatus* (Iharos, 1966) comb. nov. et nom. inq., *U. mihelcici* (Iharos, 1964) comb. nov. et nom. inq., *U. montanus* (Mihelčič, 1938) comb. nov. et nom. inq., *U. neoundulatus* (Durante Pasa & Maucci, 1975) comb. nov., *U. nodosus* (Murray, 1907) comb. nov., *U. novaeguineae* (Iharos, 1967) comb. nov., *U. pappi* (Iharos, 1966) comb. nov., *U. pilatoi* (Durante Pasa & Maucci, 1979) comb. nov., *U. pratensis* (Iharos, 1964) comb. nov. et nom. inq., *U. qinlingensis* (Li et al., 2005) comb. nov., *U. roberti* (Biserov, 1996) comb. nov., *U. ronsisvallei* (Binda & Pilato, 1969) comb. nov., *U. rudescui* (Iharos, 1966) comb. nov., *U. septentrionalis* (Thulin, 1928) comb. nov., *U. silvicola* (Iharos, 1966) comb. nov. et nom. inq., *U. theresiae* (Iharos, 1964) comb. nov. et nom. inq., *U. torulosus* (Mihelčič, 1959) comb. nov., *U. truncorum* (Iharos, 1964) comb. nov., *U. tucumanensis* (Claps & Rossi, 1984) comb. nov., *U. woodsae* (Kathman, 1990) comb. nov.

Remarks: Within the genus many species need to be revisited, and their diagnoses updated, but, in contrast to *Dianea* gen. nov., the majority of them seems to be valid. Correct reanalyses of the gibbosity arrangement are crucial for the taxonomy of *Ursulinius* gen. nov.

Composition of Doryphoribiidae fam. nov.

Genus: *Apodibius* Dastych, 1983

Species: *A. confusus* Dastych, 1983, *A. nuntius Binda*, 1984, *A. richardi* Vargha, 1995

Genus: *Doryphoribius* Pilato, 1969

Species (divided into ecomorphological groups distinguished in this paper):

– two macroplacoids and dorsolateral gibbosities present, terrestrial (the *D. flavus* group): *D. amazzonicus* Lisi, 2011, *D. barbarae* Beasley & Miller, 2012, *D. bindae* Lisi, 2011, *D. dawkinsi* Michalczyk & Kaczmarek, 2010, *D. dupliglobulatus* Ito, 1995, *D. flavus* (Iharos, 1966), *D. huangguoshuensis* Wang et al., 2007, *D. maranguensis* Binda & Pilato, 1995, *D. mcinnesae* Meng et al., 2014, *D. monstruosus* (Maucci, 1991) comb. nov., *D. niedbalai* Zawierucha et al., 2012, *D. picoensis* Fontoura et al., 2008, *D. quadrituberculatus* Kaczmarek & Michalczyk, 2004, *D. rosanae* Daza et al., 2017, *D. solidunguis* Lisi, 2011, *D. tessellatus* Meyer, 2011, *D. zyxiglobus* (Horning et al., 1978);

– two macroplacoids and dorsolateral gibbosities present, aquatic (the *D. evelinae* group): *D. evelinae* (Marcus, 1928);

– two macroplacoids present, dorsolateral gibbosities absent, terrestrial (the *D. doryphorus* group = *Doryphoribius s.s.*): *D. doryphorus* (Binda & Pilato, 1969), *D. macrodon* Binda et al., 1980, *D. neglectus* Pilato & Lisi, 2004, *D. pilatoi* Bertolani, 1984, *D. smokiensis* Bartels et al., 2007;

– two macroplacoids present, dorsolateral gibbosities absent, aquatic (the *D. koreanus* group): *D. koreanus* Moon et al., 1994, *D. polynettae* Biserov, 1988, *D. tergumrudis* Bartels et al., 2008;

– three macroplacoids and dorsolateral gibbosities present, terrestrial (the *D. vietnamensis* group): *D. elleneddiei* Haefke et al., 2014, *D. gibber* Beasley & Pilato, 1987, *D. maasaimarensis* Fontoura et al., 2013, *D. mariae* Pilato & Binda, 1990, *D. minimus* Bartels et al., 2008, *D. vietnamensis* (Iharos, 1969);

– three macroplacoids present, dorsolateral gibbosities absent, terrestrial (the *D. bertolanii* group): *D. bertolanii* Beasley & Pilato, 1987, *D. chetumalensis* Pérez-Pach et al., 2017, *D. korganovae* Biserov, 1994, *D. mexicanus* Beasley et al., 2008, *D. qinlingense* Li et al., 2004, *D. taiwanus* Li & Li, 2008, *D. turkmenicus* Biserov, 1999;

– three macroplacoids present, dorsolateral gibbosities absent, aquatic (the *D. zappalai* group): *D. longistipes* Bartels et al., 2008, *D. zappalai* Pilato, 1971.

Remarks: Currently, *Doryphoribius* comprises the most diverse group of taxa in terms of claw morphology within the entire Isohypsibioidea (see Discussion), which suggests that the genus is polyphyletic (Bertolani et al., 2014a; Cesari et al., 2016; Gąsiorek et al., 2019). In the light of our findings regarding the polyphyly of *Isohypsibius*, it seems very likely that *Doryphoribius* may comprise a number of new genera.

Given that *Isohypsibius monstruosus* Maucci, 1991 exhibits the ventral lamina (fig. 17A), which conforms with the current diagnosis of *Doryphoribius*, the new combination *D.*

monstruosus (Maucci, 1991) comb. nov. is proposed. The transfer confirms the supposition of Michalczyk & Kaczmarek (2010), that some *Isohypsibius* spp. may in fact represent *Doryphoribius s.l.*

Genus: *Grevenius* gen. nov.

Species: *G. annulatus annulatus* (Murray, 1905) comb. nov., *G. annulatus minor* (Ramazzotti, 1945) comb. nov., *G. asper* (Murray, 1906) comb. nov., *G. baicalensis* (Ramazzotti, 1966) comb. nov., *G. baldii* (Ramazzotti, 1945) comb. nov., *G. baldiioides* (Tumanov, 2003) comb. nov., *G. brevitubulatus* (Rho et al., 1997) comb. nov., *G. deconincki* (Pilato, 1971) comb. nov., *G. deflexus* (Mihelčič, 1960) comb. nov., *G. fuscus* (Mihelčič, 1971/72) comb. nov. et nom. inq., *G. granditintinus* (Chang & Rho, 1996) comb. nov., *G. granulifer* (Thulin, 1928) comb. nov., *G. hydrogogianus* (Ito & Tagami, 1993) comb. nov., *G. irregibilis* (Biserov, 1992) comb. nov., *G. karenae* (Zawierucha, 2013) comb. nov., *G. kenodontis* (Kendall-Fite & Nelson, 1996) comb. nov., *G. koreanensis* (Iharos, 1971) comb. nov. et nom. inq., *G. kotovae* (Tumanov, 2003) comb. nov., *G. kristenseni* (Pilato et al., 1989) comb. nov., *G. ladogensis* (Tumanov, 2003) comb. nov., *G. laevis* (McInnes, 1995) comb. nov., *G. lineatus* (Mihelčič, 1969) comb. nov. et nom. inq., *G. longiunguis* (Pilato, 1974) comb. nov., *G. malawiensis* (Jørgensen, 2001) comb. nov., *G. marii* (Bertolani, 1981) comb. nov., *G. monoicus* (Bertolani, 1981) comb. nov., *G. myrops* (du Bois-Reymond Marcus, 1944) comb. nov., *G. nipponicus* (Sudzuki, 1975) comb. nov. et nom. inq., *G. pulcher* (Mihelčič, 1971/72) comb. nov. et nom. inq., *G. pushkini* (Tumanov, 2003) comb. nov., *G. rugosus* (Guidi & Grabowski, 1996) comb. nov. et nom. inq., *G. rusticus* (Pilato et al., 2015) comb. nov., *G. sismicus* (Maucci, 1978) comb. nov., *G. tubereticulatus* (Pilato & Catanzaro, 1989) comb. nov., *G. verae* (Pilato & Catanzaro, 1989) comb. nov., *G. zappalai* (Pilato et al., 2015) comb. nov.

Remarks: All former aquatic *Isohypsibius* species are ascribed to the new genus. Although *Grevenius* gen. nov. comprises taxa dwelling in similar habitats, clear peculiarities can be observed in claw morphology of some limnic and intertidal species (*G. brevitubulatus* comb. nov., *G. deflexus* comb. nov., *G. granditintinus* comb. nov., *G. hydrogogianus* comb. nov., *G. irregibilis* comb. nov., *G. myrops* comb. nov.). Considering that even slight differences in claw anatomy seem to hold very strong phylogenetic signal in Isohypsibioidea, it will not be surprising if these species turn out to represent separate genera when more accurate morphological data and DNA sequences are available.

G. karenae comb. nov., *G. rugosus* comb. nov. and *G. sismicus* comb. nov. were found in hydrophilic substrata, in close vicinities of lakes or ponds, but not strictly in an aquatic habitat. However, their close affinity to *Grevenius* gen. nov. seems certain, especially that the most similar species to which they were compared in their original descriptions were all exclusively limnic taxa.

Due to morphological differences with *G. granulifer* comb. nov. (pink body colour and cuticular tubercles of identical size on the whole body in *Isohypsibius granulifer koreanensis vs* white body colour and cuticular tubercles of different sizes on the dorsum and on the venter in *G. granulifer* comb. nov.), *I. granulifer koreanensis* is elevated to a species rank as *Grevenius koreanensis* comb. nov.

Genus: *Paradiphascon* Dastych, 1992

Species: *P. manningi* Dastych, 1992.

Remarks: Given the peculiar apomorphy in the form of flexible pharyngeal tube and disproportionally widened bases of external and posterior claws, the genus requires an integrative redescription to verify its systematic position.

Genus: *Pseudobiotus* Nelson, 1980

Species: *P. hirsutellus* Pilato et al., 2010, *P. kathmanae* Nelson et al., 1999, *P. longiunguis* (Iharos, 1968) sp. dub., *P. matici* (Pilato, 1971), *P. megalonyx* (Thulin, 1928), *P. spinifer* Chang et al., 2007, *P. vladimiri* Biserov et al., 2001.

Remarks: The oldest species in the genus, *P. megalonyx*, requires an integrative redescription to aid species discovery in *Pseudobiotus*.

Genus: *Thulinius* Bertolani, 2003

Species: *T. augusti* (Murray, 1907), *T. itoi* (Tsurusaki, 1980), *T. romanoi* Bertolani et al., 2014, *T. ruffoi* (Bertolani, 1981), *T. saltursus* (Schuster et al., 1978), *T. stephaniae* (Pilato, 1974).

Remarks: The oldest species in the genus, *T. augusti*, requires an integrative redescription to allow for the verification of alleged numerous records of the species throughout the world and to aid species discovery in *Thulinius*.

Composition of Hexapodibiidae

Genus: *Haplohexapodibius* Pilato & Beasley, 1987

Species: *Haplohexapodibius seductor* Pilato & Beasley, 1987.

Genus: *Haplomacrobiotus* May, 1948

Species: *H. hermosillensis* May, 1948, *H. utahensis* Pilato & Beasley, 2005.

Genus: *Hexapodibius* Pilato, 1969

Species: *H. bindae* Pilato, 1982, *H. boothi* Dastych & McInnes, 1994, *H. christenberryae* Pilato & Binda, 2003, *H. micronyx* Pilato, 1969, *H. pseudomicronyx* Robotti, 1972, *H. reginae* Vargha, 1995.

Genus: *Parhexapodibius* Pilato, 1969

Species: *P. bactrianus* Biserov, 1999, *P. castrii* (Ramazzotti, 1964), *P. lagrecai* (Binda & Pilato, 1969), *P. pilatoi* (Bernard, 1977), *P. ramazzottii* Manicardi & Bertolani, 1987

Composition of Halobiotidae fam. nov.

Genus: *Halobiotus* Kristensen, 1982

Species: *H. appelloefi* (Richters, 1908) comb. nov. et nom. inq., *H. arcturulius* Crisp & Kristensen, 1983, *H. crispae* Kristensen, 1982, *H. geddesi* (Hallas, 1971) comb. nov. et nom. inq., *H. stenostomus* (Richters, 1908).

Remarks: Although comparative analyses of Hallas (1971) confirmed the validity of *Macrobiotus appelloefi* Richters, 1908 and *Hypsibius geddesi* Hallas, 1971, the two species are designated as synonyms of *Halobiotus stenostomus* in the current tardigrade species checklist (Degma et al., 2009–18). However, given that both species are insufficiently described, we consider them as awaiting redescriptions to verify the synonymy, staying in agreement with the opinion of the genus authority (R.M. Kristensen, pers. comm.).

Isohypsibioid genera *incertae sedis*

Genus: *Ramajendas* Pilato & Binda, 1990

Species: *R. dastychi* Kaczmarek et al., 2013, *R. frigidus* Pilato & Binda, 1990, *R. heatwolei* Miller et al., 1995, *R. renaudi* (Ramazzotti, 1972).

Genus: *Thalerius* Dastych, 2009

Species: *T. konradi* Dastych, 2009.