Article

Phylum Tardigrada: A re-evaluation of the Parachela

NIGEL J. MARLEY[1,3], SANDRA J. MCINNES[2] & CHESTER J. SANDS[2]

[1]*Marine Biology and Ecology Research Centre, School of Marine Science and Engineering, University of Plymouth, Drake Circus, Plymouth, PL4 8AA, United Kingdom*

[2]*British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom*

[3]*Corresponding author. E-mail: nigel.marley@plymouth.ac.uk*

Abstract

We assessed the available morphological evidence to see if this corroborates the paraphyly in the Parachela (Tardigrada) as suggested by recent molecular data. We reconcile molecular phylogenetics with alpha morphology, focusing on claw and apophysis for the insertion of the stylet muscles (AISM). We combine molecular and morphological evidence to define six new taxa within the Parachela Schuster et al 1980. These include two new families of Isohypsibiidae **fam. nov.** and Ramazzottidae **fam. nov.** along with four new superfamilies of Eohypsibioidea **superfam. nov.,** Hypsibioidea **superfam. nov.,** Isohypsibioidea **superfam. nov.,** and Macrobiotoidea **superfam. nov.**

Key words: Tardigrade, Eohypsibioidea **superfam. nov.**, Hypsibioidea **superfam. nov.**, Isohypsibioidea **superfam. nov.**, Macrobiotoidea **superfam. nov.**, Isohypsibiidae **fam. nov.**, Ramazzottidae **fam. nov.**, Morphology, Molecular, Systematics

Introduction

Familial level taxa that have separated into distinct lineages over many millions of years are usually clearly identifiable via a unique suite of morphological characters. In some groups, particularly the "lesser-known" or "minor" phyla, basic morphology may be so strongly conserved that deep divergences are often difficult to detect or resolve. The application of genetic techniques has frequently uncovered unexpected diversity in such groups. For example, while 13 Australian species were recognised previously within the phylum Onychophora Grube, 1853 (Reid 1996), allozyme electrophoresis has identified deep cryptic divergences within these recognised groups (Brisco and Tait 1995), leading to the description of 22 new genera and 41 new species (Reid 1996). Onychophora are relatively large, usually between 50 and 200 mm long, with an average of *ca.* 50 mm, (C.J. Sands, personal obs.), and possess many characters that are clearly visible without the aid of a microscope. As the complexity and size of the animals within a taxonomic group diminishes the number of available characters usually becomes restricted and the ability to detect subtle morphological variation technologically limited. So, while recent molecular work has indicated cryptic lineages in the phylum Tardigrada Spallanzani, 1776 (e.g. Kiehl *et al.* 2007; Sands *et al.* 2008a, b, Jørgensen *et al.* 2010) there are relatively few morphological features to corroborate such morpho-crypsis.

In this paper we discuss the current systematics of the phylum Tardigrada in light of the growing body of molecular phylogenetic evidence. We argue that despite a lack of clear morphological apomorphies, there is sufficient evidence for a considerable re-arrangement of currently accepted systematics. The re-arrangement we propose results in the establishment of four superfamilies corroborated by both alpha taxonomy and molecular analyses.

Dedication: In memoriam Prudence de Villiers, friend of the first author, who died whilst this paper was in review.

Phylum Tardigrada

Tardigrades are a phylum consisting of small (*c*. 50 to 2110 µm), roughly cylindrical, bilaterally symmetrical metazoans. The body has five distinct pseudo-segments: a head, and four body segments (three trunk and one caudal), each bearing a pair of short, stubby lobopodal limbs that terminate in either claws, toes or adhesive discs. Internal features include a fluid-filled haemocoel, a nervous system consisting of a dorsal lobed brain and ventral nerve cord with fused paired ganglia. The digestive system comprises a complex buccal-pharyngeal apparatus (buccal tube, armed with stylets, tri-radiate and muscular pharynx), an oesophagus, midgut and hindgut. The cuticle, which also lines both the fore- and hindgut, is composed of chitin, protein and lipid, and is moulted several times throughout life. This apparently simple body plan provides few characters with which to define both species and higher taxonomic hierarchy within the Tardigrada (Ramazzotti and Maucci 1983; Kinchin 1994; Nelson and Marley 2000; Nelson and McInnes 2002; Bertolani *et al*. 2009; Pilato and Binda 2010).

Recent work has elucidated many new tardigrade taxa, virtually doubling the diversity over the last 25 years (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Degma *et al*. 2010). Much of this expansion results from finding new species in newly explored regions, more detailed studies of known tardigrade localities, and 'species complex' revisions which have identified both new species and new or revised taxonomic criteria (e.g. Biserov 1990a, b; 1997/8; Claxton 1998; Bertolani and Rebecchi 1993; Guidetti and Bertolani 2001).

The two currently verified classes (Figure 1A) of Heterotardigrada Marcus, 1927 and Eutardigrada Marcus, 1927, based on the oldest described genera, *Echiniscus* Schultze, 1840 and *Macrobiotus* Schultze, 1834 respectively, are based entirely on alpha-morphology, and attributed with modifications to Marcus (1927; 1929; 1936). The Heterotardigrada includes two orders, the largely marine Arthrotardigrada Marcus, 1927, and the Echiniscoidea Richters, 1926, containing the Echiniscidae Thulin, 1928, a large family of terrestrial "armoured" tardigrades plus a few marine taxa. The Eutardigrada, containing the majority of terrestrial and freshwater taxa, comprises two orders: Apochela Schuster, Nelson, Grigarick and Christenberry, 1980, with three carnivorous genera in the Milnesiidae; and Parachela Schuster, Nelson, Grigarick and Christenberry, 1980, harbouring *ca*. 68% of the *ca*. 1100 known tardigrade species. A third class, Mesotardigrada, containing a single species (Rahm 1937) from a hot spring in Japan is now considered either dubious, because the description was very limited and neither the type locality nor type specimen have survived (e.g. Nelson 2002; Nelson and McInnes 2002), or related to Carphaniidae Binda and Kristensen, 1986 (Echiniscoidea) (Binda and Kristensen 1986). For a comprehensive taxonomic summary of all known tardigrade taxa see Guidetti and Bertolani (2005), Degma and Guidetti (2007), Degma *et al*. (2010), and Pilato and Binda (2010).

Molecular systematics

Molecular analysis, initially used to explore the phylogenetic relationship between the Tardigrada and arthropods (e.g. Garey *et al*. 1996; Giribet *et al*. 1996; Moon and Kim 1996; Mallatt *et al*. 2004), has subsequently expanded to explore the higher (ordinal level) classification (Garey *et al*. 1999; Jørgensen and Kristensen 2004, Jørgensen *et al*. 2010) and generic/species relationships (e.g. Guidetti *et al*. 2009), within the phylum.

Recent studies have uncovered apparent inconsistencies in the familial level systematics within the order Parachela. Kiehl *et al*. (2007) used 18S rDNA sequence data to examine relationships between various species of Hypsibiidae Pilato, 1969 in the context of other eutardigrades. Their results define four Parachela clades: (1) *Isohypsibius* Thulin, 1928, *Thulinius* (Bertolani, 2003), and *Halobiotus* Kristensen, 1982; (2) *Macrobiotus* and *Richtersius* (Pilato and Binda, 1989); (3) *Hebesuncus* Pilato, 1987 and *Ramazzottius* Binda and Pilato, 1986; while (4) comprises four *Hypsibius* (Ehrenberg, 1848) species. This study, suggesting polyphyletic relationships within the family Hypsibiidae, questions the evolutionary status of key morphological characters. Similarly, Sands *et al*. (2008a) amplified three (18S rDNA, Cytochrome c Oxidase subunit I and *Wingless*) gene regions from individual tardigrades and identified polyphyly in all three analyses. This prompted a more detailed molecular investigation of the phylum (Sands *et al*. 2008b) which identified three well supported superfamily lineages within the Parachela, including one which isolated *Isohypsibius*, *Thulinius* and *Halobiotus* from the rest of the Hypsibiidae (see figure 2 in Sands *et al*. 2008b). Jørgensen and Kristensen (2004) concluded that Macrobiotidae Thulin, 1928 and Hypsibiidae are monophyletic, yet the limited data can also be read as indicating an isohypsibid group (*Halobiotus stenostomus* and *Thulinius* sp. [*Hypsibius* [Z93337] now attributed to *Thulinius*]), a hypsibid line (*Ramazzottius*
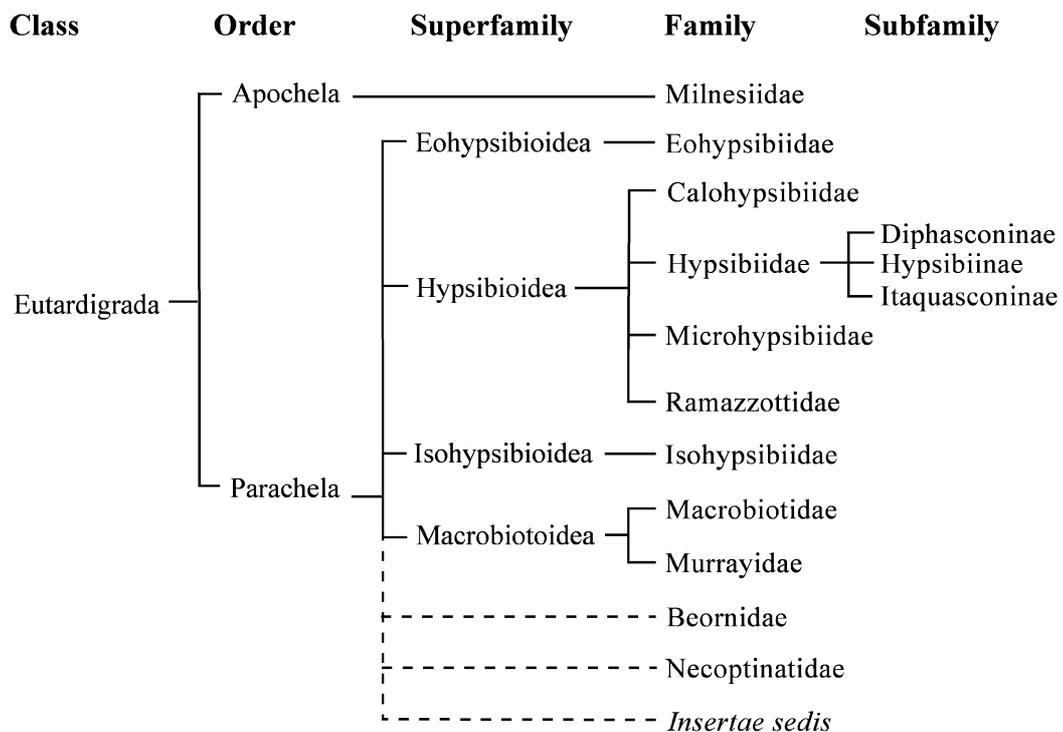
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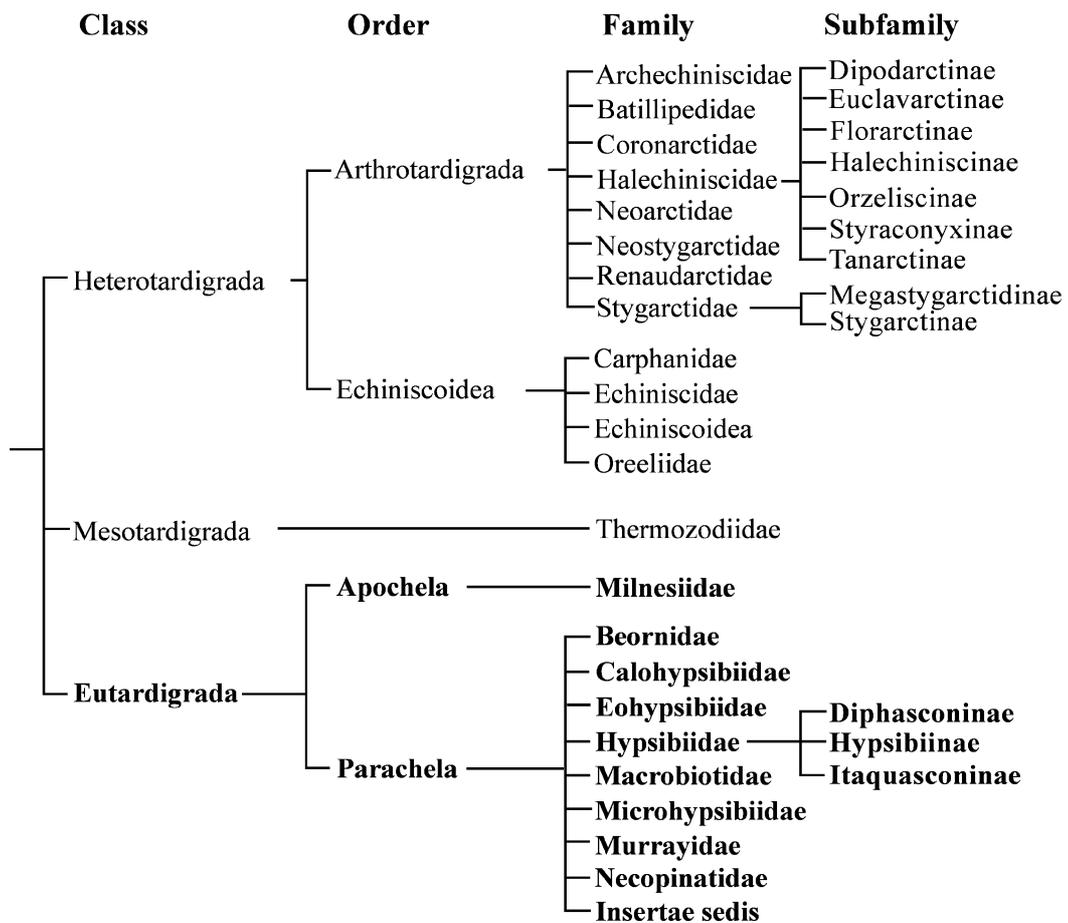
B

FIGURE 1. Phylogenetic topology of the Tardigrada. A. Phylogenetic topology of the Tardigrada based on the current systematic knowledge. B. Proposed phylogenetic topology of the Parachela with the new super families.
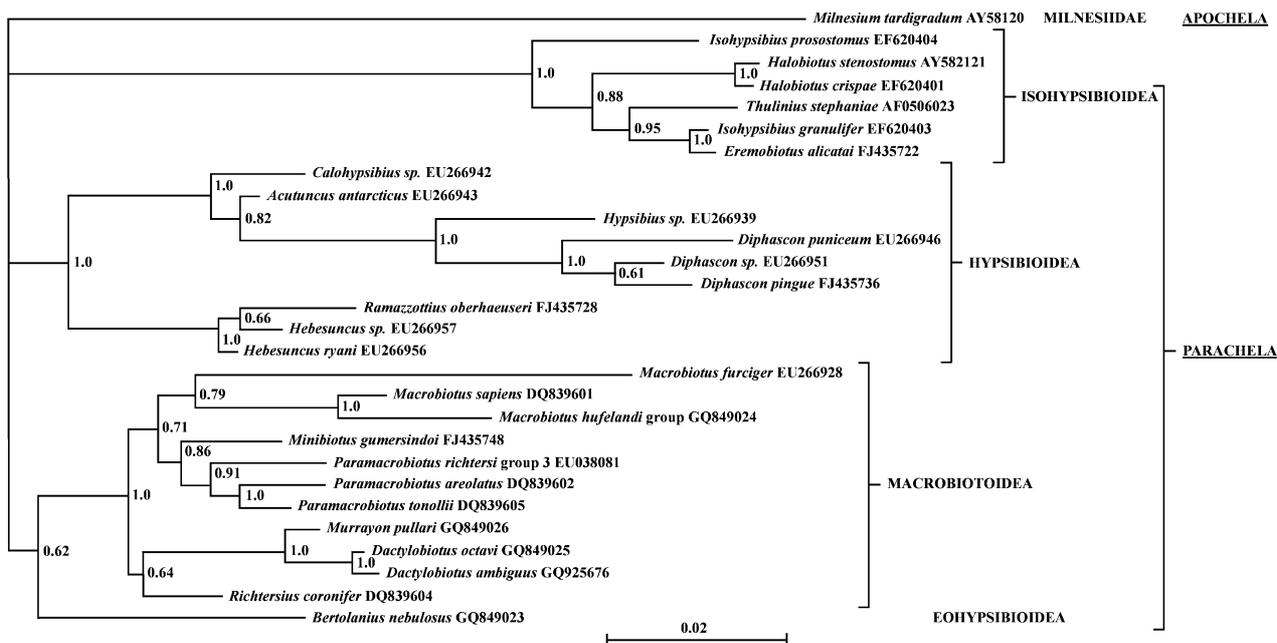
FIGURE 2. Tardigrade Bayesian phylogeny estimated using nearly complete 18S rRNA sequence showing superfamily groups within the Parachela using the Milnesiidae (Apochela) as the outgroup. All data are available from GENBANK. The analysis was conducted in 'MrBayes' (Ronquist & Huelsenbeck 2003) using a 6 parameter model; node support values are 'Posterior Probabilities' based on 2×10^6 MCMC chains with a 25% burn-in; line lengths are proportional to genetic distances.

oberhauseri) and macrobiotid (*Richtersius coronifer, Macrobiotus hufelandi*) (see figure 2 in Jørgensen and Kristensen 2004).

With the addition to GenBank of new 18S rRNA data, which included *Bertolanius nebulosus* (Dastych, 1983) (in Dastych 1983a), Eohypsibiidae Bertolani and Kristensen, 1987 (sequenced by Jørgensen *et al.* 2010 [GQ849023]) and *Eremobiotus alicatai* (Binda 1969), currently Hypsibiidae (sequenced by Guil and Giribet unpublished [FJ435722]), we re-analysis the Bayesian phylogeny to review the superfamily groups within the Parachela using the Milnesiidae (Apochela) as the outgroup. The results (Figure 2) verified the original three lineages and provided evidence of a fourth, which was also proposed by Jørgensen *et al.* 2010.

Morphological re-appraisal

Support for the higher taxa. The strength of molecular taxonomy is well recognised and is used to understand both deep evolutionary relationships, such as issues fundamental to the 'Tree of Life', as well as teasing out microevolutionary processes and identifying morpho-crypsis. Morphological appraisal however remains the fundamental tool for identification and classification. This raises the issue of reconciling conflicting molecular and morphological lines of evidence. Here we re-examine the morphological characters used in classifying families of the Parachela in search of support for the robustly supported clades identified by molecular studies.

The currently accepted systematic relationships (Figure 1A) divide the phylum into two principal classes of Heterotardigrada and Eutardigrada which are differentiated by (a) lateral cirri, (b) continuous placoid structure and (c) separate gonopore and anus. The eutardigrades lack cirri, have differentiated placoid structures and a cloaca.

Within the Eutardigrada, the Apochela are differentiated from the Parachela by claw structure and cephalic appendages. All Apochela possess four claws per leg (two simple and two compound/branched, arranged in a quadrangle) and cephalic papillae, which are thought to be homologous to the heterotardigrade external cephalic cirri (Wiederhöft and Greven 1996, Dewel *et al*. 1993, Dewel and Dewel 1996). By contrast, all Parachela have two claws per leg (each comprising a basal section, secondary and primary branches) but without cephalic papillae. Claw format is a strong ordinal-discriminating character, while the cephalic papillae of Apochela suggest a closer alignment with the Heterotardigrada, a conclusion strongly supported by molecular evidence (Jørgensen and Kristensen 2004; Kiehl *et al*. 2007; Sands *et al*. 2008b; Jørgensen *et al*. 2010).

Claw symmetry and structure are considered to be important evolutionary characters (Thulin 1928, Pilato 1969a, b). Indeed, all post-1970 studies on morphological systematics have used the subtleties of claw morphology to underpin familial descriptions (e.g. Pilato and Binda 2010).

Case for the Parachela claw (Figure 3A–D). Claw symmetry is defined by claw branch arrangement on each leg pair of which there are two types. First, 'symmetrical' with respect to the median plane of the leg, sequence of external claw secondary branch (2), primary branch (1), and internal claw primary branch (1), secondary branch (2) (shorthand - 2112) (Figure 3B and Pilato and Binda 2010, figures 1f, 10a–f, 25b–d). The alternative 'asymmetric' sequence is external claw secondary branch (2), primary branch (1), internal claw secondary branch (2), primary branch (1) (shorthand - 2121) (Figure 3C and see Pilato and Binda 2010, figures 1b–e, 7a–d, 9a, 10a–c, 11a–c, 14a, 17b and 24a).

Symmetric (2112) claw branching provides a synapomorphy that clearly unites the families Macrobiotidae and Murrayidae (Guidetti *et al.* 2000; Guidetti *et al.* 2005), and corresponds to one of the three well supported molecular lineages (Sands *et al.* 2008b). Murrayidae primary and secondary claw branches diverge immediately from a short basal section; while the Macrobiotidae, by contrast, have branches fused over a common tract of variable length (Guidetti *et al.* 2005).

Other Parachela exhibit variations of the asymmetric (2121) claw branching pattern. In its least complex form, the asymmetric pattern is demonstrated by external and internal claws of relatively similar size and shape that have a rigid structure of basal section, secondary and primary branches, as found in the Calohypsibiidae Pilato 1969 (in Pilato 1969b) and Microhypsibiidae Pilato, 1998.

Ramazzotti and Maucci (1983) described the basic external hypsibid claw as: "*il ramo basale comune (più o meno lungo) si continua direttamente nel ramo secondario, mentre il ramo principale, spesso più lungo, sempre più sottile, è inserito sul ramo basale mediante una giunzione flessibile;*" «the common (more or less long) basal section is directly continuous to the secondary branch, while the, often longer, always more slender principal branch is inserted on the basal section by means of a flexible junction». *Hypsibius* and *Isohypsibius* claw types are different, the *Hypsibius*-type being described as a continuous curve from the bottom of the basal section to the tip of the secondary branch (Figure 3Dh) while the *Isohypsibius*-type forms a right-angle between basal section and secondary branch (Figure 3Di), which Ramazzotti and Maucci regarded as being unimportant (Ramazzotti and Maucci 1983, pp 41–42 and figure 11; Pilato and Binda 2010 pg 19). However, the Hypsibiidae also demonstrate two different claw pair patterns. In the first, both internal and external claws have an 'articulation' between the primary and secondary branches (the basal section and secondary branch form a solid unit with the primary claw articulating with the secondary branch either as a flexible hinge-like link or the branch can rotate, twist or fold along its proximo-distal plane). In the second, the external claw exhibits articulation while the internal claw has a rigid structure and forms a solid unit of a basal section to the secondary branch and primary branch. Molecular systematics groups *Isohypsibius*, *Thulinius* and *Halobiotus* together in a single clade (Sands *et al.* 2008b, figure 1). All three genera exhibit an *Isohypsibius*-type claw for external and internal claws. Pilato and Binda (2010) also described *Pseudobiotus* Nelson 1980 (in Schuster *et al.* 1980) and *Mixibius* Pilato, 1992 as having two *Isohypsibius*-type claws per leg. Sequences from these groups would make a good independent test of this character as diagnostic for a larger taxonomic grouping. The remaining members of the Hypsibiidae have external and internal *Hypsibius*-type claws. This character alone would reconcile taxonomy with molecular data.

A fourth asymmetrical (2121) claw type was recognised (Bertolani, 1981b), and now known as the *Bertolanius*-type or Eohypsibiidae-type, (*sensu* Pilato and Binda 2010), in which the external and internal claws are clearly delineated by septa into basal section, secondary branch and primary branch (Figure 3E). The angle between basal section, secondary branch and primary branches are different between claws on the same leg.

Case for the apophysis for the insertion of the stylet muscles (AISM) (Figure 4A–O). The shape of the apophysis for the insertion of the stylet muscles (AISM) was, until recently, a relatively undervalued eutardigrade taxonomic character, with very few papers including descriptions or illustrations to describe both dorso-ventral and lateral perspectives. The basic shape of the AISM, described as hooks, ridges or a combination of these, is relatively straight forward and, as an accepted taxonomic criteria (e.g. Bertolani *et al.* 1999; Nelson *et al.* 1999; Guidetti *et al.* 2005; Pilato and Binda 2010), is deemed constant within the genera showing very little inter-specific variation (Binda and Pilato 1986; Pilato 1987; Pilato and Binda 1987). However, detailed inspection of the variants on the basic hook- or ridge-shaped AISM, could identify trends. Thus, *Isohypsibius*, *Thulinius* and *Pseudobiotus* (genera we propose for inclusion in the Isohypsibioidea) show long ridges on both ventral and dorsal AISM, com-

bining the apophysis with a longitudinal thickening (Figure 4K–M; Pilato and Binda 2010, Fig 12c). *Hypsibius, Acutuncus* Pilato and Binda, 1997 and *Ramazzottius* (genera we propose for inclusion in the Hypsibioidea) show variants on a prominent hook shaped AISM followed by a less distinct longitudinal thickening (Figure 4A–C; Pilato and Binda 2010, Fig 12d). Macrobiotoidea have ridged AISM accentuated by the ventral lamina and have lateral caudal processes (Figure4H–I); the Eohypsibioidea also have ridged AISM, without ventral lamina, but with lateral caudal processes (Figure 4J).
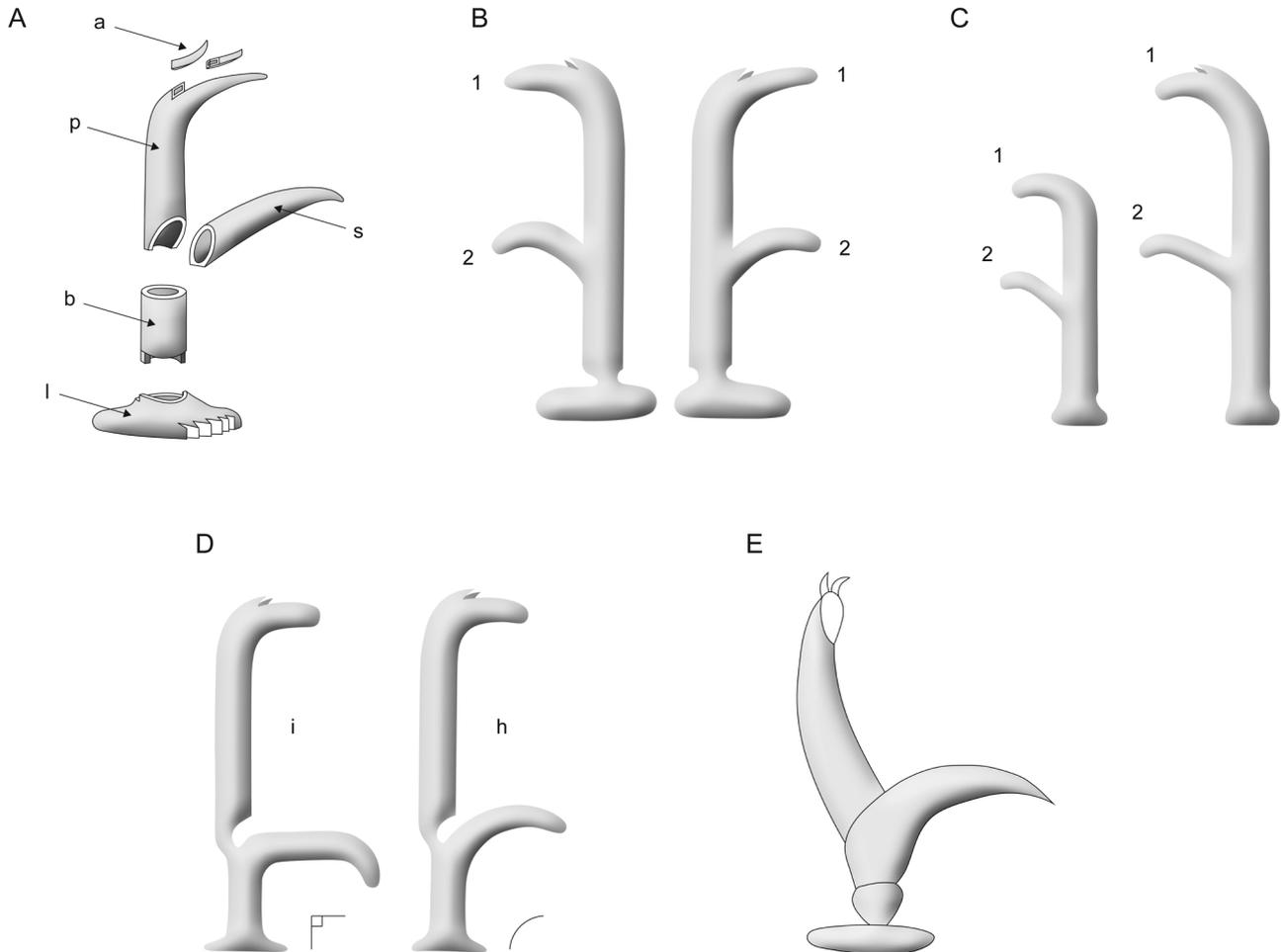

FIGURE 3. Diagrammatic representation of Parachela claw structure and nomenclature. A. Exploded diagram of a parachelan claw to explain component parts. a—accessory spines; b—basal tract; l—lunule (edge may be smooth or dentate); p—primary branch; s—secondary branch. B. Stylised diagram of macrobitid claw (2112). Secondary branch—2; primary branch—1; primary branch—1; secondary branch—2. C. Stylised diagram of a hypsibid claw (2121). Secondary branch—2; primary branch—1; secondary branch—2; primary branch—1. D . Stylised diagram of: i—isohypsibid (basal section and secondary branch at right angles) and h—hypsibid (basal section and secondary branch forming continuous arc) claws. E. Stylised diagram of Eohypsibiidae-type claw—claws are clearly delineated by septa into basal section, secondary branch and primary branch.

Balancing the two cases. Discussions in the early 1980's (Schuster *et al*. 1980, Pilato 1982) give contrasting accounts of the relative importance of claw structure versus buccal apparatus structure, though it is now necessary to accept that both sides have their merits and, as a result of data limitations, we should not dismiss one over the other. Both claw and buccal apparatus are primary characters, but it is also necessary to bear in mind the importance of environmental factors which may exert secondary (phenotypic) impacts on the shapes of both claws and buccal apparatus. Taxa from wet or more aquatic habitats, e.g. *Pseudobiotus*, *Thulinius*, *Macroversum* Pilato and Catanzaro, 1988 and *Dactylobiotus* Schuster, 1980 (in Schuster *et al*. 1980) tend to have longer more flexible claws, while edaphic (soil inhabiting) *Xerobiotus* Bertolani and Biserov, 1996, Calohypsibiidae, and Microhypsibi-idae have highly reduced claws. The buccal apparatus is similarly shaped by food preference. Predators (e.g. *Adorybiotus granulatus* (Richters, 1903), which use heavy musculature to propel a prey-penetrating stylet will require

larger, more robust AISM compared with the more delicate stylet of the algal/detritivore *Hypsibius dujardini* (Doyère, 1840). Therefore while ecological constraints can greatly modify some aspects of claw physiology, the signals affecting the buccal apparatus may be more conserved but are more difficult to interpret and describe.
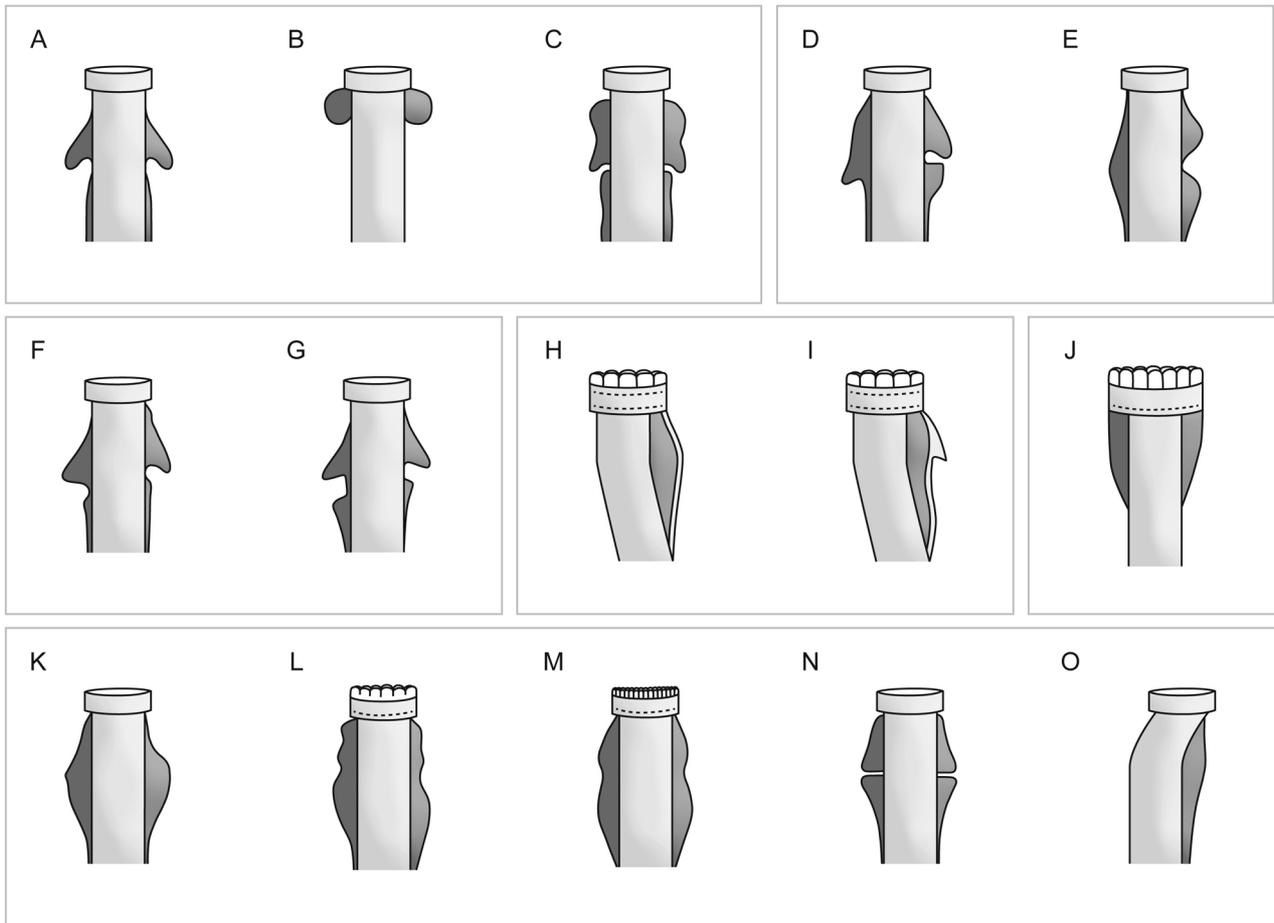

FIGURE 4. Stylised diagrams of the apophysis for the insertion of the stylet muscles (AISM) (in lateral view, left —dorsal, right—ventral). Hook shaped AISM of Hypsibioidea, Hypsibiidae, Hypsibiinae: A. *Hypsibius*, simple hooks; B. *Borealibius*, modified spherical hooks; C. *Acutuncus*, accentuated hooks. Modified hook shaped AISM of Hypsibioidea, Microhypsibiidae: D. *Microhypsibius*, accentuated hook and hook with ridge. Modified hook shaped AISM of Hypsibioidea, Calohypsibiidae: E. *Calohypsibius*, triangular hook and triangular hook with triangular ridge. Modified hook shaped AISM of Hypsibioidea, Ramazzottidae: F. *Hebesuncus*, asymmetric triangular and basic hook, G. *Ramazzottius*, asymmetric hooks. Ventral crest ASIM of the Macrobiotoidea, Macrobiotidae: H. *Macrobiotus*, asymmetric crest, 10 peribuccal lamellae present. Ventral crest ASIM of the Macrobiotoidea, Murrayidae: I. *Dactylobiotus*, asymmetric crest with hook, 10 peribuccal lamellae present. Ridge shaped AISM of Eohypsibioidea, Eohypsibiidae: J. *Eohypsibius*, Broad ridges widening mouth with 14 peribuccal lamellae. Ridge shaped AISM of Isohypsibioidea, Isohypsibiidae: K. *Isohypsibius*, simple triangular ridges, L. *Thulinius*, simple undulating ridges, 12 peribuccal lamellae present, M. *Pseudobiotus*, simple undulating ridges, 30 peribuccal lamellae present, N. *Mixibius*, modified ridges with small gap, and O . *Doryphoribius*, asymmetric crest ridge.

Taxonomic considerations

The Parachela can be subdivided into four groups, based on claw shape and AISM, that correspond with the four lineages (Figure 1B), which we propose as new superfamilies of Eohypsibioidea, Hypsibioidea, Isohypsibioidea and Macrobiotoidea.

Some elements do not fit the proposed superfamily groupings, i.e. Beornidae Cooper, 1964, Necopinatidae Ramazzotti and Maucci, 1983, and Apodibius Dastych, 1983 (in Dastych 1983b). Details for Beornidae are so poor as to preclude specific identification, while supporting molecular data remains unavailable. Bertolani and Grimaldi (2000) contended that the claws were similar to *Isohypsibius* group or *Dactylobiotus*, placing the Beornidae in

either Isohypsibioidea or Macrobiotoidea. Necopinatidae have a single cuticular tooth instead of two composite and branched claws, while *Apodibius* have no claws to help place them within the phylogeny. Molecular data would be pivotal in indicating the potential links of all three of these contentious taxa. Pilato and Binda (2010) placed *Apodibius* within the Necopinatidae but did not provide a rationale. We reject this apparent new familial position for *Apodibius*, which we still consider as *insertae sedis*.

The proposed higher taxa subdivisions within the Tardigrada systematic are shown in Figure 1B. However, the proposed superfamily designations require amendments to several taxonomic descriptions outlined below.

Amended Taxonomic descriptions

Class EUTARDIGRADA Marcus

Cephalic cirri absent; dorsal plates absent; claws composite and branched.

Order PARACHELA Schuster, Nelson, Grigarick and Christenberry

Cephalic papillae absent. Two, external and internal, claws per leg; each delineated into basal section, secondary branch and primary branch.

Superfamily: Eohypsibioidea, superfam. nov. Bertolani and Kristensen

Remarks. Under the International Code of Zoological Nomenclature (ICZN), Fourth Edition (1999), Rule 36.1 the Statement of the Principle of Coordination for family-group names applies to this new taxon and therefore the authorship and date will be Bertolani and Kristensen 1987.

Diagnosis. Parachela; claws asymmetric (2121); Eohypsibiidae-type claw pairs; apophysis for the insertion of the stylet muscles (AISM) ridged with lateral caudal processes of crests and hooks.

Type Family. Eohypsibiidae Bertolani and Kristensen, 1987

Composition. Eohypsibiidae

Etymology. Superfamilial name derived from the type genus *Eohypsibius* Kristensen, 1982.

Family: Eohypsibiidae, Bertolani and Kristensen

Diagnosis. Eohypsibioidea. Eohypsibiidae-type claws, which are clearly delineated by septa, in linear order, from basal section, secondary branch and primary branch. The angle between basal section, secondary branch and primary branches are different between claws on the same leg and the internal claw can rotate on its base by 180°.

Type genus. *Eohypsibius* Kristensen, 1982.

Composition. *Eohypsibius* and *Bertolanius* Özdikmen, 2008.

Etymology. Familial name derived from the type genus *Eohypsibius*.

Superfamily: Isohypsibioidea, superfam. nov.

Diagnosis. Parachela. Claws asymmetrical (2121); *Isohypsibius*-type claw pairs; AISM ridged.

Type family. Isohypsibiidae **fam. nov.**

Composition. Isohypsibiidae **fam. nov.**

Etymology. Superfamilial name derived from the type genus *Isohypsibius* Thulin, 1928.

Family: Isohypsibiidae fam. nov.

Remarks. With the establishment of the superfamily, Isohypsibioidea, a familial title is required under ICZN (1999) and we propose the familial name of Isohypsibiidae derived from the genus *Isohypsibius*.

At the core of this group *Isohypsibius, Thulinius* and *Halobiotus* directly match the clade clearly demonstrated in Kiehl *et al.* (2007), Sands *et al.* (2008b) and Figure 2. While the AISM of *Halobiotus* have been described as hooks (Pilato and Binda 2010), original descriptions and images suggest modified ridges with dorsal hook and ventral fenestra (Kristensen, 1982; Crisp and Kristensen, 1983). Pilato and Binda (2010) ascribe *Isohypsibius*-type claws to *Pseudobiotus* and *Doryphoribius* Pilato 1969 (in Pilato, 1969b), the former having a ridged AISM but the latter an elongated ventral ridge AISM extended into a ventral lamina. They also describe *Isohypsibius*-type claws and ridged AISM for *Eremobiotus* Biserov, 1992, which is aligned with *Isohypsibius* in Figure 2, and the elongated ridged AISM to *Ramajendas* Pilato and Binda, 1990, though this genus has a combination of *Isohypsibius*- and *Hypsibius*-type claws (Pilato and Binda 2010). We agree with these points and therefore include these genera within the new superfamily and family.

We are less certain as to the familial position of *Mixibius* (type species, formally *Isohypsibius saracenus* Pilato, 1973). This taxon was described as having modified *Isohypsibius*-type claws which would support moving it into the new family and superfamily. The AISM was however described as a modified hook (Pilato 1992, Pilato and Binda 2010), but other evidence suggests this modified hook is markedly different from the *Hypsibius* form and is, *sensu stricto*, actually a small gap between the apophysis and the elongated longitudinal thickening. We therefore consider the overall shape of the AISM to be a modified ridge rather than hook and tentatively place *Mixibius* in the Isohypsibiidae, but advocate only molecular evidence can confirm this position. Similarly, claw morphology would also place *Thalerius* Dastych, 2009 in this group, though AISM data are not yet available for this taxon.

We envisage further morphological and molecular work will be required to clarify the interrelationships between these seven (or eight) genera.

Diagnosis. Isohypsibioidea. Claw pairs of similar size and shape. External and internal claws exhibiting articulation (the basal section and secondary branch form a solid unit while the primary branch and secondary branch articulate). Claws *Isohypsibius*-type, forming a right-angle between basal section and secondary branch. AISM ridge-like.

Composition. Based on the criteria discussed above: *Isohypsibius* Thulin, 1928, *Doryphoribius* Pilato, 1969, *Pseudobiotus* Nelson, 1980 (in Schuster, Nelson, Grigarick and Christenberry 1980), *Thulinius* (Bertolani, 2003), *Halobiotus* Kristensen, 1982, *Ramajendas* Pilato and Binda, 1990, and *Eremobiotus* Biserov, 1992 (*Mixibius* Pilato, 1992 and *Thalerius* Dastych, 2009).

Etymology. As the *Isohypsibius*-type claw structure defines the superfamily, we propose the familial name of Isohypsibiidae, with the type genus of *Isohypsibius*.

Superfamily: Hypsibioidea superfam. nov. Pilato

Remark. Under the ICZN (1999), Rule 36.1 the Statement of the Principle of Coordination for family-group names applies to this new taxon and therefore the authorship and date will be Pilato 1969(a).

Diagnosis. Parachela; claws asymmetrical (2121); *Hypsibius*-type claw pairs; AISM hooked (or, if the buccal tube is elongated, AISM can be broad ridges).

Type family. Hypsibiidae Pilato, 1969(a).

Composition. Calohypsibiidae Pilato, 1969(a); Hypsibiidae Pilato, 1969(a); Microhypsibiidae Pilato, 1998; and Ramazzottidae **fam. nov.**

Etymology. Superfamilial name derived from the type family and genus *Hypsibius* Ehrenberg, 1848.

Family: Hypsibiidae Pilato <amended description>

Remarks. With the removal of the Isohypsibid types from this group the original familial description of the Hypsibiidae requires modification. Using Thulin (1928), Pilato (1969b; 1987) and Pilato and Binda 2010, this becomes:

Diagnosis. Hypsibioidea. The claw pairs are usually dissimilar; the external claw exhibiting articulation (the basal section and secondary branch form a solid unit with the primary claw articulating with the secondary branch) while the internal claw has a rigid structure (a solid unit of basal section, secondary branch and primary branch). Claws *Hypsibius*-type forming a continuous arc between basal section and secondary branch. AISM hook shaped or (if the buccal tube is elongate) broad ridges.

Type genus. *Hypsibius* Ehrenberg, 1848 (amended by Thulin, 1928).

Composition. Subfamilies Hypsibiinae Pilato, 1969(a), Diphasconinae Dastych, 1992 and Itaquasconinae Rudescu, 1964.

Etymology. Family name derived from the type genus *Hypsibius*.

Subfamily: Hypsibiinae Pilato (sensu Pilato and Binda 2010 amended)

Remark. With the removal of the Isohypsibid types the group composition has changed.

Diagnosis. Hypsibiidae. Buccal-pharyngeal apparatus of the Hypsibiinae model (see Pilato and Binda 2010).

Type genus. *Hypsibius* Ehrenberg, 1848.

Composition. Three genera are ascribed to the subfamily: *Acutuncus* Pilato and Binda, 1997, *Borealibius* Pilato, Guidetti, Rebecchi, Lisi, Hansen and Bertolani, 2006, and *Hypsibius*.

See Pilato and Binda (2010) for descriptions of Diphasconinae and Itaquasconinae.

Family*: Ramazzottidae* fam. nov.

Remarks. Another group requiring familial status comprises the genera *Hebesuncus* and *Ramazzottius*, which molecular data highlights as a distinct, well supported clade (Kiehl *et al*. 2007; Sands *et al*. 2008b), and which we propose elevating to a familial rank of Ramazzottidae **fam. nov.**, type genus *Ramazzottius* Binda and Pilato, 1986. Both *Hebesuncus* and *Ramazzottius* have the Parachela asymmetrical double-claws (2121) with a variant on the *Hypsibius*-type claw, often referred to as *Ramazzottius*-type claw. *Ramazzottius* has the more extreme variant, where the external claw has a long basal section forming an arc with the secondary branch and a long, thin, straight primary branch. The external claws of *Hebesuncus* are less extreme. However, the AISM of both genera form blunt hooks, the dorsal being different from the ventral apophysis causing slight asymmetry with respect to the frontal plane (Binda and Pilato 1986; Pilato 1987; Pilato and Binda 2010). In addition, both genera deposit eggs with a sculptured chorion, though this may not be a definitive familial character. The elongated buccal-pharyngeal tube of *Hebesuncus* is, in our opinion, of lesser systematic importance as it only defines the genus (see Kristensen 1987 vis-à-vis the Echiniscidae).

The presence in *Ramazzottius* of the cephalic elliptical dorso-lateral sense organs could be symplesiomorphic with similar organs in *Calohypsibius* Thulin, 1928, *Paradiphascon* and *Halobiotus*. These structures are possible analogues of the Apochela cephalic papillae (Prof. R.M. Kristensen, pers. comm.; see also Wiederhöft and Greven 1996; 1999); though their function(s) in the Parachela remain unclear.

Diagnosis. Hypsibioidea. The AISM comprises asymmetric, dissimilar dorsal and ventral "blunt hooks". Claw pairs asymmetric (2121), external claw primary branch joins the secondary claw and basal section with flexible junction; primary branch may be very long and slender; the internal claw branches and basal section unified into a single rigid element. Eggs have a sculptured chorion and are deposited free in the environment.

Type genus. *Ramazzottius* Binda and Pilato, 1986.

Composition. *Ramazzottius*, *Hebesuncus* Pilato, 1987.

Etymology. Family name derived from the generic name *Ramazzottius* Binda and Pilato, 1986.

Superfamily: Macrobiotoidea, superfam. nov.

Remark. Under the ICZN (1999) Rule 36.1 the Statement of the Principle of Coordination for family-group names applies to this new taxon and therefore the authorship and date will be Thulin 1928.

Diagnosis. Parachela; claw pairs symmetrical (2112); AISM generally asymmetrical, due to the ventral lamina, with lateral caudal processes of crests and hooks.

Type family. Macrobiotidae Thulin, 1928.

Composition. Macrobiotidae and Murrayidae (Guidetti, Rebecchi and Bertolani, 2000).

Etymology. Superfamilial name derived from the type family Macrobiotidae Thulin, 1928.

Conclusion

New molecular data presented us with the opportunity to re-assess the morphological characters that form the basis of familial, and higher, taxonomy within the Eutardigrada. We present two new families and four new superfamilies to encompass (with few exceptions) all the familial taxa of the Parachela. This major revision of the higher taxa within the Eutardigrada is based on combinations of structural differences in both claws and the apophysis for the insertion of the stylet muscles (AISM) where we recognise three principal characters. First, paired claw branch symmetry (2112) *versus* asymmetry (2121); second a basal claw section rigidly joined to secondary and primary branches *versus* a rigid basal section/secondary branch and a flexible primary branch; third, the development of ridge- or hook-like AISM, which may be modified *via* a ventral lamina.

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