Ongoing revision of Echiniscoididae (Heterotardigrada: Echiniscoidea), with the description of a new interstitial species and genus with unique anal structures

NADJA MØBJERG[1*,•], ASLAK JØRGENSEN[1,•] and REINHARDT M. KRISTENSEN[2,•]

[1]*Department of Biology, August Krogh Building, University of Copenhagen, Denmark*
[2]*The Natural History Museum of Denmark, University of Copenhagen, Denmark*

Marine tidal heterotardigrades (Echiniscoididae) have gained increasing interest owing to their unique adaptations and evolutionary position, bridging marine and limnoterrestrial taxa. Echiniscoididae was established to accommodate the marine genera *Anisonyches* and *Echiniscoides*. However, it has become apparent that *Anisonyches*, with its claw configuration, median cirrus and seminal receptacles, clearly has little or no affinity to tidal echiniscoidids with supernumerary claws. Consequently, we establish **Anisonychidae fam. nov.** to accommodate *Anisonyches* in a paraphyletic Arthrotardigrada and discuss its affinity to other heterotardigrade taxa. We recently split *Echiniscoides* into *Isoechiniscoides* and *Echiniscoides* s.l. The latter remains a miscellany of species complexes and undescribed genera, and it has become evident that a larger number of echiniscoidids belonging to *Echiniscoides*, *Isoechiniscoides* and undescribed genera coexist in intertidal sediments. Here, we erect **Neoechiniscoides aski** gen. nov., sp. nov. from Roscoff, France, which has a unique anal system, characterized by prominent lateral lobes with a set of wing-like structures. Phylogenetic analyses based on *COI* sequences infer a close relationship between *N. aski*, an undescribed species from Roscoff and unidentified species from Maine, USA. We propose that the new genus includes the former *Echiniscoides* species *Echiniscoides pollocki* and *Echiniscoides horningi*, which we hereby transfer.

ADDITIONAL KEYWORDS: *Anisonyches* – *COI* – marine – morphology – *Neoechiniscoides* – species complexes – Tardigrada – tidal.

INTRODUCTION

Tardigrades (phylum Tardigrada) are divided into two major evolutionary lineages represented by the monophyletic clades Eutardigrada (including the newly erected Apotardigrada) and Heterotardigrada (Guil *et al.*, 2019). At present, the diverse Heterotardigrada is composed of two orders, Echiniscoidea Richters, 1926 and Arthrotardigrada Marcus, 1927, but recent molecular studies have demonstrated that the highly successful marine, limnic and semiterrestrial Echiniscoidea evolved from the marine and thus paraphyletic Arthrotardigrada (Jørgensen *et al.*, 2010; Guil & Giribet, 2012; Guil *et al.*, 2013; Bertolani *et al.*, 2014; Fujimoto *et al.*, 2016; Jørgensen *et al.*, 2018).

Here, we focus on an ongoing revision of echiniscoidean tardigrades, which currently comprise four families, i.e. the limnoterrestrial Echiniscidae Thulin, 1928, the marine Echiniscoididae Kristensen & Hallas, 1980, the limnoterrestrial Oreellidae Ramazzotti, 1962 and the limnic Carphanidae Binda & Kristensen, 1986.

The marine family Echiniscoididae was originally established by Kristensen & Hallas (1980) to accommodate the genera *Anisonyches* Pollock, 1975 and *Echiniscoides* Plate, 1889, with the latter including two species: *Echiniscoides hoepneri* Kristensen & Hallas, 1980 and the cosmopolitan *Echiniscoides sigismundi* (M. Schultze, 1865). However, it has become apparent that *Anisonyches* (see Pollock, 1975), with its unique claw configuration, median cirrus and seminal receptacles, does not belong to Echiniscoididae (Kristensen *et al.*, 2012; Fujimoto *et al.*, 2016; Møbjerg *et al.*, 2016). Grimaldi de Zio *et al.* (1987) noted the erroneous systematic position of *Anisonyches*, but

the issue was not reinvestigated, and *Anisonyches* was not removed from Echiniscoididae (see Chang & Rho, 1998). Below, we erect **Anisonychidae fam. nov.** to accommodate *Anisonyches* in the paraphyletic Arthrotardigrada and discuss its affinity to other heterotardigrade taxa.

Currently, the genus *Echiniscoides* consists of eight species, with *E. sigismundi* divided into eight subspecies (Kristensen & Hallas, 1980; Guidetti & Bertolani, 2005; Degma *et al.*, 2019; Perry *et al.*, 2018). Notably, the extensive molecular variation described by Faurby *et al*. (2011, 2012) and Faurby & Barber (2015) indicates that *Echiniscoides* s.l. is composed of a much larger number of distinct clusters that represent species or genera. Faurby *et al.* (2012) argued that these clusters represent cryptic species complexes, i.e. multiple evolutionary lineages, with almost identical morphology. However, our recent investigation shows that morphological variation is indeed present (Møbjerg *et al.*, 2016).

From *Echiniscoides*, the former *Echiniscoides higginsi* group was recently removed, for which the subfamily Isoechiniscoidinae Møbjerg et al., 2016 was established. Using integrative data from morphology and molecular analysis, Isoechiniscoidinae was identified, with *Isoechiniscoides* Møbjerg et al., 2016 as the type genus. Isoechiniscoidinae consists of interstitial species with isonych claws and pillars in the epicuticle, and the species differ considerably from the members of the genus *Echiniscoides*. Specifically, our analyses revealed that isoechiniscoid tardigrades form a sister-group relationship with all other taxa in Echiniscoididae (Møbjerg *et al.*, 2016). We also presented molecular data from seven- or eight-clawed, undetermined and morphologically undescribed species belonging to the *E. sigismundi* complex collected from the lichen *Lichina pygmaea* (Lightf.) C.Agardh and interstitially from sand. In our inferred *COI* phylogeny, these specimens were positioned among specimens from Maine, USA (Møbjerg *et al.*, 2016). In the study by Faurby *et al.* (2011), these Maine specimens were found to represent evolutionarily independent units related to specimens from the Azores (Portugal), Roscoff and Le Croisic (both in France). Here, we erect a new genus based on molecular analyses and thorough morphological investigation of the above-mentioned new interstitial species. Below, we provide a brief outline of morphological characters traditionally used in echiniscoidid taxonomy.

The morphological characters traditionally used to differentiate species of *Echiniscoides* include epicuticular pillars, number of claws, size and shape of cirri and clavae, presence and shape of dorsal mouth plates, the buccal tube, leg sense organs and the anal system. Epicuticular pillars were used in the original *Echiniscoides* taxonomy (Hallas & Kristensen, 1982),

but with the removal and generic elevation of the former *E. higginsi* group to *Isoechiniscoides*, the remaining *Echiniscoides* species lack epicuticular pillars. Cuticular sculpture is variable in *Echiniscoides*, from fine punctuation or slightly wrinkled to circular elements arranged like roofing tiles (Kristensen & Hallas, 1980). Cuticular sculpture was used to differentiate between subspecies by Kristensen & Hallas (1980); these subspecies probably represent species, but cuticular sculpture has not yet been compared with molecular data. The number of claws is supernumerary, but highly variable from seven to 13 claws in adult *Echiniscoides*. Generally, the fourth pair of legs has one less claw than the first three leg pairs (Kristensen & Hallas, 1980). It is currently unclear how decisive claw number is with regard to species status, because the number of claws has not yet been coupled to molecular data, with the notable exception of the six-clawed *Isoechiniscoides* species (Møbjerg *et al.*, 2016). Within echiniscoidid tardigrades, primary clavae range from oval to spiny-tip papillae and, notably, they are often similar to the sensory organ on the fourth leg (p4). Papilla cephalica (secondary clavae) are generally dome shaped and of variable size. However, in *Echiniscoides horningi* Miller & Kristensen, 1999 they are split into a double structure that might represent secondary and tertiary clavae (Miller & Kristensen, 1999). Cirri represent good taxonomic characters within *Echiniscoides* and range in shape from cones to elongated spines with fibres (tufts) protruding from the terminal opening. The median cephalic cirrus is always lacking in echiniscoidids. Dorsal mouth plates can be missing, but are then represented by ring-like structures (*E. sigismundi* and *E. hoepneri*). In *E. horningi*, one dorsal mouth plate is present, whereas in *Isoechiniscoides sifae* Møbjerg et al., 2016 two plates are present. The presence of a flexible buccal tube has recently been used by Perry & Miller (2015) to describe *Echiniscoides wyethi* Perry & Miller, 2015. Leg sense organs are important taxonomic structures, with a range of different sizes and shapes. The leg sense organs (p1) on the first pair of legs can be absent or dome shaped, the sense organ (p2) on Leg II can range from dome shaped to spine shaped, the sense organ (p3) on Leg III is always spine shaped (pointed papillae) with minor differences in length, and the sense organs (p4) on the fourth leg pair often look like the primary clavae. The anal system has not previously been used as a character of phylogenetic importance within Echiniscoididae, although Hallas & Kristensen (1982) used the presence of cuticular anal flaps (here referred to as 'wings') as a distinguishing characteristic for *Echiniscoides pollocki* Hallas & Kristensen, 1982.

In the present study, we use the structure of the anal system as the morphological basis for erecting a new

genus, *Neoechiniscoides*, and we consequently compare this system with the anal systems of *Isoechiniscoides* and distinct members of *Echiniscoides*.

MATERIAL AND METHODS

SAMPLING AND MICROSCOPY

Material of *Anisonyches* was available from the collection at the Zoological Museum (ZMUC), Natural History Museum of Denmark, mounted on microscope slides. We have investigated a specimen of *Anisonyches eleutherensis* Bartels et al., 2018 from Bermuda (ZMUC-TAR 1204) and three specimens of *Anisonyches diakidius* Pollock, 1975 collected by J. Renaud-Mornant in 1979 from Guadeloupe (ZMUC-TAR 1200–1202). The holotype of *A. diakidius* was recently re-examined by Bartels *et al.* (2018).

Sediments consisting of sand mixed with stone pebbles were collected at L'Aber de Roscoff, France during low tide in the summers of 2011, 2012 and 2013 and in April 2019. Specimens of *I. sifae* and an undescribed species of *Neoechiniscoides* (reported as *Echiniscoides* sp. B by Møbjerg *et al.*, 2016) were extracted from the sediment using freshwater shocking and subsequent sieving through a 30 μm net (Jørgensen *et al.*, 1999). Additionally, specimens of a genetically similar *Neoechiniscoides* species (reported as *Echiniscoides* sp. A by Møbjerg *et al.*, 2016) were collected from the lichen *L. pygmaea*. Owing to the lack of specimens for thorough morphological analyses, this last species will not be dealt with here in further detail. After extraction, the tardigrades were transferred to seawater from the locality and stored at 5 °C (Halberg *et al.*, 2013). Living specimens were observed and photographed (using up to ×40 objectives) and either transferred to aldehyde fixatives for morphology (permanent mounts and scanning electron microscopy) or used for DNA extractions.

Four specimens of the new species, described below as *Neoechiniscoides aski*, were used for DNA extractions (see Møbjerg *et al.*, 2016: *Echiniscoides* sp. B), and a total of nine specimens have been mounted in glycerol on microslides. Permanent slides were photographed using a DP20 camera on an Olympus BX51 compound microscope with differential interference contrast optics. At present, seven specimens of the new species have been processed and analysed via scanning electron microscopy, conducted according to Halberg *et al.* (2013) and Hygum *et al.* (2016). Twenty-six slides of *E. pollocki* (Rhode Island, USA) and 12 specimens of *E. horningi* (Macquarie Island, sub-Antarctic) from the Reinhardt Møbjerg Kristensen collection at the Natural History Museum of Denmark, University of Copenhagen were consulted for the morphological analyses.

SEQUENCE ALIGNMENT, PHYLOGENETIC INFERENCE AND GENETIC VARIATION

All available *COI* sequences for *Isoechiniscoides* and *Echiniscoides* in GenBank were aligned with MUSCLE (Edgar, 2004). The full phylogeny using *COI* is presented in the Supporting Information (Fig. S1). Compared with our recent study (Møbjerg *et al.*, 2016), the present analyses used a more closely related outgroup (*Isoechiniscoides* instead of *Milnesium* Doyère, 1840 and *Echiniscus* C.A.S. Schultze, 1840), a conservatively trimmed nucleotide matrix of the *COI* sequences and an analysis with the exclusion of the variable third codon position nucleotide to avoid extensive homoplasy. Specifically, 261 *COI* sequences were trimmed to 600 bp, and phylogenetic analyses using Bayesian (MrBayes v.3.2; Ronquist *et al.*, 2012) and maximum likelihood (MEGA7; Kumar *et al.*, 2016) inferences were conducted, with *Isoechiniscoides* as the outgroup taxon. Maximum likelihood analyses conducted with RAxML Blackbox (Stamatakis *et al.*, 2008) and MEGA gave similar topologies and bootstrap support. In MrBayes, the Bayesian inference analyses were conducted with the GTR+I+G substitution model and run for 5 000 000 replicates, with a sample frequency of 100 and 10 000 trees discarded as burn-in. In MEGA, the maximum likelihood analyses were conducted with the Nearest-Neighbor-Interchange heuristic method, using the GTR+I+G substitution model. The branch support in the maximum likelihood analyses was evaluated with 1000 bootstrap replicates. In a second analysis in MEGA, the third codon position was excluded from the maximum likelihood analysis to investigate the effect of substitutional saturation on unsupported deeper branches. Uncorrected p-distances with pairwise deleted alignment gaps were calculated in MEGA to investigate the genetic distances in *COI* between the *Echiniscoides* taxa most closely related to the new genus, *Neoechiniscoides*. In MEGA, the transition/transversion bias was calculated to investigate substitutional saturation in *COI* that might affect the resolution of deeper nodes.

For the nuclear gene *28S*, which is a more slowly evolving gene than *COI*, the data matrix consisted of 36 taxa and 650 nucleotides, with *Isoechiniscoides* as the outgroup (see Supporting Information, Fig. S2). Phylogenetic inference was conducted with the same settings as in the *COI* analyses.

RESULTS

In our ongoing revision of Echiniscoididae, we have recently described *Isoechiniscoides* and erected the subfamily Isoechiniscoidinae for it (Møbjerg *et al.*, 2016). Thus, Echiniscoididae currently comprises

three genera, i.e. *Anisonyches*, *Echiniscoides* and *Isoechiniscoides*. However, this represents an outdated classification, because *Anisonyches* species have a median cirrus, seminal receptacles and a unique claw configuration, showing a closer phylogenetic relationship with groups of the paraphyletic Arthrotardigrada than with Echiniscoidea (Grimaldi de Zio *et al*., 1987; Kristensen *et al*., 2012; Fujimoto *et al*., 2016; Møbjerg *et al*., 2016; Bartels *et al*., 2018; Fig. 1). Notably, two cuticular seminal receptacles, as found in all species of *Anisonyches*, are typical for female arthrotardigrades, and seminal receptacles are never present in echiniscoidids, i.e. *Echiniscoides*, *Isoechiniscoides* and *Neoechiniscoides*. In Echiniscoidea, the family Oreellidae also possesses seminal receptacles (Kristensen, 1987), clearly opening a future discussion on the affinity of this family. Here, we suggest that *Anisonyches* is removed from Echiniscoididae and that a new family, Anisonychidae, is established to accommodate *Anisonyches*. We place this new family in the order Arthrotardigrada, but at the same time emphasize that Arthrotardigrada is currently paraphyletic (or perhaps even polyphyletic) and thus in need of a revision. We also provide an amended diagnosis of Echiniscoididae and formally erect subfamily Echiniscoidinae. Within the latter, we erect a new genus and species, *Neoechiniscoides aski*.

TAXONOMIC ACCOUNTS

ORDER ARTHROTARDIGRADA MARCUS, 1927

FAMILY **ANISONYCHIDAE** MØBJERG, JØRGENSEN & KRISTENSEN **FAM. NOV.**

lsid:zoobank.org:act:6A303AC8-EFA1-4639-BDE8-CB5CB32813EB

Diagnosis: Unplated, interstitial, marine heterotardigrades. Four claws inserted directly on each of the first three leg pairs, and three claws inserted directly on the fourth pair of legs (in adults). Paired basal hooks on all claws, with the two hooks oriented at right angles to one another. Median cephalic cirrus is always present, but can be reduced. Cephalic appendages include internal and external cirri, lateral cirri (cirri A) and generally also primary clava and flat, often indistinct, secondary clava. Cirri E is always present. Papillae on leg IV are often present, and sensory structures on legs I–III may also be present (presently seen in only one species, *Anisonyches mauritianus* Grimaldi de Zio, D'Addabbo Gallo, Morone De Lucia & D'Addabbo, 1987). Black eyes are present. Stylets are long, with stylet sheaths; stylet supports are

lacking. The three placoids are $CaCO_3$ incrusted. Paired seminal receptacles are present in females. The coiled receptacles open relatively distant from the six-lobed female gonopore. The anal system consists of two lateral lobes connected by a median zig-zag structure, with the anus formed by a small terminal pore.

Type genus: Anisonyches Pollock, 1975.

Generic diagnosis (amended): The same diagnosis as for the family.

Type species: Anisonyches diakidius Pollock, 1975.

Other species in the genus: Anisonyches mauritianus Grimaldi de Zio, D'Addabbo Gallo, Morone De Lucia & D'Addabbo, 1987; *Anisonyches deliquus* Chang & Rho, 1998; and *Anisonyches eleutherensis* Bartels, Fontoura & Nelson, 2018.

Discussion: For the present study, we have examined three specimens of the type species, *A. diakidius* (Fig. 1A–E). These specimens (ZMUC-TAR 1200–1202) were collected by J. Renaud-Mornant in 1979 from Guadeloupe (French Antilles) and sent to our museum along with a drawing of one of the specimens (Fig. 1F). Furthermore, we have examined a specimen of *A. eleutherensis* from the Bahamas, present in our collection (ZMUC-TAR 1204), with a distinct *Anisonyches* claw configuration as described by Bartels *et al.* (2018). From our recent investigations, and that of colleagues (Bartels *et al.*, 2018), it is apparent that *Anisonyches*, with its seminal receptacles (Fig. 1B), median cirrus (Fig. 1C) and unique claw configuration (Fig. 1D), does not belong among the echiniscoidids. Consequently, based on the presence of a median cephalic cirrus and seminal receptacles, we place *Anisonyches* in its own family, Anisonychidae, in the order Arthrotardigrada, but at the same time strongly emphasize that Arthrotardigrada is paraphyletic and thus in need of taxonomic revision. We note that the claw configuration of *Anisonyches* bears some resemblance to the limnic Carphanidae Binda & Kristensen, 1986 currently retained in Echiniscoidea. Within Echiniscoidea, the family Oreellidae has retained seminal receptacles as in *Anisonyches*, but the claw configuration in *Oreella* is different from *Anisonyches* and *Carphania* with regard to the position of the basal hooks, putative indications of toes and four claws on all legs (Binda & Kristensen, 1986). Here, we emphasize the fact that both caphanids and oreellids lack a median cirrus. Grimaldi de Zio *et al.* (1987) suggested that *Anisonyches* is more closely related to the arthrotardigrade taxa Coronarctidae or Stygarctidae than to Echiniscoididae. We note that claws
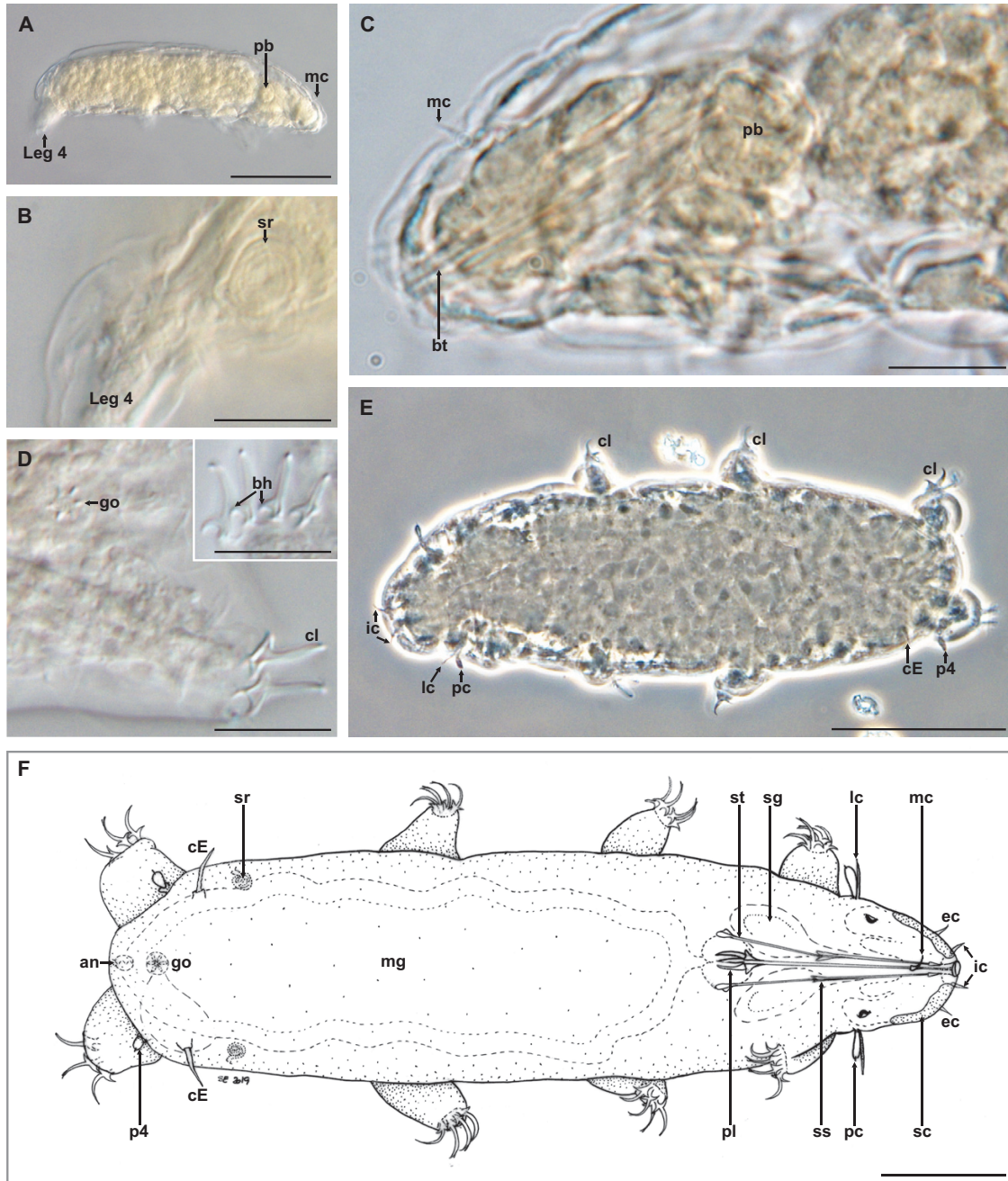
Figure 1. Light microscopy and habitus drawing of *Anisonyches diakidius* from Guadeloupe collected by Jeanne Renaud-Mornant, 1979 and deposited at the Natural History Museum of Denmark (ZMUC-TAR 1200–1202). A, lateral view of a female (ZMUC-TAR 1202) with indistinct median cirrus. Scale bar: 50 µm. B, close-up of seminal receptacle of the same female (ZMUC-TAR 1202). Lateral view. Scale bar: 10 µm. C, close-up of head region (ZMUC-TAR 1202) revealing the buccal tube, the thin median cirrus and the pharyngeal bulb. Scale bar: 10 µm. D, ventral view (ZMUC-TAR 1201) of female gonopore and two of the claws of the fourth leg. The inset shows the claws of the second leg. Note the basal hook of the claws. Scale bars: 10 µm. E, dorsal view of female (ZMUC-TAR 1200) showing various sensory structures and claws. Scale bar: 50 µm. F, habitus drawing of female (ZMUC-TAR 1202) in dorsal position, before it moved into a lateral position, made by J. Renaud-Mornant in 1979, redrawn by S. Elle in 2019. Scale bar: 20 µm. Abbreviations: an, anus; bh, basal hook of claw; bt, buccal tube; cE, cirrus E; cl, claw; ec, external cirrus; go, gonopore; ic, internal cirrus; lc, lateral cirrus (cirrus A); Leg 4, terminal leg; mc, median cirrus; mg, midgut; p4, sensory papilla of fourth leg; pb, pharyngeal bulb; pc, primary clava; pl, placoid; sc, secondary clava, sg, stylet gland; sr, seminal receptacle; ss, stylet sheath; st, stylet.

inserted directly on the leg is a characteristic of both Coronarctidae and Stygarctidae, clearly underlining the need for thorough future investigations into limnic and marine heterotardigrades.

ORDER ECHINISCOIDEA MARCUS, 1927

FAMILY ECHINISCOIDIDAE KRISTENSEN & HALLAS, 1980

Diagnosis (amended): Unplated, marine Echiniscoidea. Adults with supernumerary claws (six to 13) on each leg. Secondary clava (papilla cephalica) dome shaped or almost flat (indistinct). Median cephalic cirrus always absent. Other cephalic appendages and leg appendages small (reduced). Cirri A and E similar in both structure and length. The fourth leg sensory structure is papillar, as is the primary clava. Stylet sheath present; stylet supports always absent. Seminal receptacles lacking. External fertilization of the smooth eggs. Anal system consists of two lateral lobes and a terminal lobe. The anal opening is located in front of the terminal lobe.

Type genus: Echiniscoides Plate, 1889.

Discussion: The family Echiniscoididae consists of two subfamilies: Echiniscoidinae and Isoechiniscoidinae. The presence of supernumerary (six to 13) claws in adults is not seen in any other heterotardigrades. Seminal receptacles are never present in Echiniscoididae, but within Echiniscoidea seminal receptacles are found in *Oreella* Murray, 1910 (Binda & Kristensen, 1986; Dastych *et al.*, 1998).

SUBFAMILY ECHINISCOIDINAE MØBJERG, KRISTENSEN & JØRGENSEN, 2016

Diagnosis (amended): Unplated, marine Echiniscoidea. Adults with supernumerary anisonych claws on each leg. Secondary clava (papilla cephalica) dome shaped or almost flat (indistinct). Other cephalic appendages and leg appendages small (reduced). Cirri A and E similar in both structure and length. First leg sensory structure absent or small and dome shaped; a seta may be present on the second leg. The third leg sensory structure is a pointed papilla or seta. The fourth leg sensory structure is papillar, as is the primary clava. Epicuticular pillars are absent. Seminal receptacles are lacking. Fertilization of eggs is external. The anal system is relatively large and can be seen from the dorsal side as two large, nearly terminal lateral lobes. The terminal anal lobe is relatively small.

GENUS *NEOECHINISCOIDES* MØBJERG, JØRGENSEN & KRISTENSEN **GEN. NOV.**

(FIGS 2–7)

urn:lsid:zoobank.org:act:8FA86B31-39B9-4523-AFAF-E0DB7DE91562:

Diagnosis: Primarily interstitial Echiniscoidinae, with seven to eight, occasionally six or nine, anisonych claws (generally more than six claws on each leg in adults). Epicuticular pillars absent. The dorsal cuticle is strongly sculptured. Cirri A and E are present and relatively short in both sexes. First and second leg sensory structures are small and dome shaped. The third leg sensory structure is a seta, and the fourth leg sensory structure is papillar, as is the primary clava. Secondary clavae (cephalic papilla) are more distinct and appear relatively larger in males than in females. Cephalic papillae may be subdivided into secondary and tertiary clavae. Seminal receptacles are lacking. The anal system appears large, with two wings associated with the lateral lobes. The distance between the anus and the gonopore is larger in females than in males. A small ventral cuticular plate is present in front of the female gonopore (not observed in *N. pollocki*).

Etymology: From the Greek νέος, neos (new or young) combined with the generic name *Echiniscoides*.

Type species: Neoechiniscoides aski; described below.

Other members of the genus: Neoechiniscoides horningi (Miller & Kristensen, 1999) **comb. nov.** (basionym: *Echinsicoides horningi* Miller & Kristensen, 1999), sub-Antarctic (Macquarie Island), collected from the lichen *Caloplaca* sp. on rock in lower supralittoral zone. *Neoechiniscoides pollocki* (Hallas & Kristensen, 1982) **comb. nov.** (basionym: *Echiniscoides pollocki* Hallas & Kristensen, 1982), Rhode Island, USA, found interstitially, from tidal sediments.

Differential diagnosis of the genus: Neoechiniscoides leg sense organs are of the *Echiniscoides sigismundi* type, i.e. p1 and p2 are dome shaped, p3 is a short setae, and p4 is similar to the primary clava, consisting of a small papilla with a spiny tip. Cirri (internal, external, A and E) are shorter in *Neoechiniscoides* (range unadjusted for body size 3.0–8.7, 3.0–7.6, 10.0–17.0 and 10.0–18.0 µm, respectively) compared with *Isoechiniscoides* (range unadjusted for body size 7.3–12.0, 6.6–9.7, 14.5–21.0 and 24.0–33.0 µm, respectively), but generally longer than cirri of *Echiniscoides*. Specifically, cirri are longer in *N. aski* and *N. pollocki* than in *Echiniscoides*, but *N. horningi* falls within the

range of *Echiniscoides*, which has an internal, external, A and E cirri length unadjusted for body length of 3.1–4.8, 2.2–3.8, 8.3–13.0 and 7.5–12.0 μm, respectively, according to Kristensen & Hallas (1980). The anal system (Fig. 6) of *Neoechiniscoides* differs from that of *Echiniscoides* and *Isoechiniscoides*, specifically in the paired wings associated with the lateral lobes of the anus (wl in Figs 2, 4). Using light microscopy, the lateral anal lobes thus appear as a double structure. In scanning electron microscopy, these anal lobes appear as a double swollen structure, which can be observed when viewed dorsocaudally. Interestingly, during sampling we observed that the movements of the new interstitial *Neoechiniscoides* species were more similar to slowly moving eutardigrades than to other members of Echiniscoididae. Notably, specimens of the new species, *N. aski*, were highly responsive to freshwater shocking, which resulted in a fast gain of body water and associated swelling.

Generic discussion: The molecular phylogenetic investigation of Echiniscoididae reveals several possibly undescribed taxa. Here, we establish the new genus *Neoechiniscoides*, with its members morphologically clearly distinguishable from the other taxa in Echiniscoididae. The wing structure of the lateral anal lobes is not present in other heterotardigrades. We acknowledge that a cautious approach should be taken to morphological structures with regard to their significance at a specific taxonomic level at this early stage of echiniscoid revision. Hence, characteristics of the anal system might, in the future, have significance at a higher taxonomic level.

DESCRIPTION OF THE TYPE SPECIES

NEOECHINISCOIDES ASKI MØBJERG, JØRGENSEN & KRISTENSEN **SP. NOV.**

Diagnosis: Interstitial, medium-sized *Neoechiniscoides* with six to nine claws on each leg and 8,8,8,7 as the most common claw formula. Large black to dark brown eyes. Dorsal cuticle with star-like, polygonal structures arranged in transverse rows that continue ventrally on the head. Abdomen with a smooth cuticle, but polygonal structures are present on lateral anal lobes. The mouth is subterminal and lacks dorsal mouth plates; instead, three circular structures are present surrounding the mouth opening. The stylets and buccal tube are relatively short. Stylet supports are not observed using light microscopy. Calcium carbonate incrustations are found only in the anterior part of the pharyngeal placoids. Cirrus A and E have a single spiral sculpture at the basis. Internal and external cirri have swollen bases. Spine-shaped setae

are present on leg III. Legs I and II have small dome-shaped sensory organs, and leg IV has a papilla similar to the primary clava. Females have a flat and males a more raised, nearly papillary secondary clava. The male gonopore is a five-lobed, nearly penis-like structure (not ovoid). The female gonopore is a six-lobed rosette located on a ring-shaped protrusion. A small ventral plate is present in front of the female gonopore, and two small depressions (interpreted as muscle attachment sites) are present lateral to the female gonopore. Males lack this ventral plate; the muscle attachments are present, but placed more anteriorly with respect to the male gonopore. Both sexes have enlarged lateral anal lobes (neochiniscoid anal system).

Etymology: Named for Ask Møbjerg Jørgensen, who in turn is named for the Nordic mythological 'Tree of Life', Ask Yggdrasil.

Holotype (Figs 2D, F, 4; Table 1)*:* Female, 301 μm long, with mature ovary (three oocytes) and well-developed six-lobed gonopore. The animal is in moult. Deposited at The Natural History Museum of Denmark (NHMD-230293).

Type locality: Collected 17 July 2013 in the L'Aber de Roscoff, Brittany, France (48°43′17.251″N, 04°00′26.522″W) by Nadja Møbjerg and Aslak Jørgensen. Collected from oxic, sandy sediment with few stones.

Allotypic paratype (Figs 2E, 5; Table 1)*:* Male, 228 μm long, with large secondary clavae, mature spermatozoa in the seminal vesicles and well-developed papillary gonopore. Deposited at The Natural History Museum of Denmark (NHMD-230297). Collected 6 July 2012 in the L'Aber de Roscoff, Brittany, France (48°43′17.251″N, 04°00′26.522″W) by Nadja Møbjerg and Aslak Jørgensen. Collected from oxic, sandy sediment with few stones.

Other paratypes (Figs 2C, 2G, 2H, 3; Table 1)*:* In total, 14 additional paratypes were collected at the same locality as the holotype and allotype in the period from 6 July 2012 to 17 July 2013. Seven specimens (six females and one male) were mounted on microslides in glycerol and sealed with Glyceel (NHMD-230294 to 230302). Seven paratypes (four females and three males) were mounted on three SEM-stubs (NHMD-230303 to 230305) and used for scanning electron microscopy. All specimens were deposited at The Natural History Museum of Denmark. Additionally, digital images of four voucher specimens that have been used for molecular work exist (Fig. 2B; see Møbjerg *et al.*, 2016: *Echiniscoides* sp. B/T16, GenBank accession
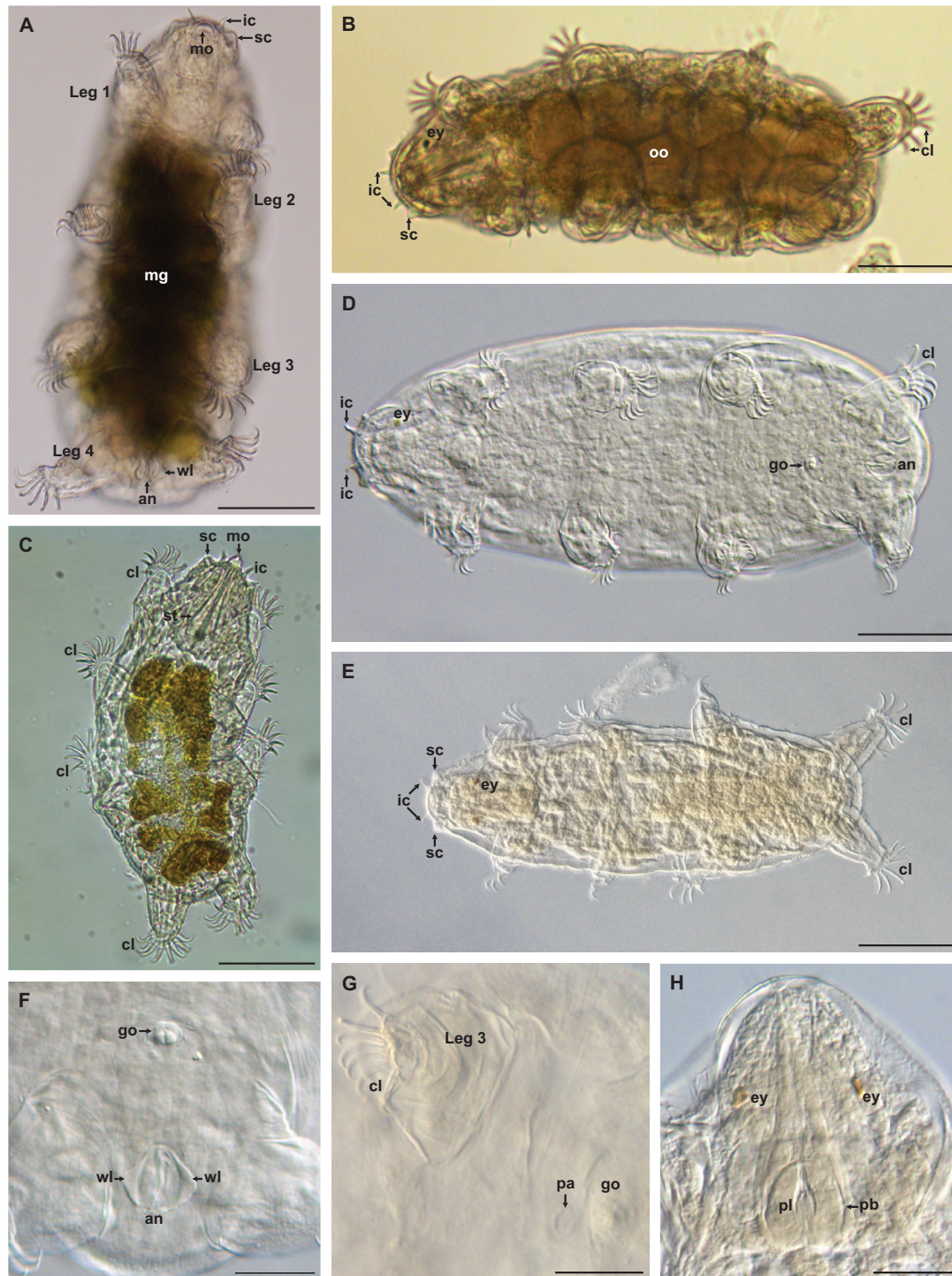
Figure 2. Light microscopy of *Neoechiniscoides aski*. A–C, images of live, moving specimens. A, ventral view of a live adult female, showing the anal system with lateral wings. In live animals, the conspicuous anal complex moves from side to side when the animal walks/runs. Scale bar: 50 µm. B, dorsal view of live adult female used for DNA extraction (*28S* GenBank accession number KX363645; *COI* GenBank accession number KX363656). Note the eight to nine mature oocytes. Scale bar: 50 µm. C, dorsal view of live adult male. Scale bar: 50 µm. D, holotype of *N. aski* (female). Scale bar: 50 µm. E, paratypic allotype of *N. aski* (male). Scale bar: 50 µm. F, ventrocaudal view of holotypic female showing anal system and the gonopore, which is raised on an elevation (note that seminal receptacles are lacking). Scale bar: 20 µm. G, ventral view of paratypic

numbers KX363644 and KX363655; *Echiniscoides* sp. B/T18, GenBank accession numbers KX363645 and KX363656; *Echiniscoides* sp. B/T20, GenBank accession number KX363657; and *Echiniscoides* sp. B/T21, GenBank accession number KX363658).

Description (Figs 2–6; Table 1)*:* The collected specimens have a measured body length ranging from 207 (smallest males) to 301 µm (holotypic female). The most common claw formula of *N. aski* seems to be 8,8,8,7, but the number of claws varies significantly between and within specimens, as illustrated by the holotype, which has a different number of claws on the left (8,9,8,7) and the right sides (8,8,8,8), respectively (Table 1). The dorsal cuticle is characterized by a polygonal granulation that continues ventrally on the head. The polygons are between 2 and 5 µm in size, with the size of the granulation decreasing laterally and in depressions of the cuticle. Each polygon has several fibres that connect to neighbouring polygons. The dorsal cuticle is modified in connection with muscle attachment sites (Figs 3F, 5). Specifically, five dorsal cuticular attachment sites (without polygon structure) were observed on the caudal segment. The buccopharyngeal tube is relatively short, and the buccopharyngeal apparatus appears similar to that of *Echiniscoides sigismundi sigismundi* (M. Schultze, 1865). The sensory structures are also of *E. s. sigismundi* type: black to dark brown eyes, two small domes (p1 and p2) on leg I and II, respectively, and p3 is a seta. The sensory structure on leg IV is similar to the primary clava; both are a papillary structure with a tiny spine. Cirri A and E are short, with a few spiral striations at the base. The secondary clava appear different in males compared with females. In females, they seem flatter and are surrounded by a ring of unsculptured cuticle (Figs 3C, 4), whereas the male has a slightly more protruding secondary clava that can be seen from the dorsal side (Figs 3H, 5). In both sexes, the secondary clava attach directly on the head (not as a projection as in *E. s. sigismundi*). The gonopore of the female is unique within the Echiniscoididae and is formed of six large units positioned on a ring-shaped cuticular protrusion. Two small depressions (Figs 3B, 4) are situated close to the gonopore; we interpret these as muscle attachment sites. A small cuticular plate is present in front of the gonopore (Figs 3B, 4); males lack this structure. The anal system consists of three lobes: two lateral and a terminal lobe. The anus appears as an opening at the junction between the three lobes. The lateral lobes of the anal systems appear as a double structure, with two wings (Figs 2A, F, 3B, G, 4, 5, 6). We have no transmission electron microscopy of these lobes, but hypothesize that they might be glandular.

DIFFERENTIAL DIAGNOSIS OF THE SPECIES

Neoechiniscoides aski is a medium- to large-sized neoechiniscoid, as revealed by a comparison between the holotypic females and allotypic males of *N. aski* and *N. pollocki* (Table 1). Allotypic female length is 234 µm in *N. horningi*, whereas the length of the holotypic male was reported to be 216 µm (Miller & Kristensen, 1999). In contrast to *N. aski*, it seems that both *N. pollocki* and *N. horningi* have a fixed claw formula of 8,8,8,7 in adults and 6,6,6,5 in juveniles. Juveniles of *N. aski* were not found. The dorsal cuticle is strongly sculptured in all three *Neoechiniscoides* species, and in *N. pollocki* and *N. aski* the granulation continues ventrally on the head. In *N. horningi*, the ventral cuticle of the head is smooth. *Neoechiniscoides aski* has a unique dorsal cuticular structure (lacking polygons) on the caudal segment composed of two paired and a single unpaired attachment site (Figs 3F, 5). These sites have not been observed in other species of *Neoechiniscoides*. However, two similar middorsal attachment sites were observed caudally in *I. sifae*. In *N. pollocki*, the internal and external cirri have been observed to terminate in a small bundle of eight or nine rays, whereas in *N. aski* they seem to terminate in a single hair-like structure, and in *N. horningi* the cirri are cone-like structures. Secondary clavae appear more distinct and larger in males than in females of *N. pollocki* and *N. aski*. Importantly, cephalic papillae in *N. horningi* split up into secondary and tertiary clavae. Two small dorsal mouth plates are present in both *N. horningi* and *N. pollocki*. These plates are lacking in *N. aski*, which instead has three annulated oral rings. In live specimens of *N. aski*, it seems that these annulated oral rings might be involved in telescoping of the mouth cone.

PHYLOGENETIC INFERENCE

The phylogenetic relationships of *Neoechiniscoides* to *Echiniscoides* groups were resolved only with

female revealing the cuticular plate anterior to the female gonopore. Scale bar: 20 µm. H, anterior view of paratypic female showing the brown eyes as they appear after glycerol preparation. The placoids of the pharyngeal bulb are still present (they eventually dissolve in glycerol preparations). Scale bar: 20 µm. Abbreviations: an, anus; cl, claw; ey, eye; go, gonopore; ic, internal cirrus; mg, midgut; mo, mouth opening; oo, oocyte; pa, ventral cuticular plate; pb, pharyngeal bulb; pl, placoid; sc, secondary clava; wl, lateral wing of the anal structure.

Table 1. Measurements of selected characters on holotypes and paratypes of *Neoechiniscoides aski* and *Neoechiniscoides pollocki*

	Neoechiniscoides aski					*Neoechiniscoides pollocki*	
	Holotype	Allotype	Paratypes			Holotype	Allotype
	Female	Male	Male	Female	Female*	Female	Male
Body length (µm)	300.8	227.5	206.9	265.2	244	281	216
Body width (µm)	123.5	73.6	63.2	111. 9	85	119	76
Buccal canal length (µm)	41.8	30. 9	35.4	42.4	n/a	48	43
Bulb length (µm)	19.1	18.1	16.4	19.9	n/a	23	18
Placoid length (µm)	10.1	9.5	7.6	9.8	n/a	19	11
Internal cirrus length (µm)	8.7	7.5	7.1	7.5	7.2	7.6	5.4
External cirrus length (µm)	6.2	6.1	4.7	5.6	4.8	7.6	5.4
Lateral cirrus/cirrus A length (µm)	13.1	13.9	12.9	11. 8	15	17	15
Cirrus E length (µm)	14.8	10.7	10.6	15.7	n/a	18	16
Primary clava length (µm)	4.9	5.1	6.0	5.1	5	5.4	5.9
Secondary clava (height × width; µm)	8.3 × 6.8	10.3 × 5.9	10.1 × 8.7	9.5 × 5.8	8 × 5	11 × 9	16 × 11
p1 length, leg I sensory organ (µm)	2.8	2.8	2.1	2.9	2	3.2	2.5
p2 length, leg II sensory organ (µm)	2.7	2.8	2.1	2.9	3	3.5	2.9
p3 length, leg III sensory organ (µm)	7.8	7.5	6.0	8.3	6	12	10
p4 length, leg IV sensory organ (µm)	5.1	4.6	4.8	5.7	5	5.4	6.5
Distance from anus to gonopore (µm)	26.6	13.5	11.7	22.7	26	27.5	12.7
Number of claws left side/right side	8,9,8,7 8,8,8,8	7,7,7,7 7,8,7,8	7,7,7,7 7,7,7,6	8,8,8,8 8,8,8,7	8,8,8,7 8,8,8,7	8,8,8,7 8,8,8,7	8,8,8,7 8,8,8,7
Date collected	17 July 2013	6 July 2012	6 July 2012	9 July 2012	9 July 2012	11 May 1978	11 May 1978
ZMUC and NHMD no.	NHMD-230293	NHMD-230297	NHMD-230298	NHMD-230300	NHMD-230303	ZMUC TAR-758	ZMUC TAR-759

*Scanning electron microscopy preparation (NHMD-230303) includes several specimens. n/a, non-applicable.

some branch support by Bayesian inference (Fig. 7; Supporting Information, Figs S1, S2). The full phylogeny using *COI* is presented in the Supporting Information (Fig. S1). It was not possible to achieve congruence between phylogenetic methods for deeper nodes, despite the use of a more optimized data matrix of *COI* and additional phylogenetic analyses compared with Møbjerg *et al*. (2016). In the *COI* phylogeny, as inferred by Møbjerg *et al*. (2016), *N. aski* is monophyletic and forms a clade (*Neoechiniscoides*) with closely related species from Maine and Roscoff. Notably, a specimen marked as *E. pollocki* has a *COI* sequence in GenBank (JQ689505). This sequence was not closely related to *Neoechiniscoides* in any analyses. It is our view (and that of colleagues) that this sequence does not belong to *N. pollocki*. Sequencing of the specific specimen was performed with no/little prior identification effort, and no voucher photographs exist of the animal. It has become evident that a larger number of echiniscoidid species belonging to all three genera (*Echiniscoides*, *Isoechiniscoides* and *Neoechiniscoides*) coexist in intertidal sediments; a fact that was not known at the time of sampling of the given specimen. Importantly, a colleague recently performed a sequence analysis of various *Echiniscoides* specimens from Japan with different morphologies in comparison to the type material of *N. pollocki* and found that the '*N. pollocki*' *COI* sequence from GenBank was similar to these Japanese *Echiniscoides* (S. Fujimoto, personal communication). Thus, to recapitulate, the '*N. pollocki*' *COI* sequence from GenBank is likely to belong to an unidentified *Echiniscoides* species with a different morphology from that of *N. pollocki*. No sequence data exist for *N. horningi*.
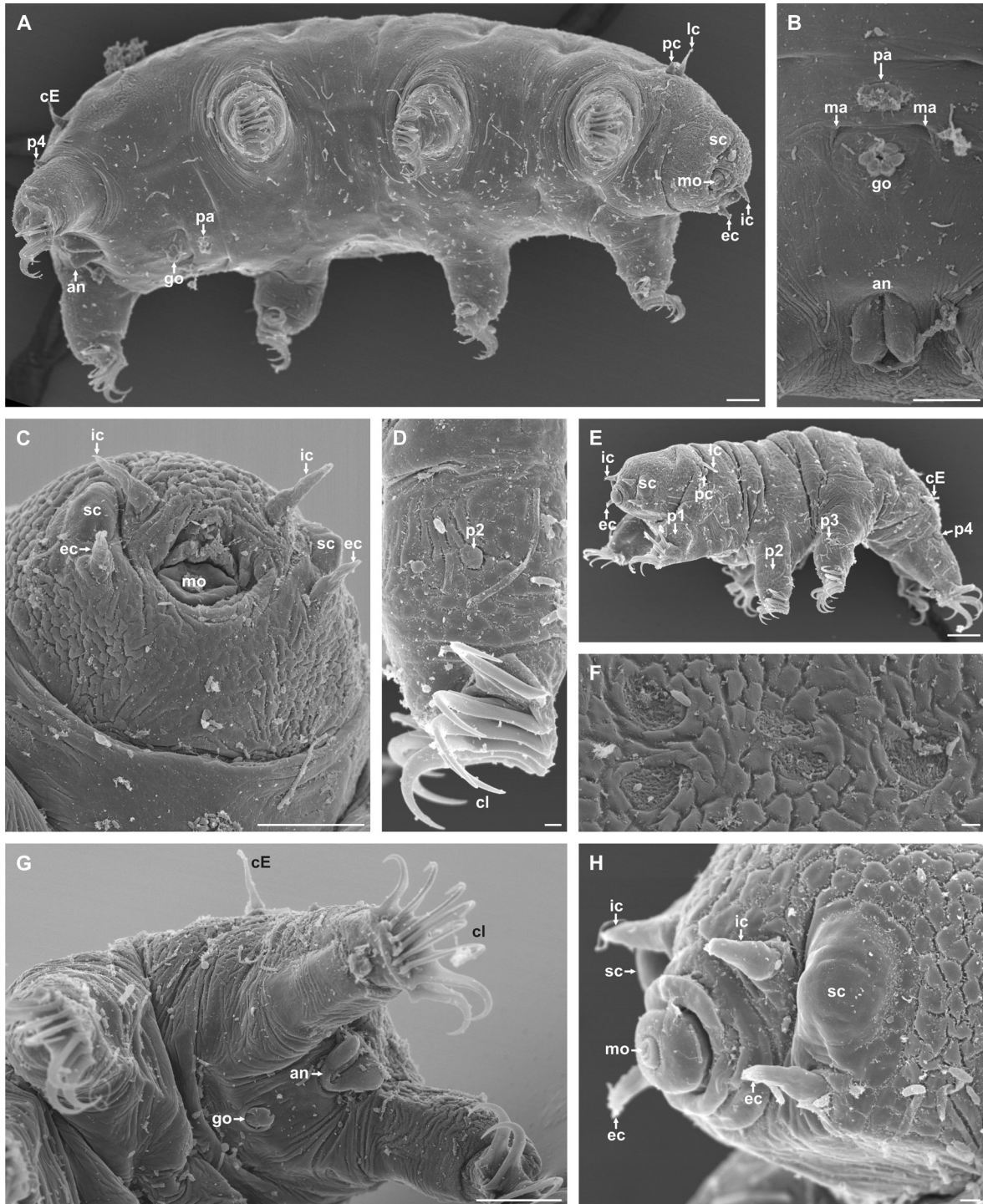
Figure 3. Scanning electron microscopy of *Neoechiniscoides aski*. A–C, female. A, habitus of mature female. Scale bar: 10 µm. B, close-up of gonopore with associated cuticular plate and anus. Note muscle attachment sites located lateral to the female gonopore. Scale bar: 10 µm. C, close-up of head region. Note the cuticular sculpture of the head. Scale bar: 10 µm. D–H, male. D, second leg, with dome-shaped sense organ (p2). Scale bar: 1 µm. E, habitus of adult male. Scale bar: 10 µm. F, prominent dorsal cuticular attachment sites of the caudal segment. Scale bar: 1 µm. G, posterioventral part of a male, showing the fourth leg pair, anal system and gonopore. Anterior is to the left. Scale bar: 10 µm. H, close-up of head region of male. Note the relatively large secondary clavae. Scale bar: 1 µm. Abbreviations: an, anus; cE, cirrus E; cl, claw; ec, external

The inferred phylogeny using *28S* was largely unresolved and unsupported between Bayesian and maximum likelihood analyses (Supporting Information, Fig. S2).

GENETIC DIVERSITY

A relatively small uncorrected p-distance of *COI* (1.43%) suggests that the clades *N. aski* and *Neoechiniscoides* sp. A from Roscoff are closely related despite inhabiting different substrates. Furthermore, they are closely related to *Neoechiniscoides* specimens from Maine, USA (0.46–1.22%). In fact, *Neoechiniscoides* sp. A has a greater similarity in *COI* sequence to the specimens from Maine than *N. aski*.

When comparing *N. aski* with other clades in Figure 7, the genetic variation is of a much higher level (13.53–18.41%), perhaps reflecting the variation between related genera. The maximum likelihood estimate of transition/transversion bias (2.15) suggests substitutional saturation, because it deviates from the unbiased condition (0.5).

DISCUSSION

The present study focuses on an ongoing revision of echiniscoidid tardigrades, highlighting the enigmatic genus *Anisonyches* with its unique claw configuration, median cirrus and seminal receptacles. We erect a new family, Anisonychidae, for the genus and place Anisonychidae in the paraphyletic order Arthrotardigrada, but strongly emphasize that Arthrotardigrada is in need of taxonomic revision. We further highlight the exceptional diversity among tardigrades inhabiting tidal zones and discuss morphological features that have not yet received much attention; specifically, the anal system and the presence of a cuticular plate and muscle attachment sites surrounding the female gonopore.

The intertidal zone at Roscoff seems to be a hotspot for echiniscoidid tardigrades (Møbjerg *et al.*, 2016). This could be attributable to the wide range of different habitats, i.e. a fluctuating intertidal zone, with hard rock surface ideal for barnacles, lichens and algae, combined with sandy beaches mixed with various degrees of stony rubble. We erect a new genus *Neoechiniscoides* with unique anal structures containing species that seem to coexist with other echiniscoidids (*Echiniscoides and Isoechiniscoides*) living in intertidal sediments. The new species, *Neoechiniscoides aski* has a dorsal

cuticle with polygonal structures arranged in transverse rows and most often the claw formula 8,8,8,7. The relatively large females have two muscle attachment sites flanking the gonopore and a small anterior plate. There is a clear difference in body size between sexes, and it seems that there is a sexual dimorphism with regard to the presence of the small ventral plate in front of the gonopore. The females in both *N. aski* and *N. horningi* have this small cuticular plate. Whether the plate is also present in *N. pollocki* awaits further investigations. The males of all three species lack this plate.

Current knowledge of the phylogenetic relationships within Echiniscoididae makes any hypotheses on the evolution of the anal system tentative. The echiniscoidid anal system is built by three anal lobes (two lateral and a terminal) of varying size and form. As holds for other echiniscoidids, the anal system of *Isoechiniscoides* consists of two lateral lobes and a terminal lobe. The terminal lobe is relatively large compared with other echiniscoidids, giving the anal system of this genus an ovoid appearance, with the three lobes clearly visible using light microscopy. Although the species in *Echiniscoides* generally exhibit a simple trilobed anal system, it is clear that *Neoechiniscoides* has evolved unique additional structures that increase the size of the lateral anal lobes. Specifically, in neoechiniscoidids the lateral lobes have an extra structure that we refer to as 'wings'. An equally complex anal system is not present in any other heterotardigrades, although they possess variable trilobed anal systems (Jørgensen *et al.*, 2014).

Currently, the phylogenetic relationships between the genera of *Echiniscoides* s.l. are unresolved, because congruence between methods could not be achieved. It is possible that the observed substitutional saturation makes the rapidly evolving *COI* a poor candidate for resolving the deeper nodes in Echiniscoididae. In the study by Møbjerg *et al.* (2016), a clade similar to Figure 7 was inferred by *28S*, but phylogenetic inference with this slowly evolving gene also resulted in poor resolution, as did the combination of *28S* and *COI*. An explanation could be that the evolution of the genera in Echiniscoididae might have happened rapidly, resulting in unresolved relationships. Currently, no time line has been established with regard to echiniscoidid evolution, and the poor resolution might be a result of gene and taxon sampling.

Other specimens from Maine and Roscoff previously regarded as *Echiniscoides* s.l. are similar to *N. aski*

cirrus; go, gonopore; ic, internal cirrus; lc, lateral cirrus (cirrus A); ma, lateral attachment sites associated with the female gonopore; mo, mouth opening; p1–p4, sensory structures of the legs I–IV; pa, ventral cuticular plate; pc, primary clava; sc, secondary clava.
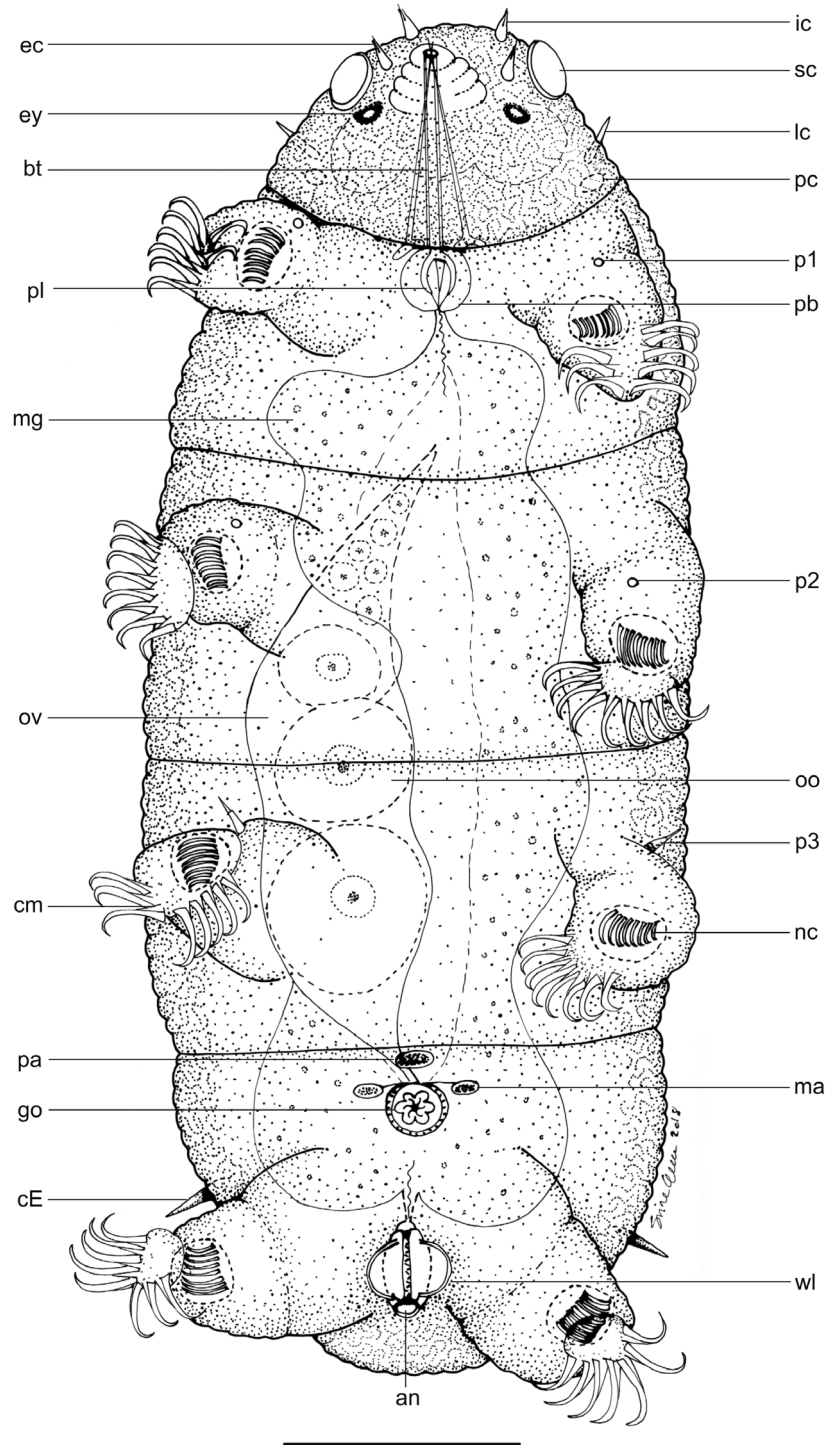
Figure 4. *Neoechiniscoides aski.* Drawing of holotypic female (NHMD-230293), ventral view. Scale bar: 50 µm. Abbreviations: an, anus; bt, buccal tube; cE, cirrus E; cm, claw membrane; ec, external cirrus; ey, eye; go, gonopore; ic, internal cirrus; lc, lateral cirrus (cirrus A); ma, lateral attachment sites associated with the female gonopore; mg, midgut; nc, new claws forming inside claw gland; oo, oocyte; ov, ovary; p1–p3, sensory structures of legs I–III, respectively; pa, ventral cuticular plate; pb, pharyngeal bulb; pc, primary clava; pl, placoid; sc, secondary clava; wl, lateral wing of the anal structure.
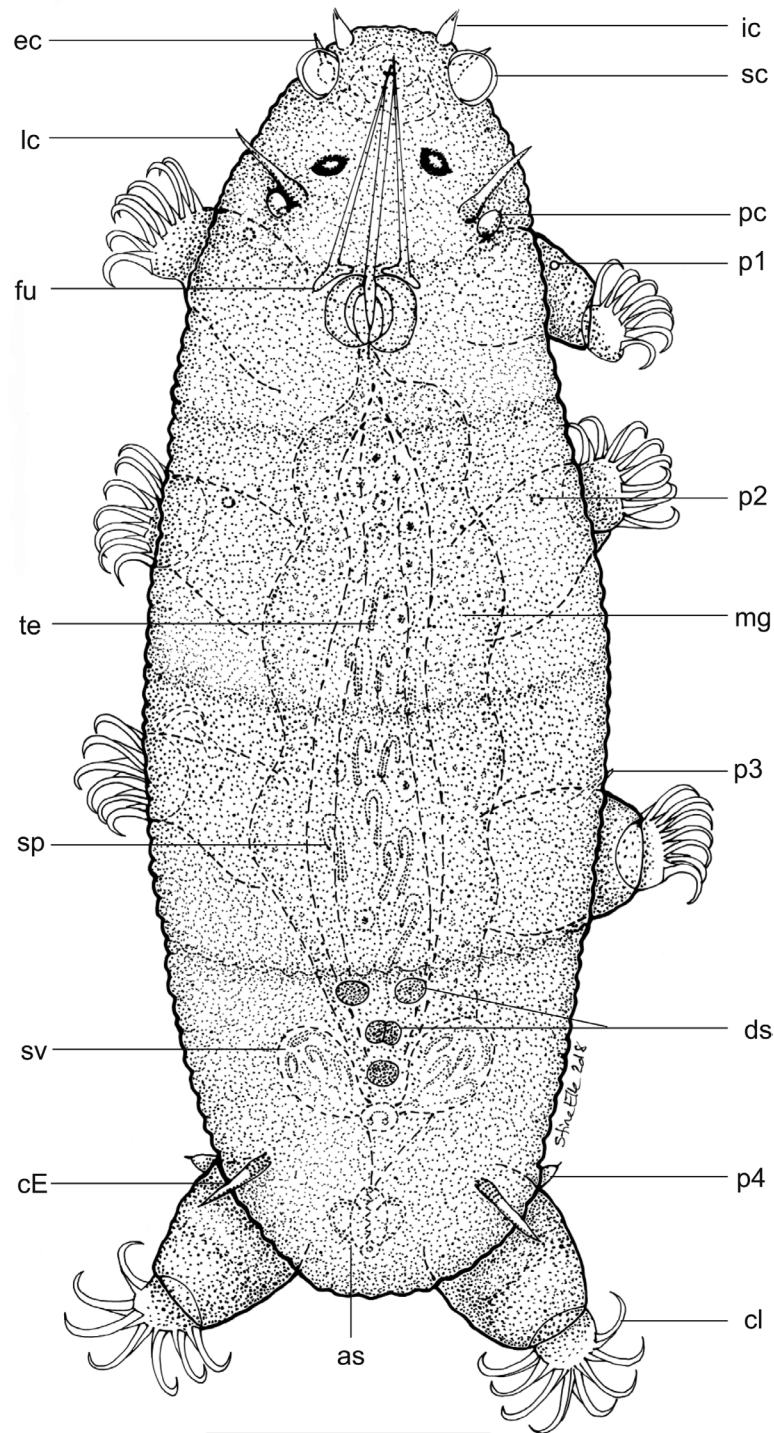
Figure 5. *Neoechiniscoides aski.* Drawing of allotypic male (NHMD-230297), dorsal view. Scale bar: 50 µm. Abbreviations: as, anal system; cE, cirrus E; cl, claw; ds, dorsal cuticular attachment sites; ec, external cirrus; fu, furca of the stylet; ic, internal cirrus; lc, lateral cirrus (cirrus A); mg, midgut; p1–p4, sensory structures of legs I–IV, respectively; pc, primary clava; sc, secondary clava; sp, spermatozoa; sv, seminal vesicle; te, testicle.
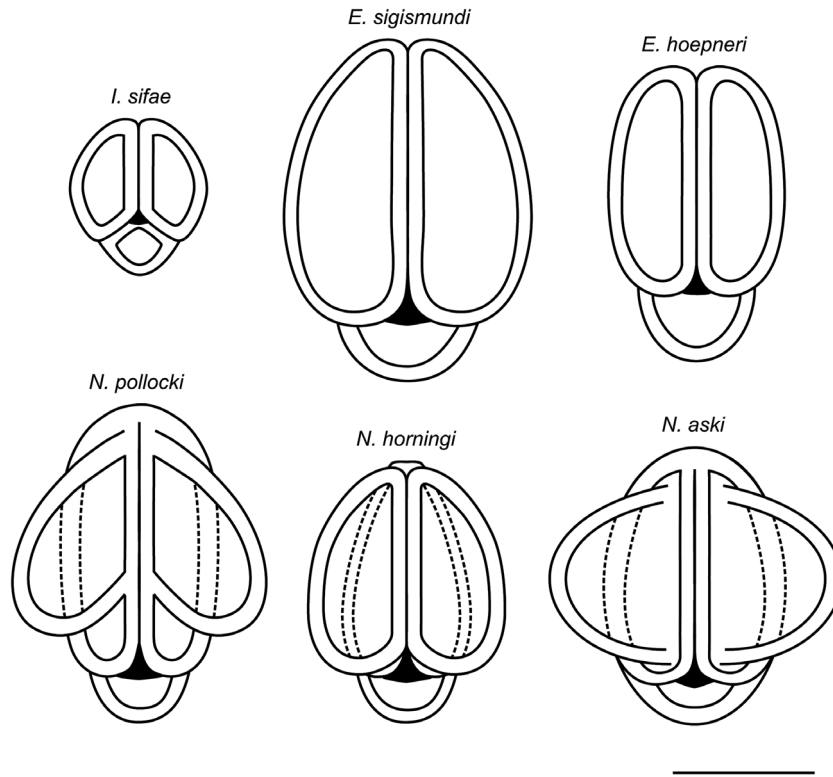
Figure 6. Drawing of anal system in *Isoechiniscoides*, *Echiniscoides* and *Neoechiniscoides* based on investigations of live animals and preparations on slides for light microscopy. In all three genera, a three-lobed anal system is present and consists of two lateral lobes and a terminal lobe of variable size. The anal opening is indicated in black. In *Neoechiniscoides*, two wings are present on the lateral lobes. These wings are easily visualized in live animals and using light microscopic preparations, whereas this double structure of the lateral lobes is difficult to visualize in specimens prepared for scanning electron microscopy. Scale bar: 10 μm.

with regard to *COI* sequence variation and are closely related to *N. aski* as inferred by the phylogenetic analyses of *COI*. Although the specimens all belong to *Neoechiniscoides*, the low genetic variation can be interpreted in two different ways with regard to the taxonomic status: (1) the specimens constitute a single species of *Neoechiniscoides* collected at different localities and substrates, i.e. they might be interpreted as belonging to different populations of *N. aski*; or (2) the specimens constitute different species with low *COI* divergence, representing ongoing speciation events supported by barriers to interbreeding, i.e. long distance (Maine–Roscoff) and/or different substrates (Roscoff–Roscoff). There is no obvious solution to their current taxonomic status.

Interestingly, our voucher pictures of the *Lichina* specimens (*Echiniscoides* sp. A of Møbjerg *et al.*, 2018) show that they generally are smaller and with much longer cirrus E and stylets than *N. aski*. Unfortunately, the defining anal system structures

of *N. aski* are not clear in the light microscopy photographs of the specimens from *Lichina*. We are unable to characterize the Maine specimens morphologically, because the sequences were generated in another study before the consistent use of digital images as vouchers before DNA extraction. The low *COI* sequence variation (0.46–1.22%) between *N. aski* from Roscoff and the undetermined *Neoechiniscoides* species from Maine raises the possibility of transatlantic Gulf Stream dispersal. Our analyses showed no indication that *Neoechiniscoides* has used the Mid-Atlantic Islands (the Faroe Islands, Iceland and Greenland) as stepping stones to facilitate transatlantic dispersal. This is in accord with the results described by Faurby *et al.* (2011), who found no indication of Mid-Atlantic Islands stepping stones in the dispersal of *Echiniscoides*. Likewise, the low *COI* variation (1.43%) between the interstitial *N. aski* and *Neoechiniscoides* specimens from Roscoff collected from the lichen *Lichina* raise
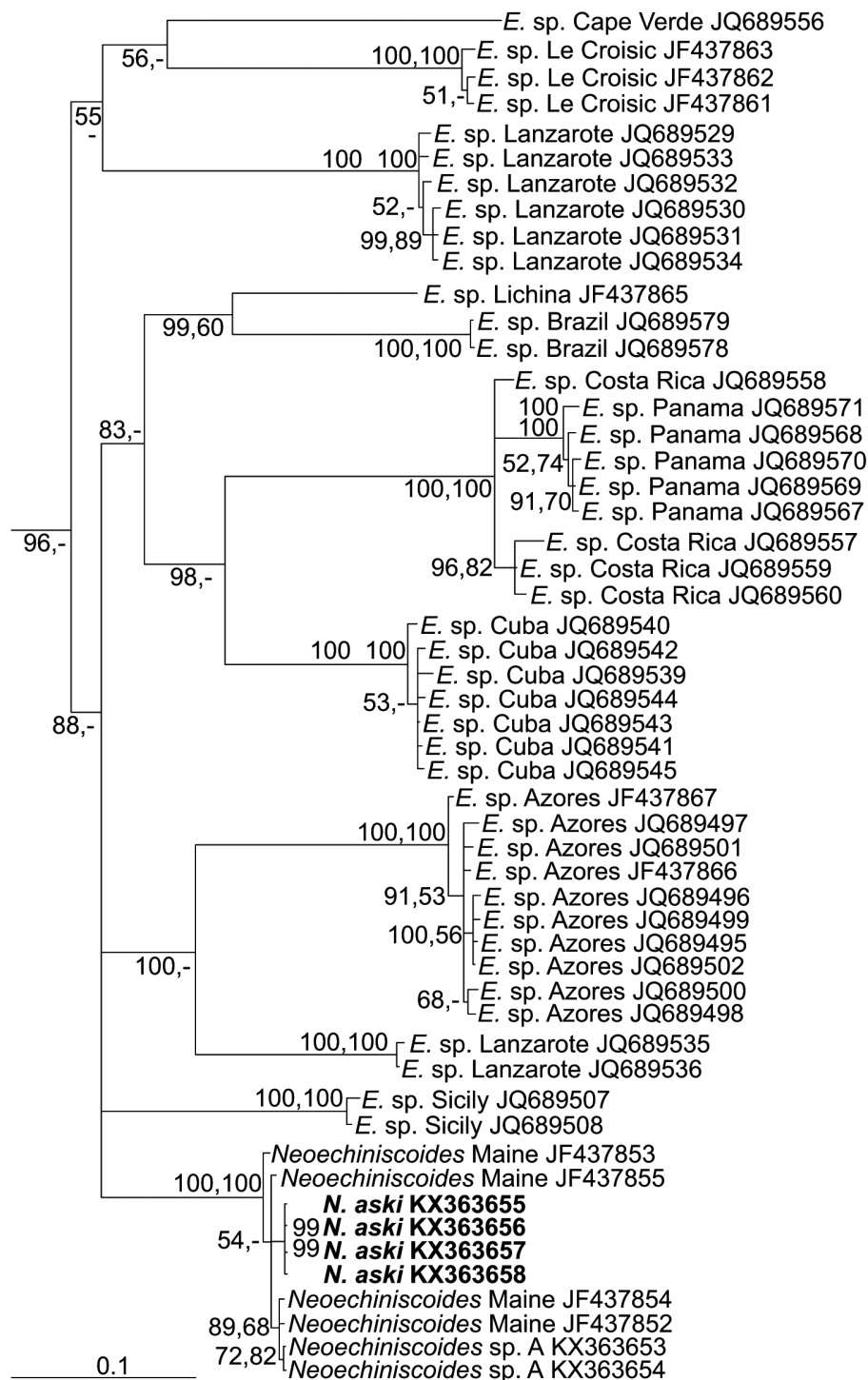
Figure 7. Phylogenetic position of *Neoechiniscoides aski* inferred by Bayesian inference of *COI*. The figure represents the smallest supported clade informative with regard to the phylogenetic relationships of *Neoechiniscoides*. Posterior probabilities (first) are shown as percentages, and bootstrap values are indicated.

questions regarding the flexibility of habitat choice in closely related echiniscoidid tardigrades. The *Neoechiniscoides* species from Maine were collected

from barnacles, and in this context the phylogeny indicates an invasion of the interstitial habitat within *Neoechiniscoides* by *N. aski*. Møbjerg *et al.* (2016)

showed that *Isoechiniscoides* might be the sister group to *Echiniscoides* s.l. + *Neoechiniscoides*, raising the possibility that the echiniscoidid heterotardigrades evolved from an interstitial ancestor.

Our investigations of Echiniscoididae suggest that the molecular and morphological diversity within this family might be of a magnitude comparable to the heavily investigated Eutardigrada. Notably, as previously reported, a rough estimate of uncorrected *COI* variation is ~28% within Eutardigrada and 38% between Eutardigrada and Apotardigrada, whereas variation in the *COI* sequences of Echiniscoididae is ~30% (Møbjerg *et al.*, 2016). The latter finding clearly calls for an increased focus on the fascinating echiniscoideans inhabiting tidal habitats worldwide.

ACKOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Phylogeny of Echiniscoidinae inferred from *COI*. Posterior probability and bootstrap values are shown.
Figure S2. Phylogeny of Echiniscoidinae inferred from *28S*. Posterior probability and bootstrap values are shown.