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Halanonchus scintillatulus sp. nov. from New Zealand and a review of the suborder Trefusiina (Nematoda: Enoplida)

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Abstract. We provide a review of the enoplid suborder Trefusiina Siddiqi, 1983, based on morphological considerations and analyses of new and published 18S rDNA sequences. We also describe *Halanonchus scintillatulus* Leduc sp. nov. from the Hauraki Gulf, northern New Zealand, as well as females of *Trefusialaimus idrisi* Leduc, 2013 from the continental slope of New Zealand. We show for the first time that the structure of the female reproductive system of *Trefusialaimus* Riemann, 1974 consists of two opposed and outstretched ovaries, an unusual feature for the Enoplida. The Trefusiina did not form a monophyletic group in the 18S rDNA phylogeny due to the placement of *Lauratonema* Gerlach, 1953 and *Trefusialaimus* sequences well away from the main Trefusiina clade. However, due to generally weak Maximum Likelihood support values, we refrain from changing the classification of these taxa until more comprehensive analyses can be conducted. Our phylogenetic analysis supports the inclusion of the Trischistomatidae Andrássy, 2007 within the Trefusiina, meaning that all of the enoplid suborders now include at least some terrestrial/freshwater representatives. The Trefusiina currently comprises five families, 14 genera and 92 valid species.

Keywords. *Trefusialaimus*, Halanonchinae, Trefusiidae, Triplonchida, continental slope.

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Introduction

Knowledge of phylogenetic relationships within the nematode order Enoplida Filipjev, 1929 has progressed substantially in the last two decades, largely as a result of molecular phylogenetic analyses of

18S rDNA sequences. For example, the Alaimina Clark, 1961 was moved to the Enoplida by De Ley & Blaxter (2002) from the Dorylaimida Pearse, 1942, where it was previously classified by Lorenzen (1981), the Campydorina Jairajpuri, 1983 was placed with the Enoplida by Mullin *et al.* (2003), and the order Trefusida Lorenzen, 1981 was ranked as a suborder within the Enoplida by De Ley & Blaxter (2004) based on analyses presented by Rusin *et al.* (2001). These and other changes have led to the current division of Enoplida, which now comprises seven suborders: the Enoplina Chitwood & Chitwood, 1937, Trefusiina Siddiqi, 1983, Tripyloidina De Coninck, 1965, Oncholaimina De Coninck, 1965, Ironina Siddiqi, 1983, Campydorina Jairajpuri, 1983 and Alaimina (De Ley & Blaxter 2004). Two of these suborders, the Campydorina and Alaimina, mainly comprise terrestrial species, with some freshwater species also found in Alaimina and some marine species in Campydorina (Holovachov 2019). The other five suborders mainly comprise marine species, with the suborder Trefusiina currently the only suborder comprised exclusively of marine species (Smol & Coomans 2006).

The classification of the Enoplida will likely keep evolving as more comprehensive molecular analyses are conducted. The SSU phylogenetic analyses of Bik *et al.* (2010), for example, suggest that most of the enoplid suborders listed in the widely used classification of De Ley & Blaxter (2004) are not monophyletic. The only two exceptions are the closely-related Trefusiina and Tripyloidina; these two suborders, however, were found to form a larger monophyletic clade with the terrestrial/freshwater genera *Trischistoma* Cobb, 1913 and *Tripylina* Brzeski, 1963, which were previously classified within the order Triplonchida Cobb, 1920 (family Tripylidae de Man, 1876; Zullini 2006; Andrássy 2007). Several other SSU phylogenies and molecular studies have shown a close relationship between *Trischistoma*, *Tripylina* and the Enoplida, and the family Trefusiidae in particular (Holterman *et al.* 2006; Meldal *et al.* 2007; Zhao & Buckley 2009; van Megen *et al.* 2009; Zhao *et al.* 2012).

In order to take into account this new evidence regarding the placement of the genera *Tripylina* and *Trischistoma*, Zhao (2011) proposed an updated classification where these two genera are placed within the family Trischistomatidae Andrássy, 2007 in the suborder Tripyloidina. This placement was considered conservative, as the Tripylidae had previously been classified together with the Tripyloididae by Lorenzen (1981) and Siddiqi (1983), and the Trefusiina was exclusively marine. The classification of the Tripylidae with the Enoplida by Lorenzen (1981) was partly based on his observation of metanemes in *Tripylina glomerans* Bastian, 1865; Zhao (2011) later also observed metanemes in four *Trischistoma* species he described from New Zealand. The presence of metanemes in *Tripylina* and *Trischistoma* appears to suggest closer affinities with the Tripyloididae (Tripyloidina), which are characterised by the presence of metanemes, than the Trefusiidae (Trefusiina), which do not possess metanemes. The two genera also resemble Tripyloidina by the presence of teeth in the buccal cavity (absent in Trefusiina) and a monorchic male reproductive system (diorchic in most of Trefusiina). In addition, *Tripylina* is characterised by the outer labial and cephalic setae in a single circle, a feature of Tripyloidina, but which differs from the Trefusiina (mostly two separate circles). The arrangement of outer labial and cephalic setae in separate circles in *Trischistoma*, on the other hand, is the same as in most of the Trefusiina. Moreover, both *Trischistoma* and *Tripylina* have non-spiral (pocket-shaped) amphids and a monodelphic female reproductive system, which differ from the Tripyloidina (spiral amphids, didelphic female reproductive system) but are consistent with some of the Trefusiina. Therefore, while there are morphological (presence of metanemes) and molecular grounds (based on SSU phylogenies) to classify *Trischistoma* and *Tripylina* within the Enoplida, the morphological data is equivocal as to whether they should be placed with the Tripyloidina or Trefusiina. The molecular evidence, however, strongly suggests a closer relationship between *Trischistoma* and *Tripylina* and the Trefusiina than with the Tripyloidina (Van Megen *et al.* 2009; Bik *et al.* 2010).

The Trefusiina currently comprises four families: Simpliconematidae Blome & Schrage, 1985, Xennellidae De Coninck, 1965, Lauratonematidae Gerlach, 1953 and Trefusiidae Gerlach, 1966 (De

Ley & Blaxter 2004). The Trefusiidae comprises two subfamilies, six genera and over 30 valid species (Bezerra *et al.* 2020). The Trefusiidae is common in shallow marine sediments worldwide and has also been found as deep as the abyssal plain (Miljutin *et al.* 2010). Changes to the classification of the family were recently proposed by Shi & Xu (2017) based on analyses of 18S rDNA sequences, which led them to argue that the structure of the female reproductive system is a more meaningful taxonomic character for defining subfamilies than buccal morphology.

A reassessment of the Trefusiina is timely given the recent and rapid developments in molecular phylogenetics and considering that the latest comprehensive taxonomic treatment of the Trefusiina based on morphological characteristics was conducted decades ago (Lorenzen 1981, 1994). Here, we provide a review of the Trefusiina based on an overview of each family, subfamily and genus of the suborder and review relationships based on morphological considerations and phylogenetic analysis of new and published 18S rDNA sequences of the Trefusiina and other Enoplida. We also describe *Halanonchus scintillatulus* Leduc sp. nov. (family Trefusiidae) from the Hauraki Gulf, northern New Zealand, as well as females of *Trefusialaimus idrisi* Leduc, 2013 from the continental slope off southeastern New Zealand.

Material and methods

Sampling and morphological analyses

A multicorer was used to obtain samples from the Firth of Thames in the Hauraki Gulf, a large bay in the north of New Zealand's North Island, in December 2003. Samples were fixed in 10% formalin and stained with Rose Bengal. Samples were subsequently rinsed on a 1 mm sieve to remove large particles and on a 45 µm mesh to retain nematodes. *Halanonchus scincillatulus* sp. nov. and other nematodes were extracted from the remaining sediments by Ludox flotation and transferred to pure glycerol and mounted onto permanent slides (Somerfield & Warwick 1996).

A sediment sample was obtained in April 2007 using a multicorer on the eastern Chatham Rise off the east coast of New Zealand's South Island at a depth of 1029 m. The sample, which consisted of sandy silt sediment, was fixed in 10% buffered formalin and stained with Rose Bengal. Samples were subsequently rinsed on a 45 µm mesh and extracted using the Ludox flotation method. *Trefusialaimus idrisi* specimens were transferred to pure glycerol and mounted onto permanent slides (Somerfield & Warwick 1996).

A one litre sediment sample (0–10 cm sediment depth) was obtained by hand at low tide from the upper subtidal zone at Sesoko Beach, Okinawa, Japan on 13 December 2017. The sediment consisted of coarse carbonate sand. Nematodes were extracted by decantation on a 63 µm mesh immediately after sampling, and live nematodes were sorted under a dissecting microscope. One male *Trefusialaimus* specimen and one morphologically similar juvenile specimen were mounted in a drop of seawater on a temporary slide to confirm their identity, and images of key morphological features were taken prior to molecular analyses (see below). Visual inspection of the male specimen at 400 × magnification showed similarities with *Trefusialaimus idrisi* due to the presence of numerous round, golden inclusions along the body, the length and arrangement of cephalic sensilla and the structure of the spicular apparatus, but species identity could not be confirmed without risking damaging or losing the specimen.

Descriptions were made from glycerol mounts using differential interference contrast microscopy and drawings were made with the aid of a camera lucida. The terminology used for describing the arrangement of morphological features such as setae follows Coomans (1979). All measurements are in µm, and all curved structures are measured along the arc. Type specimens are held in the National Institute of Water and Atmospheric Research (NIWA) Invertebrate Collection, Wellington, and the National Nematode Collection of New Zealand (NNCNZ), Landcare Research New Zealand Ltd, Auckland.

Abbreviations

a = body length/maximum body diameter
b = body length/pharynx length
c = body length/tail length
c′ = tail length/anal or cloacal body diameter
cbd = corresponding body diameter
ceph. = cephalic
cs = cephalic seta
g = granule
ils = inner labial sensilla
L = total body length
n = number of specimens
ND = no data
ols = outer labial sensilla
V = vulva distance from anterior end of body
%V = V/total body length × 100

DNA extraction, PCR and sequencing

Following observation and digital imaging under a compound microscope, one *Trefusialaimus* male specimen and one morphologically similar juvenile specimen were transferred to 50 µl of a guanidinium thiocyanate solution prepared following Sinniger *et al.* (2010). The DNA was further extracted as described in Sinniger *et al.* (2010), adjusting the volumes accordingly (i.e., using 50 µl of isopropanol for precipitation and eluting in 30 µl of ultrapure water). The rDNA small subunit (SSU) was amplified using the primers from Holterman *et al.* (2006): 1096F, 5'-GGTAATTCTGGAGCTAATAC-3' and 1912R, 5'-TTTACGGTCAGAACTAGGG-3' for the first SSU fragment and 1813F, 5'-CTGCGTGAGAGGTGAAAT-3' and 2646R, 5'-GCTACCTTGTTACGACTTTT-3' for the second fragment. The LSU fragment was amplified using the primers D2A (5' ACAAGTACCGTGAGGGAAAGT 3') and D3B (5' TGCGAAGGAACCAGCTACTA 3') (Nunn, 1992) with thermal cycles as described in Leduc & Zhao (2018). The PCR products were sequenced bi-directionally using the amplification primers by Macrogen Japan (Kyoto, Japan). Sequences were assembled and edited in Geneious ver. 10.2.2 (Kearse *et al.* 2012).

Sequence alignment and phylogenetic inference

The ribosomal DNA SSU and D2-D3 of LSU sequences of *Trefusialaimus* sp. were deposited in GenBank under accession numbers MN689267, MN689268 and MN689269, MN689270, respectively. SSU phylogenetic analyses were conducted using sequences of representative genera of the Enoplida and rooted using Triplonchida sequences. The initial D2-D3 of LSU analyses confirmed that the LSU rDNA gene is only informative at the species to family levels (De Ley *et al.* 2005) and could not determine the placement of *Trefusialaimus*. The D2-D3 of LSU sequences were therefore not used to determine phylogenetic relationships. The SSU of DNA sequences were aligned using the MUSCLE algorithm (Edgar 2004a, 2004b) with default parameters, and then the alignment was modified by using Gblocks (Castresana 2000; Talavera & Castresana 2007) with relaxed gap setting (only positions where 50% or more of the sequences have a gap are treated as a gap position) to remove the sites of questionable alignment. After removing sites of questionable alignment, Gblocks gave a 1445 bp site alignment from the original SSU rDNA with 1638 bp alignment.

Phylogenies were built in Geneious ver. 10.2.6 (http://www.geneious.com, Kearse *et al.* 2012). MrModelTest ver. 2.3 (Nylander 2004) in conjunction with PAUP* ver. 4.0b10 (Swofford 2002) and jModelTest ver. 2.1.10 software (Darriba *et al.* 2012) were used to select the best model using the

Akaike Information Criterion. The substitution model [GTR (general time-reversible) + I (proportion of invariable sites) + G (gamma distribution)] was selected by MrModelTest in conjunction with PAUP* as the best-fit model, whereas the substitution model TVM+I+G was selected as the best model by jModelTest ver. 2.1.6. Because the model TVM+I+G cannot be implemented in Geneious ver. 10.2.6, Bayesian trees were constructed with MrBayes under the most similar best-fit model [GTR+I+G] (Huelsenbeck & Ronquist 2001), which is not expected to have a significant impact on tree topology. The trees were run with chain length of 1 100 000, and burn-in length of 100 000. The perimeter files from multiple runs were inspected for chain convergence in Tracer ver. 1.5 (Rambaut & Drummond 2007), and the trees were edited in FigTree ver. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) and PowerPoint. These analyses were also conducted with PhyML ver. 3.0 using the default settings in Geneious ver. 10.2.6. The substitution model GTR, the NNI (default, fast) topology search and 1000 bootstrap replicates (Guindon *et al.* 2010) were selected for building the tree.

Results

Molecular phylogenetic analyses

Two SSU (18S) sequences of 846 and 1587 bp and two D2-D3 of LSU (28S) sequences of 766 and 778 bp were generated from the juvenile and male *Trefusialaimus* specimens, respectively. The two SSU sequences were 100% identical over the 841 bp region of overlap. The two LSU sequences were

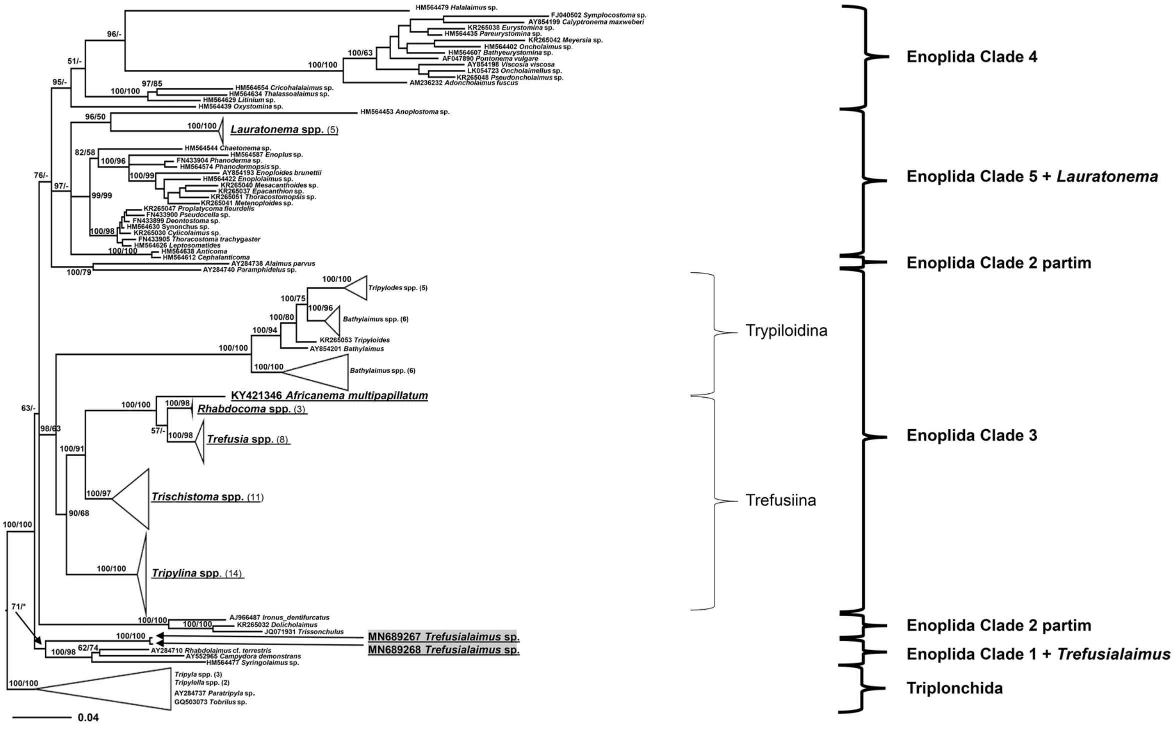

Fig. 1. Bayesian tree of the Enoplida (and Triplonchida outgroup) inferred from SSU sequences, aligned using the MUSCLE alignment algorithm and with regions of questionable alignment removed using Gblocks, under the general time-reversible (GTR) + proportion of invariable sites (I) + gamma distribution (G) model. Trefusiina sequences are shown in underlined font, and new *Trefusialaimus* sequences are highlighted in grey. The five enoplid clades described by Bik *et al.* (2010) are identified on the right. Posterior probability (left) and bootstrap values (right) are given on corresponding clades. Dashes (-) indicate no support and aserisks (*) indicate <50% support. The scale stands for substitutions per site.

largely identical, with the exception of two ambiguous base pairs (R vs A and Y vs T) and an additional guanine in a polyG stretch (5 Gs vs 4 Gs) in the juvenile sequence; however, these differences were not considered relevant as possibly resulting from PCR or sequencing artefacts. These results support the morphological observations of the male and juvenile specimens suggesting that they belong to the same species.

The SSU consensus tree recovered six main enoplid lineages, which largely correspond to the enoplid clades identified by Bik *et al.* (2010), although with some discrepancies (Fig. 1). A major difference is that in our analysis, the Ironidae de Man 1876 (*Ironus* Bastian, 1865*, Dolicholaimus* de Man, 1888 and *Trissonchulus* Cobb, 1920) and the Alaimina (*Alaimus* de Man, 1880 and *Paramphidelus* Andrássy, 1977) are not grouped together (clade 2 in Bik *et al.* 2010). However, the Maximum Likelihood support for the placement of the Ironidae and Alamina is weak or non-existent in both the present study and in Bik *et al.* (2010).

Most Trefusiidae sequences included in the analysis (i.e., *Trefusia* de Man, 1893, *Rhabdocoma* Cobb, 1920, and *Africanema* Vincx & Furstenberg, 1988) formed a well-supported clade (100% posterior probability and bootstrap support). This clade formed a moderately supported group with the Trischistomatidae sequences (*Trischistoma* and *Tripylina*; 90% posterior probability and 68% bootstrap support), although the Trischistomatidae itself did not form a monophyletic clade. The Trefusiidae+Trischistomatidae clade formed a larger, moderately- to well-supported clade with the Tripyloidina (98% posterior probability and 63% bootstrap support), which corresponds to clade 3 identified by Bik *et al.* (2010).

The Trefusiina did not form a monophyletic group in the SSU phylogeny due to the placement of *Lauratonema* Gerlach, 1953 and *Trefusialaimus* sequences well outside of enoplid clade 3 (Fig. 1). *Lauratonema* sequences were grouped with *Anoplostoma* Bütschli, 1874 with weak to strong support (enoplid clade 5; 96% posterior probability and 50% bootstrap support), whereas *Trefusialaimus* sequences were grouped with sequences of the Suborder Campydorina (*Rhabdolaimus* de Man, 1880, *Campydora* Cobb, 1920 and *Syringolaimus* de Man, 1888) with no or weak support (enoplid clade 1; 71% posterior probability and 0% bootstrap support). Although this SSU phylogeny does not provide support for the inclusion of *Lauratonema* and *Trefusialaimus* within the Trefusiina, it does not provide conclusive evidence indicating which clade they should be assigned to because of generally weak Maximum Likelihood support values.

Phylum Nematoda Diesing, 1861
Class Enoplea Inglis, 1983
Subclass Enoplia Pearse, 1942
Order Enoplida Filipjev, 1929
Suborder Trefusiina Siddiqi, 1983

Diagnosis (modified from Smol & Coomans 2006)

Cuticle smooth or striated, except in Xennellidae De Coninck, 1965 where it is annulated. Metanemes absent, except in Trischistomatidae. Amphids usually non-spiral; spiral only in some Trefusiinae Gerlach, 1966 and Halanonchinae Wieser & Hopper, 1967. Outer labial and cephalic sensilla setiform and usually positioned in two well-separated circles (except in *Trefusialaimus*, Lauratonematidae and *Tripylina*). No cephalic capsule, except in *Xennella* which has a non-annulated cephalic capsule formed by thickening of body cuticle. Buccal cavity usually without teeth (except in Trischistomatidae and Lauratonematidae). Opening of pharyngeal glands unknown in most species, near buccal cavity in *Trefusialaimus*. Male reproductive systems usually with two testes (monorchic in *Trefusialaimus* and Trischistomatidae). Female reproductive system monorchic or diorchic; ovaries reflexed except in *Cytolaimium exile* Cobb, 1920 and *Trefusialaimus idrisi* where they are outstretched. Caudal glands (when present) lie completely within the tail or position unknown.

Remarks

Lorenzen (1981) erected the order Trefusiida, which was subsequently the lowered to the level of suborder within the Enoplida in the classification of De Ley & Blaxter (2004) based on analyses of 18S rRNA sequences (Rusin *et al.* 2001) and following Siddiqi (1983). The Trefusiida was not considered monophyletic by Lorenzen (1981) because it is not characterized by any character which is apomorphic for that taxon. The order, as defined by Lorenzen (1981), originally comprised the following families: the Simpliconematidae, Xennellidae, Lauratonematidae, Trefusiidae and Onchulidae Andrássy, 1964. The Onchulidae, which is comprised of terrestrial and freshwater species but no marine species, has since been moved to the order Triplonchida Cobb, 1920 in the classification of De Ley & Blaxter (2002; 2004). The Onchulidae is characterised by spicules surrounded by a muscular pouch (or ‘capsule’), a trait which differentiates the Triplonchida from the Enoplida (De Ley & Blaxter 2002).

The family Simpliconematidae comprises a single genus with a single species described from a single male specimen. *Simpliconema aenigmatoides* Blome & Schrage, 1985 was placed within the Trefusiida by Lorenzen (1981, 1994) based on the presence of three lips, the pharyngeal glands seemingly opening in the frontal part of the pharynx, the absence of metanemes and having secretory-excretory gland located in the pharyngeal region. Lorenzen (1981, 1994), however, also noted similarities with the genus *Linhystera* Juario, 1974, family Xyalidae Chitwood, 1951, order Monhysterida Filipjev, 1929, in the arrangement of cephalic sensilla, amphid shape, location of secretory-excretory gland and single anterior testis to the left of the intestine.

The family Xennellidae comprises two genera, *Xennella* Cobb, 1920 and *Porocoma* Cobb, 1920, comprising four and one species, respectively. This group is unusual in having a cephalic capsule (*Xennella*) and an annulated cuticle with longitudinal ridges (both genera) but is similar to most Trefusiina in having outer labial and cephalic setae in separate circles and pocket-shaped amphideal fovea, as well as lacking metanemes (Lorenzen 1981).

The family Lauratonematidae comprises three genera (*Lauratonema*, *Lauratonemella* Tchesunov, 1984 and *Lauratonemoides* De Coninck, 1965, together comprising 13 species) which differ in the structure of the male copulatory apparatus and/or female reproductive system. The Lauratonematidae differ from most other Trefusiina taxa (except *Tripylina* and *Trefusialaimus*) in having the outer labial setae and cephalic setae in a single circle. The family is also characterized by unique features within the Enoplida, i.e., vulva either very close to anus or with female genital branch joining the cloaca, presence of only one posterior testis in some species, and ovary always to the left of the intestine and posterior testis always to the right of the intestine (Lorenzen 1981). The placement of this group in the SSU phylogenetic tree indicates no relationship with the Trefusiina; instead it appears to be closely related to *Anoplostoma* (see Fig. 1). However, due to the weak support for this placement, we leave the Lauratonematidae within the Trefusiina until more conclusive evidence for an alternative classification is provided.

The family Trischistomatidae comprises two genera: *Tripylina* with 22 valid species and *Trischistoma* with 17 valid species. Phylogenies based on SSU sequences consistently show that *Trischistoma* and *Tripylina* form a monophyletic clade with the Trefusiidae, which, along with morphological similarities discussed below, strongly indicate that the Trischistomatidae should be included in the Trefusiina.

The family Trefusiidae comprises two subfamilies, six genera (*Africanema*, *Cytolaimium* Cobb, 1920, *Halanonchus* Cobb, 1920, *Rhabdocoma*, *Trefusia* and *Trefusialaimus*) and 34 valid species. It is characterised mainly by plesiomorphic traits (i.e., jointed outer labial sensilla, buccal cavity without teeth) and was considered not monophyletic by Lorenzen (1981). However, Vincx & Vanreusel (1989) considered the presence of the ventral gland in the pharyngeal region as an apomorphic character for the Trefusiidae within the Trefusiina.

Superfamily Trefusioidea Gerlach, 1966
Family Simpliconematidae Blome & Schrage, 1985

Diagnosis (from Blome & Schrage 1985)

Cuticle striated. Labial region divided into three lips. Cephalic sensilla with 6+10 arrangement; setose outer labial and cephalic sensilla. Circular amphideal fovea. Male reproductive system monorchic with anterior testis to the left of the intestine; sperm cell drop-shaped. Spicules slender, elongated; gubernaculum absent. Tail filiform. Females not known.

Type genus

Simpliconema Blome & Schrage, 1985.

Remarks

Simpliconema is characterized by cephalic and caudal regions similar to those of *Marisalbinema* Tchesunov, 1990 (family Xyalidae; Fig. 2), which was described after the treatment of the Trefusiida by Lorenzen (1981, 1994), while the long slender spicules are similar to those of *Paramonohystera* Steiner, 1916 (Xyalidae). More broadly, *Simpliconema* is characterized by features which resemble the Monhysterida more closely than the Trefusiina, including a circular amphideal fovea (within the Trefusiina, the amphideal fovea is circular only in some Trefusiidae genera, namely species of *Cytolaimium*, *Trefusia*, and *Rhabdocoma*), 6+10 arrangement of the anterior sensilla (usually 6+6+4 in the Trefusiina, except in the Lauratonematidae, *Trefusialaimus* and *Tripylina*), and the presence of

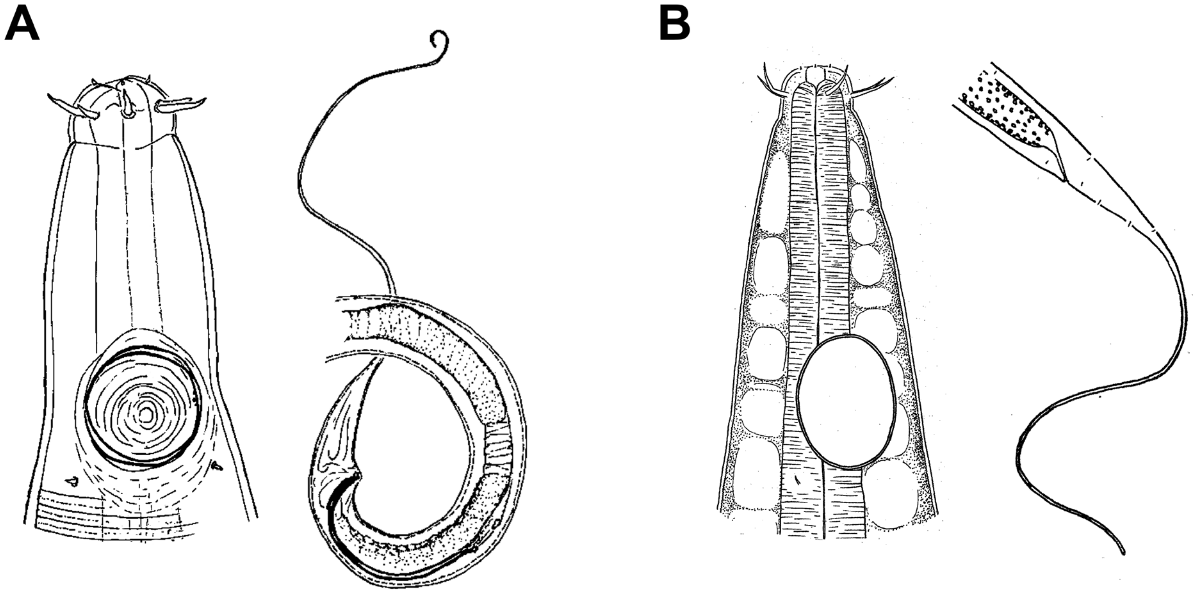

Fig. 2. Anterior and posterior body regions. **A**. *Simpliconema aenigmatodes* Blome & Schrage, 1985. **B**. *Marisalbinema galtsovae* Tchesunov, 1990. Drawings modified from Blome & Schrage (1985) and Tchesunov (1990).

only one anterior testis (usually two testes in Trefusiina, except some Lauratonematidae, *Trefusialaimus*, and Trischistomatidae). Blome & Schrage (1985) also noted differences with the Trefusiidae, such as the structure of the sperm (drop-shaped vs elongated in the Trefusiidae) and differentiated vas deferens (vs undifferentiated in the Trefusiidae). It appears likely that the taxonomic placement of the Simpliconematidae will need to be updated. Morphological information on the structure of the female reproductive system should allow us to settle the placement of this genus.

Genus ***Simpliconema*** Blome & Schrage, 1985

Diagnosis

Simpliconematidae. One species.

Type species

Simpliconema aenigmatoides Blome & Schrage, 1985.

Family Xennellidae De Coninck, 1965

Diagnosis (emended from Lorenzen 1981)

Cuticle annulated except in *X. cephalata* Cobb, 1920 where it is apparently smooth; longitudinal ridges on cuticle usually present. Amphideal fovea pocket-shaped, horseshoe-shaped or circular. Anterior sensilla arranged in a 6+6+4 pattern; long, setose outer labial and cephalic sensilla of similar length. Tapering cephalic capsule present (*Xennella*) or absent (*Porocoma*); when present, cephalic capsule offset from rest of body by constriction, cuticular discontinuity and/or thickened cuticle. Buccal cavity minute, tubular, without teeth. Female reproductive system monodelphic (*Xennella*) or didelphic (*Porocoma*). Male reproductive system monorchic (at least in *Xennella suecica* Allgén, 1935); spicules short, arcuate; gubernaculum present or absent, precloacal supplements present or absent.

Type genus

Xennella Cobb, 1920.

Remarks

De Coninck (1965) erected the subfamily Xennellinae, which originally only contained the genus *Xennella*. He placed the subfamily within the family Dasynemellidae De Coninck, 1965, order Desmodorida De Coninck, 1965. The subfamily was later raised to family by Gerlach & Riemann (1973/1974). The Xennellidae was placed within the Trefusiida by Lorenzen (1981) based on the pocket-shaped amphid and absence of metanemes. This classification was followed by De Ley & Blaxter (2004).

Within the Enoplida, an annulated cuticle is found in the genus *Cricohalalaimus* Bussau, 1993 (Oxystominidae, suborder Ironina Siddiqi, 1983), and the Lauratonematidae (suborder Trefusiina) are characterized by a "distinctly striated" cuticle. A cephalic capsule is present only within the suborder Enoplina, which is formed by the muscles of the anterior end of the pharynx attaching to the body cuticle. In *Xennella*, there is no evidence of any attachment between the pharynx and the body cuticle; instead, the cephalic capsule appears to be formed solely by the thickening of the cuticle. This would suggest that this genus may be better placed within one of the marine chromadorean orders, instead of within the Enoplida. It is possible that the placement of *Xennella* and *Porocoma* will need to be updated in the future as more morphological and molecular data become available.

Genus ***Xennella*** Cobb, 1920

Diagnosis

Xennellidae. Cuticle annulated or smooth; longitudinal ridges on cuticle present or absent. Tapering cephalic capsule offset from rest of body by constriction, cuticular discontinuity and/or thickened cuticle. Female reproductive system with reflexed anterior ovary and rudiment of posterior genital branch. Male reproductive system monorchic (at least in *X. suecica* Allgén, 1935). Spicules short, arcuate; gubernaculum present or absent, precloacal supplements present or absent. Four species.

Type species

Xennella cephalata Cobb, 1920.

Other valid species

X. filicaudata Allgén, 1954.
X. metallica Tchesunov, 1988.
X. suecica Allgén, 1935.

Remarks

In the classification of Filipjev (1925; 1934), *Xennella* was placed together with the genus *Tycnodora* Cobb, 1920, which has since been synonymized with *Halalaimus* de Man, 1888 by Lorenzen (1981), and *Schistodera* Cobb, 1920, which has since been synonymized with *Oxystomina* Filipjev, 1918 (family Oxystominidae Chitwood, 1935) by Hope & Murphy (1972). This placement reflected the apparently smooth cuticle of *X. cephalata*, although the cuticle of *X. suecica* and *X. metallica* is clearly annulated. De Coninck (1937) later provided a detailed description of the males of *X. suecica*, and indicated close similarities between *Xennella* and *Dasynemoides* Chitwood, 1936 based on the annulated cuticle with longitudinal ridges, structure of the cephalic capsule and arrangement of anterior sensilla.

The changing classification of *Xennella* partly stems from uncertainty regarding the structure of the amphids. While Cobb (1920) shows a pocket-shaped amphideal fovea in his original description of *X. cephalata* (which indicates relationships with the Enoplida), De Coninck (1965) shows a rounded amphideal fovea in *X. suecica* (which, together with other features, could indicate relationships with either the Desmodoridae Filipjev, 1922, Ceramonematidae Cobb, 1933 or Monoposthiidae Filipjev, 1934). Other authors only show an almond-shaped amphideal aperture without showing the structure of the amphideal fovea (Allgén 1935; Tchesunov 1988), which may have been obscured by the thick cuticle of the cephalic capsule (De Coninck 1937).

Genus ***Porocoma*** Cobb, 1920

Diagnosis (from Gerlach 1962 and Cobb 1920)

Xennellidae. Cuticle annulated with longitudinal ridges. Cephalic capsule absent. Secretory excretory pore lies on a setiform elevation. Amphideal fovea horseshoe-shaped. Female reproductive system with two posterior ovaries, one of which extends anterior to vulva and folds posteriorly. Number and structure of male genital branch(es) unknown. Spicules short, arcuate. Tail conicocylindrical. One species.

Type species

Porocoma striata Cobb, 1920.

Remarks

This genus was considered closely related to *Oxystomina* by Wieser (1953), presumably due to the shape and arrangement of the cephalic sensilla, the minute buccal cavity and body shape. *Porocoma* was included in the Oxystominidae in the classification of Hope & Murphy (1972) but was later moved to the family Xennellidae by Lorenzen (1981) based on similarities with *Xennella* in the shape and arrangement of cephalic sensilla and presence of longitudinal cuticular ridges.

Family Lauratonematidae Gerlach, 1953

Diagnosis (emended from Lorenzen 1981)

Cuticle distinctly striated. Metanemes absent. Outer labial setae and cephalic setae arranged in one circle. Amphideal fovea non-spiral, pocket- or club-shaped. Buccal cavity usually cuticularized; funnel-shaped pharyngostoma, sometimes with small teeth, cheilostoma cylindrical, shallow or deep. Secretory-excretory system either restricted to pharyngeal region or extends further posteriorly. Female reproductive system monodelphic with anterior reflexed ovary to the left of the intestine; vulva located very close to anus (*Lauratonemoides*) or ending in the cloaca (*Lauratonema* and *Lauratonemella*). Male reproductive system monorchic or diorchic, posterior testis always to the right of the intestine; precloacal supplements absent. Spicules short, straight or only slightly bent; gubernaculum present or absent. Caudal glands lie completely within the tail. Tail conical or conicocylindrical.

Type genus

Lauratonema Gerlach, 1953.

Remarks

Lorenzen (1981) states that this family is characterised by a monorchic male reproductive system; however, more recent species descriptions (Tchesunov 1984; Fadeeva 1989; Chen & Guo 2015) show the presence of two opposed testes in some *Lauratonema*, *Lauratonemoides,* and *Lauratonemella* species. The family was revised by Tchesunov (1984), who provided a key to species of the family.

Genus ***Lauratonema*** Gerlach, 1953

Diagnosis

Lauratonematidae. Female gonad and intestine ending in the cloaca. Gubernaculum (when present) reduced, without apophyses. Ten species.

Type species

Lauratonema reductum Gerlach, 1953.

Other valid species

L. adriaticum Gerlach, 1953.
L. dongshanense Chen & Guo, 2015.
L. hospitum Gerlach, 1956.
L. juncta Fadeeva, 1989.
L. macrostoma Chen & Guo, 2015.
L. mentulatum Wieser, 1959.
L. obtusicaudatum Murphy & Jensen, 1961.
L. pugiunculus Wieser, 1959.
L. reniamphidum Hopper, 1961.

Remarks

A recent key to valid species of the genus was provided by Chen & Guo (2015).

Genus ***Lauratonemella*** Tchesunov, 1984

Diagnosis

Lauratonematidae. Female gonad and intestine ending in the cloaca. Male reproductive system with two opposed testes. Asymmetric male copulatory apparatus with left spicule larger than right spicule and left gurbenacular apophysis larger than right apophysis. One species.

Type species

Lauratonemella spiculifer (Gerlach, 1959) Tchesunov, 1984.
= *Lauratonema spiculifer* Gerlach, 1959.

Genus ***Lauratonemoides*** De Coninck, 1965

Diagnosis

Lauratonematidae. Vulva located very close to anus. Two species.

Type species

Lauratonemoides originale (Gerlach, 1956) De Coninck, 1965.
= *Lauratonema originale* Gerlach, 1956.

Other valid species

L. minutus (Platonova, 1971) Tchesunov, 1984.
= *Lauratonema minutum* Platonova, 1971.

Family Trischistomatidae Andrássy, 2007

Diagnosis (from Zhao 2011)

Cuticle smooth, thin, not annulated. Metanemes present. Labial region divided into three lips. Outer labial setae and cephalic setae either in one circle or two separate circles. Amphideal fovea pocket-shaped with slit-like amphideal aperture. Buccal cavity narrow, surrounded by pharyngeal musculature, with three teeth (often only one is visible) in one or two stomatal chambers. Pharynx muscular, cylindrical; cardia present or absent. Female monodelphic with anterior reflexed ovary, with or without post-vulval uterine sac; vulva located at ≥ 59 % of body length from anterior extremity. Male reproductive system monorchic. Spicules narrow, may or may not be enclosed within a muscular pouch; when present, muscle pouch almost completely surrounds spicules. Papillose precloacal supplements present or absent. Spermatozoa elongated or globular, usually with visible nucleus. Tail with three glands and terminal spinneret.

Type genus

Trischistoma Cobb, 1913.

Remarks

The classification of the family was last revised by Zhao (2011). Phylogenies based on SSU sequences consistently show that while *Trischistoma* and *Tripylina* form a monophyletic clade with the Trefusiidae, the two genera do not form a monophyletic clade, and *Trischistoma* is more closely related to the Trefusiidae than *Tripylina* (Holterman *et al.* 2006; Meldal *et al.* 2007; Zhao & Buckley 2009; van

Megen *et al.* 2009; Bik *et al.* 2010, Zhao *et al.* 2012; present study). Morphologically, *Trischistoma* may be considered more similar to Trefusiina than *Tripylina* due to its having the same arrangement of the anterior sensilla (outer labial and cephalic setae in separate circles), as well as having the buccal cavity with minute denticles only (no teeth in Trefusiina); *Tripylina* differs more strongly in the arrangement of the anterior sensilla (outer labial and cephalic setae in one circle) and buccal cavity with larger teeth. *Trischistoma* also shares an unusual spermatozoa morphology (relatively large and/or elongated, elliptical or fusiform, with central rod and/or nucleus near one extremity) with several Trefusiidae species. In *Trischistoma*, spermatozoa have been described for *T. equatoriale* Andrassy, 2006 and *T. tenuissimum* Andrassy, 2011, and they both exhibit this unusual morphology. In the Trefusiidae, similar spermatozoa have been observed in species of *Trefusialaimus* (Riemann 1974; Leduc 2013), *Rhabdocoma* (Ott 1977; Vincx & Vanreusel 1989), and *Trefusia* (Bussau 1993). This kind of spermatozoa morphology has not been observed in *Tripylina*.

The Trischistomatidae is characterised by having a buccal cavity with teeth, a feature not found in any other Trefusiina family except some *Lauratonema*. We therefore propose to retain this family for the time being, despite the morphological differences between the two genera, and SSU phylogenies indicating that *Trischistoma* and *Tripylin*a do not form a monophyletic group.

Genus ***Tripylina*** Brzeski, 1963

Abunema Khera, 1971.

Diagnosis (from Brzeski 1963, Zhao 2009 and Cid del Prado-Vera *et al.* 2012)

Trischistomatidae. Body length 0.8–1.8 mm. Cuticle smooth with numerous minute pores, thin, not annulated. Six longer outer labial setae and four shorter cephalic setae in a single circle. Buccal cavity with dorsal tooth in a stomatal chamber and two subventral denticles anterior or posterior to the latter. Pharyngeal-intestinal valve composed of three glands around anterior portion of intestine. Female reproductive system monodelphic with anterior reflexed ovary, post-vulval uterine sac present or absent; vagina with or without internal cuticularised pieces; vulva at 59–83% of body length from anterior extremity. Males rare; reproductive system monorchic, sperm cells ovoid or drop-shaped. Spicules narrow, sickle-shaped; when present, muscular pouch partially or completely encloses spicules. Papillose precloacal supplements present. Tail short, anteriorly conical and posteriorly cylindrical, strongly bent. Twenty-two species.

Type species

Tripylina arenicola (de Man, 1880) Brzeski, 1963.
  = *Tripyla arenicola* de Man, 1880.
  = *Tripyla* (*Trischistoma*) *arenicola* – Schneider, 1939.
  = *Trischistoma arenicola* – Schuurmans Stekhoven, 1951.

Other valid species

T. bravoae Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo, 2012.
T. gorganensis Asghari, Pourjam, Heydari, Zhao & Ramaji, 2012.
T. iandrassyi Cid del Prado Vera, Ferris & Nadler, 2016.
T. ixayocensis Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo, 2012.
T. kaikoura Zhao, 2009.
T. longa Brzeski & Winiszewska-Ślipińska, 1993.
T. macroseta (Vinciguerra & La Fauci, 1978) Tsalolikhin, 1983.
  = *Trischistoma macroseta* Vinciguerra & La Fauci, 1978.
T. manurewa Zhao, 2009.

T. montecilloensis Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo, 2012.
T. puxianensis Xu, Zhao, Wang & Zheng, 2013.
T. rorkabanarum Cid del Prado Vera, Ferris & Nadler, 2016.
T. sheri Brzeski, 1963.
  = *Tripyla (Trischistoma) sheri* – Khera, 1970.
  = *Trischistoma ursulae* Argo & Heyns, 1973.
  = *Tripylina ursulae* – Tsalolikhin, 1983.
T. stramenti (Yeates, 1972) Tsalolikhin, 1983.
  = *Trischistoma stramenti* Yeates, 1972.
T. tamaki Zhao, 2009.
T. tearoha Zhao, 2009.
T. tlamincasensis Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo, 2012.
T. ursulae (Argo & Heyns, 1973) Tsalolikhin, 1983.
  = *Trischistoma ursulae* Argo & Heyns, 1973.
T. valiathani Tahseen & Nusrat, 2010.
T. yeatesi Zhao, 2009.
T. ymyensis Tahseen & Nusrat, 2010.
T. zhejiangensis Pham, Wang, Zhao & Zheng, 2013.

Remarks

Tripylina was revised Andrássy (1985) who synonymised *Abunema* with *Tripylina*, and more recently by Zhao (2009) and Cid del Prado-Vera *et. al.* (2012). Cid del Prado-Vera *et al.* (2016) provided an updated key to the species of the genus. The muscular pouch found in some *Tripylina* species such as *T. bravoae* and *T. longa* almost completely surrounds the spicules, unlike the muscular pouch found in *Tripyla* (Triplonchida), which surrounds the proximal half of the spicules only. In *Tripylina iandrassyi*, there appears to be no muscle pouch. Males are known only for *T. arenicola*, *T. bravoae*, *T. iandrassyi* and *T. longa*.

Genus ***Trischistoma*** Cobb, 1913

Diagnosis (from Zullini 2006, Andrássy 2007 and Zhao 2011)

Trischistomatidae. Body length 0.6–2.2 mm. Very slender, posterior portion often bent dorsally. Cuticle smooth, thin, not annulated. Six longer outer labial setae and four shorter, thinner cephalic setae in two separate circles. Pharynx strongly muscular. Buccal cavity with minute denticles. Female monodelphic with anterior reflexed ovary, with or without post-vulval uterine sac; vulva located at 67–83% of body length from anterior extremity. Tail bent dorsally, 3–7 times as long as cloacal/anal body diameter, usually more or less S-shaped. Males rare; reproductive system monorchic with single reflexed testis. Spicules not surrounded by muscular pouch. Spermatozoa ellipse-shaped or fusiform, sometimes unusually large, up to 1 cbd long. No or few (1–3) papillose precloacal supplements. Tail conical. Seventeen species.

Type species

Trischistoma pellucidum Cobb, 1913.
  = *Tripyla pellucida* – Micoletzky, 1922.

Other valid species

T. abharensis Asghari, Eskandari, Maafi, Zhao, Alvarez-Ortega & Nadirkhanloo, 2015.
T. corticulensis Cid del Prado-Vera, Ferris & Nadler, 2016.
T. equatoriale Andrássy, 2006.
T. gracile Andrássy, 1985.

= *Trefusia monodelphis* Bussau, 1990.
T. helicoformis Cid del Prado-Vera, Ferris & Nadler, 2016.
T. minor Tahseen & Nusrat, 2010.
T. monohystera (de Man, 1880) Schuurmans Stekhoven, 1951.
= *Tripyla monohystera* de Man, 1880.
= *Tripylina monohystera* (de Man, 1880).
= *Tripylina monohysteroides* Altherr, 1963.
T. otaika Zhao, 2011.
T. ripariana Cid del Prado-Vera, Ferris & Nadler, 2016.
T. subtilissimum Andrássy, 2011.
T. taiguensis Xu, Zhao & Wang, 2015.
T. tenuissimum Andrássy, 2011.
T. riregius Zhao, 2011.
T. ukorehe Zhao, 2011.
T. veracruzense Cid del Prado-Vera, Ferris & Nadler, 2010.
T. waiotama Zhao, 2011.

Species inquirenda

T. conicaudatum Schuurmans Stekhoven, 1951.

Remarks

Trischistoma was revised by Zhao (2011). Asghari *et al.* (2015) provided a table with morphometrics of all *Trischistoma* species, and Cid del Prado-Vera *et al.* (2016) provided a key to the species of the genus. *Trefusia monodelphis* Bussau, 1990 was synonymised with *Trischistoma gracile* by Andrássy (2007).

Family Trefusiidae Gerlach, 1966

Diagnosis (emended from Lorenzen 1981)

Cuticle smooth or faintly striated. Labial region generally divided into three lips. Inner labial sensilla usually papillose, rarely setose, either in separate circle or very close to outer labial sensilla. Outer labial sensilla and cephalic setae usually in separate circles (except *Trefusialaimus*); outer labial setae usually jointed, cephalic setae often located far posteriorly, sometimes posterior to amphids. Amphids either spiral or non-spiral (round or pocket-shaped). Buccal cavity without teeth, either minute to medium size, funnel-shaped and not cuticularised, or large, barrel-shaped and cuticularised. Secretory-excretory system restricted to pharyngeal region (often not observed). Spicules short, curved or straight, with or without capitulum; gubernaculum present or absent, without apophyses (except in *Africanema*). Female reproductive system monodelphic or didelphic; ovaries reflexed (except in *Cytolaimium exile* and *Trefusialaimus idrisi)*. Male reproductive system usually diorchic (monorchic in *Trefusialaimus*). Papilliform, setiform or discoid precloacal and pharyngeal supplements may be present. Caudal glands restricted to tail region.

Type genus

Trefusia de Man, 1893.

Remarks

Gerlach (1966) erected the subfamily Trefusiinae, which he placed within the Oxystominidae and which comprised the genera *Trefusia*, *Rhabdocoma*, *Cytolaimium* and *Halanonchus*. While there had been general agreement by various authors about the placement of *Trefusia* with the Oxystominidae (Filipjev 1934; De Coninck & Schuurmans Stekhoven 1933; Chitwood & Chitwood 1937), the placement of

Cytolaimium, *Rhabdocoma* and *Halanonchus* was more controversial and relationships had been proposed with the families Monhysteridae de Man, 1876, Linhomoeidae Filipjev, 1922 and Tripyloididae Filipjev, 1928 (Filipjev 1934; Chitwood 1936, 1951; Wieser 1956; de Coninck 1965; Riemann 1966). Riemann (1966) noted that although *Rhabdocoma* and *Trefusia* share many similarities, they differ in the structure of the female reproductive system (monodelphic in *Rhabdocoma* and didelphic in *Trefusia*); however, he followed the classification of Gerlach (1966). Wieser & Hopper (1967) subsequently moved *Rhabdocoma*, *Cytolaimium* and *Halanonchus* into the freshly erected subfamily Halanonchinae, which they placed within the family Tripyloididae. They justified this change based on the large buccal cavity (in *Halanonchus*), the spiral amphids (in *Cytolaimium* and *Rhabdocoma*) and the presence of jointed setae (all three genera). They also argued that the presence of deeply incised lips, a trait also found in some Tripyloididae, indicates relationships with Tripyloididae. This argument appears to be mostly based on their observations of deeply incised lips in *Halanonchus macrurus* Cobb, 1920; however, we argue that they have misinterpreted the presence of a cuticular discontinuity in the buccal cavity as deeply incised lips (see below). It is not clear why *Trefusia* was not also moved to the Halanonchinae as it is very similar to *Rhabdocoma* and *Cytolaimium* except for features of the reproductive system. Gerlach & Riemann (1973/74) modified the classification of Wieser & Hopper (1967) by bringing the Halanonchinae together with the Trefusiinae and raising the latter to family status. They also moved *Cytolaimium* and *Rhabdocoma* back to the Trefusiinae, leaving *Halanonchus* as the sole genus within the Halanonchinae. No reason was given for this change, but it seems likely that the subfamilies were re-organised to reflect differences in the buccal cavity (i.e., small and not cuticularized in Trefusiinae vs large and cuticularized in Halanonchinae). *Trefusialaimus*, a genus with a minute buccal cavity, was subsequently described by Riemann (1974) and placed within the Trefusiinae. Vincx & Furstenberg (1988) later described *Africanema*, a genus with a large cylindrical buccal cavity, which they placed within the Halanonchinae.

Shi & Xu (2017) recently proposed moving *Rhabdocoma* to the Halanonchinae based on the presence of only one ovary in both *Rhabdocoma* and *Halanonchus*, and based on the result of phylogenetic analyses of 18S rDNA sequences. They argue that the structure of the female reproductive system is a more taxonomically informative trait for determining relationships among higher taxa than the buccal cavity.

Subfamily Trefusiinae Gerlach, 1966

Diagnosis

Trefusiidae. Outer labial sensilla always setose and jointed, usually in separate circle from cephalic setae (except in *Trefusialaimus*). Amphideal fovea circular, oval, unispiral, cryptospiral, spiral, elongated or pocket-shaped. Buccal cavity small to medium size, funnel-shaped, not cuticularized. Spicules short, arcuate or straight; gubernaculum present or absent, without apophyses. Female reproductive system didelphic with reflexed ovaries, except in *Trefusialaimus* (outstretched). Male reproductive system usually diorchic (monorchic in *Trefusialaimus*).

Genus ***Cytolaimium*** Cobb, 1920

Diagnosis (modified from Cobb 1920 and Ott 1977)

Trefusiinae. Cuticle smooth. Outer labial setae jointed, much longer than cephalic setae; cephalic setae situated at posterior edge of, or posterior to, amphids. Amphids circular, cryptospiral or unispiral. Buccal cavity small to medium-sized, funnel-shaped, not cuticularized. Pharyngeal supplements absent. Pairs of discoid supplements present in pre- and post-cloacal regions. Female reproductive system didelphic with two opposed reflexed ovaries (outstretched in *Cytolaimium exile*). Male reproductive system diorchic. Tail conical, conico-cylindrical or filiform. Two species.

Type species

Cytolaimium exile Cobb, 1920.

Other valid species

C. gerlachi Ott, 1977.
    = *Cytolaimium exile* Cobb, 1920 *sensu* Gerlach, 1962.

Remarks

Cytolaimium is similar to *Trefusia* but can be distinguished from the latter by the presence of pairs of discoid supplements in both pre- and post-cloacal regions. Gerlach (1962) synonymized *C. obtusicaudatum* Chitwood, 1936 with *C. exile*. This was not accepted by Ott (1977) who considered the two species to be morphologically distinct. Gerlach & Riemann (1973/74) moved *Trefusia conica* Gerlach, 1957 to *Cytolaimium*; however, this species lacks the discoid supplements and is therefore considered to belong to *Trefusia* (Ott, 1977). Ott (1977) reviewed the genus and provided a key to species. The latter author also erected *C. gerlachi* to accommodate the specimens described by Gerlach (1962), which he deemed morphologically distinct from *C. exile.* Ott (1977) moved *Rhabdocoma articulata* to *Cytolaimium*, but because this species is known from a juvenile only, we consider it *species inquirenda*.

Genus ***Trefusia*** de Man, 1893

Bognenia Allgén, 1932.

Diagnosis (modified from Leduc 2013)

Trefusiinae. Cuticle smooth or striated. Six jointed outer labial setae situated in separate circle from the cephalic setae; the latter situated either slightly anterior to, at same level as, or posterior to amphids. Amphideal fovea circular, oval, unispiral, spiral, elongated or pocket-shaped. Buccal cavity small or minute, funnel-shaped, not cuticularized. Males with mid-ventral row of pharyngeal supplements that may be papilliform, setiform, or complex; sometimes two additional subventral rows are also present. Precloacal supplements usually present, papilliform or setiform. Arcuate or almost straight spicules, with or without capitulum; gubernaculum present or absent. Male reproductive system diorchic. Female reproductive system with two opposed and reflexed ovaries. Tail conico-cylindrical or filiform. Eighteen species.

Type species

Trefusia longicauda de Man, 1893.

Other valid species

T. americana Keppner, 1992.
T. axonolaimoides Allgén, 1953.
T. conica Gerlach, 1957.
T. cornea Gerlach, 1958.
T. curvispiculosa Vincx & Vanreusel, 1989.
T. filicauda Allgén, 1933.
    = *T. longispiculosa* Bresslau & Schuurmans Stekhoven, 1940.
T. helgolandica Riemann, 1966.
T. honessi Keppner, 1986.
T. litoralis (Allgén, 1932) De Coninck & Schuurman Stekhoven, 1933.
    = *Bognenia litoralis* Allgén, 1932.
T. longicorpa Keppner, 1986.

T. multipapillatum Bouwman, 1981.
T. piperata Leduc, 2013.
T. pseudolitoralis Vitiello, 1970.
T. schiemeri Ott, 1977.
T. spatulata Keppner, 1992.
T. varians Gerlach, 1955.
T. zostericola Allgén, 1933.

Species inquirendae

T. filum Schuurmans Stekhoven, 1942.
T. longiseta Allgén, 1947.
T. nidrosiensis Allgén, 1933.
T. obtusicaudata Allgén, 1933.

Remarks

Keppner (1992) and Leduc (2013) provided keys to the males of the genus based on Riemann (1966). De Coninck & Stekhoven (1933) synonymised *Bognenia* with Trefusia. No male specimens have yet been described for *Trefusia varians*. *Trefusia filum*, *T. longiseta*, *T. nidrosiensis* and *T. obtusicauda* were considered *species inquirendae* by Riemann (1966). The latter author also synonymised *T. longispiculosa* with *T. filicauda*. In his PhD thesis, Bussau (1993) described *T. dominatrix* Bussau, 1993 and *T. attenuata* Bussau, 1993, but these species are considered *nomina nuda* because the descriptions were not published.

Genus ***Trefusialaimus*** Riemann, 1974

Diagnosis (modified from Riemann 1974)

Trefusiinae. Cuticle smooth. Sub-cephalic and somatic setae absent. Four jointed cephalic setae and six jointed outer labial setae in one circle; amphid pocket-shaped. Buccal cavity minute, funnel-shaped, not cuticularized. Male with one anterior outstretched testis (monorchic) and peri-cloacal papillae. Elongated sperm cells with central rod and light-refractive nucleus at one extremity. Female reproductive system (known only for *T. idrisi*) with two opposed and outstretched ovaries. Tail conico-cylindrical or filiform. Three species.

Type species

Trefusialaimus monorchis Riemann, 1974.

Other valid species

T. magnus (Filipjev, 1946) Riemann, 1974.
 = *Trefusia magna* Filipjev, 1946.
T. idrisi Leduc, 2013.

Remarks

Trefusialaimus and *Tripylina* are the only genera of the suborder Trefusiina characterized by having the outer labial sensilla and cephalic setae in a single circle. This character agrees with the diagnosis of the Tripyloidina; however, *Trefusialaimus* differs from the latter in the absence of metanemes (vs metanemes sometimes present in Tripyloidina), and in having pocket-shaped amphids (vs spiral amphids in Tripyloidina) and a toothless buccal cavity (vs teeth common in Tripyloidina). The current placement of *Trefusialaimus* within the Trefusiinae, which follows Riemann (1974) and Lorenzen (1981, 1994), is only tentative and may need to be revised as suggested by SSU phylogenetic analyses (present study).

Trefusialaimus idrisi Leduc, 2013
Table 1; Figs 3–4

Material examined

NEW ZEALAND • 2 ♀♀; western Chatham Rise off the east coast of New Zealand's South Island, Tangaroa voyage TAN0705, station 157, surface (0–5 cm) sandy silt sediments; 42.785º S, 176.715º W; depth 1029 m; 16 Apr. 2007; D. Leduc leg.; NIWA 139242. • 1 ♀; same collection data as for preceding; NNCNZ 3330.

Description

Female

Body cylindrical, slender, tapering slightly towards anterior extremity, with slight golden colouration due to the presence of numerous round, ca 1 µm diameter, golden inclusions. Cuticle smooth. Cephalic region rounded, slightly set off from body due to thickened cuticle and constriction immediately posterior to cephalic setae. Three lips, each bearing two small, conical inner labial papillae. Six outer labial setae and four cephalic setae in one circle, all with single joint; cephalic setae slightly longer than outer labial setae (6–9 vs 7–10 µm). Sub-cephalic and somatic setae absent. Amphid pocket-shaped with transverse aperture, ca 6–9 µm wide by 2 µm high. Buccal cavity funnel-shaped, without teeth. Pharynx cylindrical, slightly wider posteriorly, completely surrounding buccal cavity. Nerve ring situated at 49–66% of pharynx length. Secretory-excretory system not observed. Cardia small, surrounded by intestine. Numerous sperm cells are present throughout the pseudocoelom between pharynx and anus, as well as in the uterus. Reproductive system with two opposed and outstretched ovaries, both to the right or left of intestine. Vagina at about two thirds of body length from anterior. Tail long, ca 8–10% of total body length, narrow, gradually tapering, without setae; spinneret not observed.

Remarks

The female specimens described here agree well with the male specimen described from the central Chatham Rise (350 m depth) in the arrangement of anterior sensilla, size and position of the amphids, presence of numerous golden inclusions and tail shape. The female specimens, however, were characterized by longer bodies (5004–5947 vs 4539 µm) and shorter tails (c′ = 18–21 vs 38).

This is the first time that female *Trefusialaimus* specimens are described. The structure of the female reproductive system in this species, which consists of two opposed and outstretched ovaries, is unusual for the Enoplida, although it has been observed in *Cytolaimium exile* (Trefusiidae), and *Mediolaimus* Tahseen, Sultana, Khan & Hussain, 2012 and *Rogerus* Hoeppli & Chu, 1934 (Enoplida, family Rhabdolaimidae Chitwood, 1951). It is unclear how sperm had entered the pseudocoelom of the female specimens we observed; however, the same observation was made previously for a juvenile of the same species (Leduc 2013).

Subfamily Halanonchinae Wieser & Hopper, 1967

Diagnosis (modified from Wieser & Hopper 1967)

Trefusiidae. Cuticle smooth or striated. Three lips, deeply incised in rare cases. Inner labial sensilla papillose or setose, outer labial setae and cephalic setae in separate circles, outer labial setae usually jointed. Amphideal fovea pocket-shaped, elongated, circular or unispiral. Buccal cavity without teeth; either small, funnel-shaped, not cuticularized (*Rhabdocoma*) or large, cylindrical/barrel-shaped with cuticularized walls (*Africanema*, *Halanonchus*). Male reproductive system diorchic with outstretched testes. Spicules short, arcuate or straight; gubernaculum present or absent, with or without apophyses;

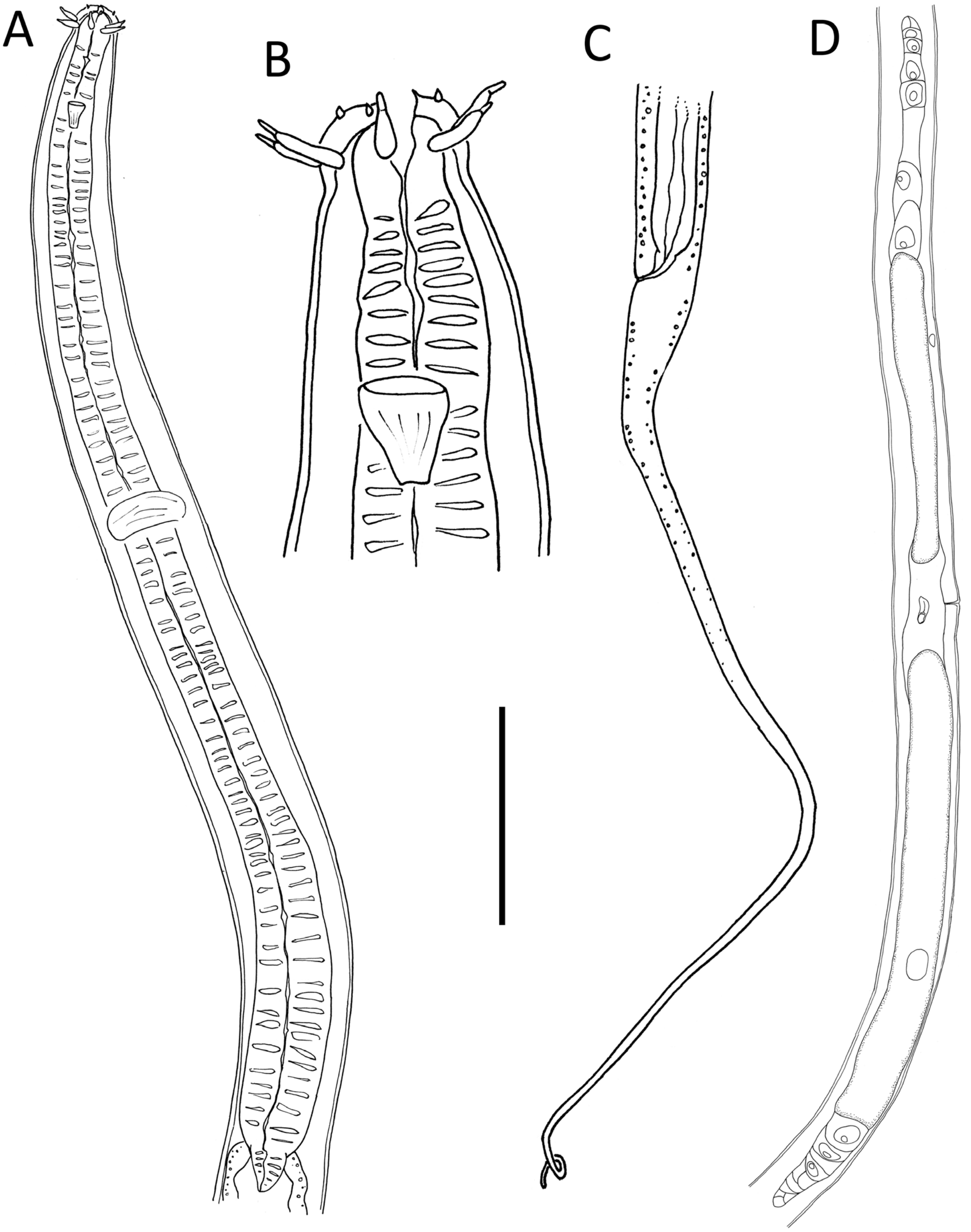

Fig. 3. *Trefusialaimus idrisi* Leduc, 2013, ♀ (NIWA139242). **A**. Anterior body region. **B**. Cephalic region. **C**. Posterior body region. **D**. Reproductive system. Scale bars: A = 50 µm; B = 20 µm; C = 90 µm; D = 125 µm.

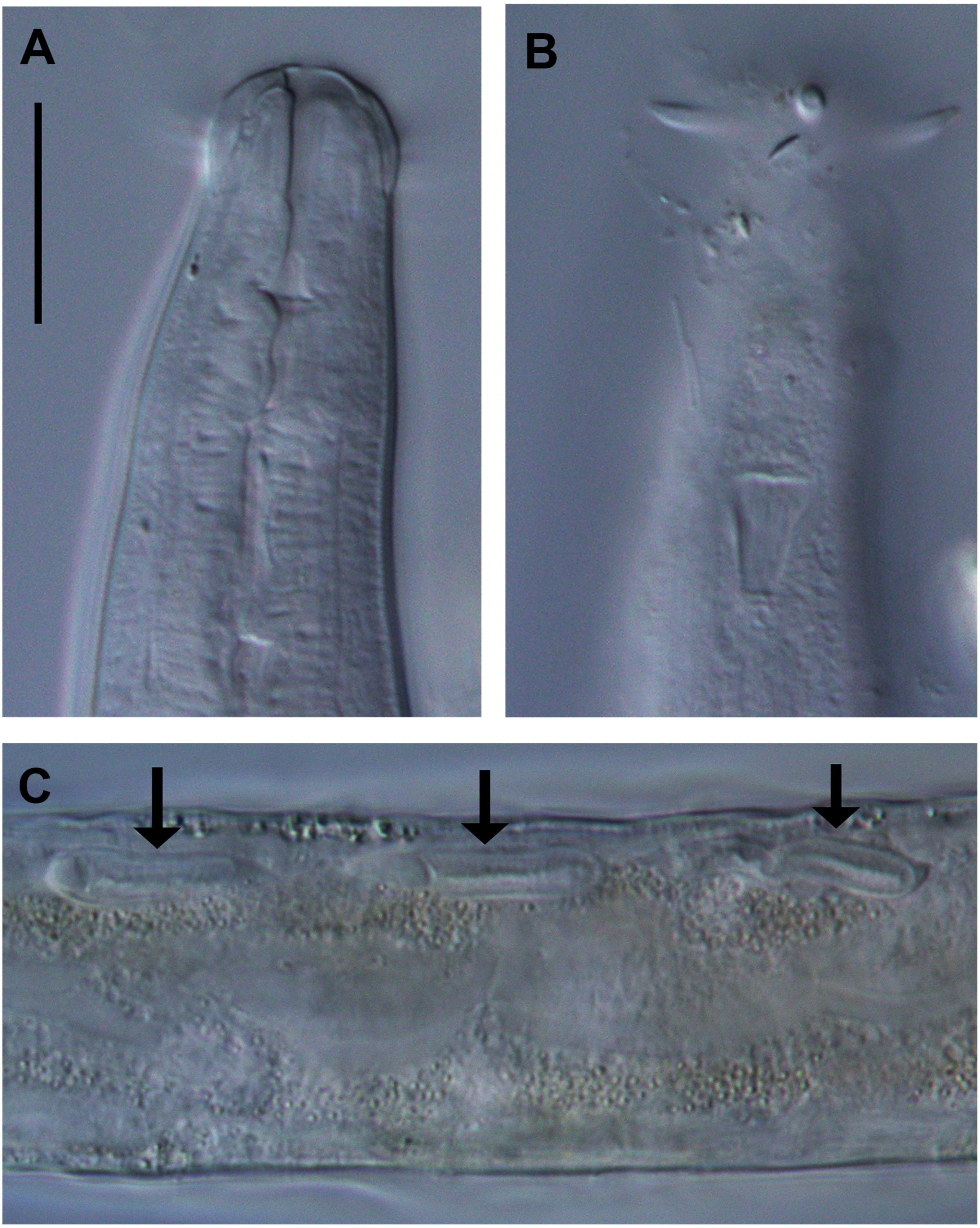

Figure 4. *Trefusialaimus idrisi* Leduc, 2013, ♀ (NIWA139242), light micrographs. **A**. Optical cross-section of cephalic region showing buccal cavity, lips and pharynx. **B**. Surface view of cephalic region showing outer labial setae and amphid. **C**. Mid-body region showing intestine and sperm cells in pseudocoelom (arrows). Scale bars: A–B = 15 µm; C = 22 µm.

Table 1. Morphometrics (µm; mean (range)) of female *Trefusialaimus idrisi* Leduc, 2013 from the continental slope of New Zealand. Abbreviations see Material and methods.

	Females
n	3
L	5517 (5004–5947)
a	143 (137–149)
b	15 (14–15)
c	11 (10–12)
c′	19 (18–21)
Head diam. at cephalic setae	14 (13–15)
Head diam. at amphids	22 (17–26)
Length of outer labial setae	8 (6–9)
Length of cephalic setae	9 (7–10)
Amphideal fovea height	10 (9–11)
Amphideal fovea width	8 (6–9)
Amphid width/cbd (%)	37 (35–41)
Amphid from anterior end	28 (27–28)
Nerve ring from anterior end	194 (173–232)
Nerve ring cbd	37 (33–40)
Pharynx length	373 (353–389)
Pharyngeal diam. at base	24 (23–26)
Pharynx cbd at base	36 (33–38)
Max. body diam.	39 (35–41)
Anal body diam.	26 (22–30)
Tail length	506 (418–557)
V	3550 (3327–3910)
%V	64 (61–66)
Vulval body diam.	39 (35–41)

papillose precloacal supplements (complex supplements in *H. bullatus* Gerlach, 1964) present or absent. Female reproductive system with single posterior reflexed ovary.

Remarks

This subfamily is not monophyletic according to the SSU consensus tree (see Fig. 1). However, it is retained at least for now because Halanonchinae is unique within the Trefusiina in having a female reproductive system with a single posterior ovary.

Genus ***Africanema*** Vincx & Furstenberg, 1988

Diagnosis (from Vincx & Furstenberg 1988 and Shi & Xu 2017)

Halanonchinae. Cuticle striated. Lips may be deeply incised. Anterior sensilla in three separate circles; jointed or simple inner labial sensilla, jointed outer labial setae at level of buccal cavity, and jointed or simple cephalic setae at level of amphids. Amphideal fovea elongate. Buccal cavity large, cylindrical, with thickly cuticularized walls, uncompartmentalized, without teeth. Papillose pharyngeal supplements may be present. Papillose precloacal supplements present. Gubernaculum present or absent. Two species.

Type species

Africanema interstitiale Vincx & Furstenberg, 1988.

Other valid species

A. multipapillatum Shi & Xu, 2017.

Genus ***Rhabdocoma*** Cobb, 1920

Diagnosis (from Ott 1977)

Halanonchinae. Amphideal fovea circular or unispiral, cephalic setae anterior to or posterior to amphids. Buccal cavity small, funnel-shaped. Papillose pharyngeal and precloacal supplements present or absent. Male reproductive system diorchic. Tail conical, conico-cylindrical or filiform. Two species.

Type species

Rhabdocoma americana Cobb, 1920.
  = *Rhabdocoma riemanni* Jayarsee & Warwick, 1977.

Other valid species

R. obtusicaudata (Chitwood, 1936) Ott, 1977.
  = *Cytolaimium obtusicaudatum* Chitwood, 1936.
  = *Cytolaimium exile* Cobb, 1920 in Ott (1972).
  = *Cytolaimium exile* Cobb, 1920 in Ott & Schiemer (1973).

***Species inquirenda*e**

R. articulata (Gerlach, 1955).
R. brevicauda Schuurmans Stekhoven, 1950.
R. cylindricauda Schuurmans Stekhoven, 1950.
R. macrura Cobb, 1920.

Remarks

Ott (1977) reviewed the genus and provided a key to species. He considered *R. macrura* to be insufficiently described and therefore *species inquirenda*. Ott (1977) also considered *R. cylindricauda* and *R. brevicauda* to have been wrongly assigned to *Rhabdocoma* and instead likely to belong to the Siphonolaimidae. *Rhabdocoma articulata* was transferred to *Cytolaimium* by Ott (1977), but the species is doubtful since it is based on a juvenile description only. *Rhabdocoma riemanni* Jayarsee & Warwick, 1977 was described subsequently to the review by Ott (1977) but it was later synonymized with *R. americana* by Vincx & Vanreusel (1989).

Genus ***Halanonchus*** Cobb, 1920

Latilaimus Allgén, 1933.

Diagnosis (modified from Pavlyuk 1984)

Halanonchinae. Cuticle smooth. Brown or golden granules often present along lateral, ventral and dorsal chords. Inner and outer labial sensilla either very close to each other or in separate circles. Inner labial sensilla papillose or setose; outer labial setae sometimes jointed; cephalic setae situated in separate circle further posteriorly but anterior to amphids. Buccal cavity large, barrel-shaped; both gymnostoma and stegostoma with cuticularized walls, often with curved cuticular discontinuity between gymnostoma amd stegostoma, which has been interpreted as “oval structures” supporting the buccal cavity. Amphideal fovea pocket-shaped, at level of buccal cavity or posterior to buccal cavity; oval or rounded amphideal aperture. Mid-ventral row of papillose pharyngeal supplements (complex supplements in *H. bullatus* Gerlach, 1964) usually present in males. Male copulatory apparatus consists of short, arcuate or straight spicules, and small gurbenaculum without apophyses; precloacal supplements present. Tail long and filiform. Seven species.

Type species

Halanonchus macrurus Cobb, 1920.

Other valid species

H. arenarius Pavlyuk, 1984.
H. bullatus Gerlach, 1964.
H. cornutus Vitiello, 1971.
H. longicaudatus (Allgén, 1935) Gerlach, 1964.
    = *Eumorpholaimus longicaudatus* Allgén, 1935.
H. papilatus Groza-Rojancovski, 1972.

Species inquirendae

H. macramphidus Chitwood, 1936.
H. renatus (Timm, 1961) Gerlach, 1964.
    = *Latilaimus renatus* Timm, 1961.
H. zosterae (Allgén, 1933) Gerlach, 1964.
    = *Latilaimus zosterae* Allgén, 1933.

Remarks

Latilaimus was synonymized with *Halanonchus* by Gerlach (1964). Pavlyuk (1984) considered *H. renatus* invalid due to the incomplete description, which did not include male specimens. We consider *H. macramphidus* and *H. zosterae* to be *species inquirendae* for the same reason. No females have yet been described for *H. longicaudatus* or *H. papilatus*.

Although the presence of pharyngeal supplements is given as a genus character by Pavlyuk (1984), this feature is absent in *H. longicaudatus*. Furthermore, only one inconspicuous pharyngeal supplement is present in *H. cornutus* and pharyngeal supplements were not observed in all male *H. scintillatulus* sp. nov. specimens. We also note that, within the genus, inner labial sensilla can be either papillose or setose, and can be situated either very close to the outer labial sensilla or in a separate circle.

Wieser & Hopper (1967) listed deeply incised lips as a diagnostic feature of the Halanonchinae. The latter authors included *Cytolaimium, Rhabdocoma* and *Halanonchus* in the subfamily, which they classified with the Tripyloididae. This family includes the genus *Bathylaimus* Cobb, 1894, which is characterized by deeply incised lips. This feature was later included in the diagnosis of *Halanonchus* provided by Pavlyuk (1984). We did not observe this feature in the *Halanonchus* specimens from the Hauraki Gulf, however, and did not see it clearly described in any description of *Halanonchus* species. We postulate that the presence of deeply incised lip was a misinterpretation of buccal cavity structures. We also believe that the description of oval structures supporting the buccal cavity resulted from a similar misinterpretation, as explained below.

Oval structures supporting the buccal cavity were first illustrated in the description of *H. macrurus* by Cobb (1920). Unfortunately, the nature of these structures was not described or interpreted by the latter author. In his description of *H. macramphidus* Chitwood, 1936, Chitwood (1936) described a buccal cavity similar to that described by Cobb (1920), but without any oval structure. Chitwood's illustration shows instead a fine line demarcating the gymnostoma and stegostoma, which is consistent with a cuticular discontinuity in the buccal cavity wall. Gerlach (1964) included oval structures in his illustration of the buccal cavity of *H. bullatus* Gerlach, 1964, the posterior edge of which coincides with a cuticular discontinuity between the gymnostoma and stegostoma as seen in cross-section. He did not describe or interpret, however, the nature of these oval structures beyond illustrating them. Oval structures were first explicitly discussed by Wieser & Hopper (1967) in their decription of *H. macrurus*. They stated that: "Each lip seems to be supported by a large oval structure which apparently was mistaken for the amphids by Allgén (1933) in his description of *Latilaimus zosterae"* (Wieser & Hopper 1967: 249). Their illustration shows a single oval structure, with a posterior margin coinciding with the limit between gymnostoma and stegostoma. Their illustration also shows two thin longitudinal lines spanning the length of the gymnostoma and stegostoma, which touch one side of the oval structure. These lines may have been interpreted by these authors as indicating the presence of deeply incised lips in this species. Our own observations, as well as the descriptions by Cobb (1920), Chitwood (1936), and Groza-Rojancovski (1972) in particular, indicate to us that the gymnostoma and stegostoma in *Halanonchus* are delimited by a curved cuticular discontinuity. In the case of *H. scintillatulus* sp. nov., the two buccal cavity compartments are also delimited by the more thickly cuticularized anterior edge of the stegostoma, which projects slightly into the buccal cavity (Fig. 5). In addition, our own observations, as well as the illustrations of Wieser & Hopper (1967) and Pavlyuk (1984), indicate that the ventrosublateral and dorsal sectors of the gymnostoma and stegostoma are delimited by longitudinal cuticular discontinuities. We hypothesise that it is the presence of these cuticular discontinuities which led previous authors to describe the presence of three oval structures supporting the buccal cavity in *Halanonchus* species, as well as the presence of deeply incised lips.

Halanonchus scintillatulus Leduc sp. nov.

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Tables 2–3; Figs 5–8

Diagnosis

Halanonchus scintillatulus sp. nov. is characterized by body length 1385–1815 µm, papillose inner labial sensilla very close to outer labial setae, cephalic setae 0.20–0.33 cbd long, amphids located posterior to buccal cavity, buccal cavity with discontinuity between gymnostoma and stegostoma walls, slight tooth-like projection of anterior edge of stegostoma wall and tail 440–623 µm long. Males with row of 6–12 pharyngeal supplements, 11–21 precloacal supplements and arcuate spicules 1.1–1.3 cbd long; females with vulva at 31–34% of body length from anterior extremity.

Differential diagnosis

Halanonchus scintillatulus Leduc sp. nov. is the smallest species of the genus, with a body length less than 2000 µm (Table 3). The new species is similar to *H. arenarius* and *H. longicaudatus* in having papillose inner labial sensilla, but differs from both species in having relatively low values of a (38–58 vs >70 in *H. arenarius* and *H. longicaudatus*) and arcuate spicules (vs straight or almost straight spicules in *H. arenarius* and *H. longicaudatus*). *Halanonchus scintillatulus* sp. nov. also differs from *H. arenarius* in amphid size (22–33% vs 14% cbd in *H. arenarius*), length of cephalic setae and outer labial setae (3–4 µm vs 5–7 µm in *H. arenarius*) and number of precloacal supplements (11–21 vs 9 in *H. arenarius*); the new species also differs from *H. longicaudatus* in amphid size (22–33% vs 50% cbd in *H. longicaudatus*) and position (posterior to buccal cavity vs at level of anterior half of buccal cavity in *H. longicaudatus*). *Halanonchus scintillatulus* sp. nov. is similar to *H. bullatus* in the arrangement and size of the cephalic and inner labial setae, amphid size and position, and spicule size and shape, but can be distinguished from the latter by the lower values of a (38–58 vs 87 in *H. bullatus*) and b (6–7 vs 10 in *H. bullatus*), shorter tail (440–623 µm vs 1270 µm in *H. bullatus*; c' = 21–26 vs 55 in *H. bullatus*),

Table 2. Morphometrics (µm; mean (range)) of *Halanonchus scintillatulus* Leduc sp. nov. from Hauraki Gulf. Abbreviations see Material and methods.

	Males		**Females**
	Holotype	Paratypes	Paratypes
n	1	5	4
L	1589	1595 (1385–1815)	1633 (1564–1708)
a	59	54 (50–58)	42 (38–45)
b	6	6 (6–7)	7 (6–7)
c	3	3	3
c′	23.3	23.1 (20.9–26.0)	25.5 (22.9–29.5)
Head diam. at cephalic setae	14	13 (12–15)	13 (12–14)
Head diam. at amphids	18	18 (16–19)	19 (18–20)
Length of cephalic setae	3–4	4 (3–4)	4 (3–4)
Amphideal aperture height	4	4 (4–5)	4
Amphideal fovea width	5	5 (4–6)	4 (4–5)
Amphid width/cbd (%)	28	28 (22–33)	25 (22–26)
Amphid from anterior end	21	21 (18–22)	19 (18–20)
Nerve ring from anterior end	117	110 (104–117)	112 (106–115)
Nerve ring cbd	27	27 (23–29)	30 (29–30)
Pharynx length	259	248 (234–256)	244 (242–248)
Pharynx diam. at base	18	19 (18–20)	20 (18–21)
Pharynx cbd at base	27	28 (24–30)	31 (30–32)
Pharyngeal supplements #	12	7 (0–11)	–
Max. body diam.	27	30 (27–34)	39 (38–42)
Spicule length	30	26 (23–28)	–
Gubernaculum length	9	8 (7–9)	–
Precloacal supplements #	21	12 (9–16)	–
Cloacal/anal body diam.	23	23 (21–24)	21 (20–23)
Tail length	536	524 (440–623)	533 (480–589)
V	–	–	356 (338–365)
%V	–	–	33 (31–34)
Vulval body diam.	–	–	33 (31–34)

fewer pharyngeal supplements (6–12 vs 13–16 in *H. bullatus*) and vulva position (%V = 31–34 vs 16 in *H. bullatus*).

Etymology

The species name is derived from the latin term '*scintillula'*, diminutive of '*scintillo'* (= sparkle, glitter), and refers to the numerous small, light refractive granules present along the body of most specimens of this species.

Table 3. Key morphological characters of all valid *Halanonchus* species. Abbreviations: N = no; ND = no data; Y = yes; otherwise, see Material and methods.

	H. arenarius	***H. bullatus***	***H. cornutus***	***H. longicaudatus***	***H. macrurus***	***H. papilatus***	***H. scintillatulus* Leduc sp. nov.**
Type locality	Sea of Japan	Red Sea	Rhone River mouth	Baltic Sea	Biscayne Bay	Black Sea	Hauraki Gulf, NZ
Body length (µm)	4400–4600	2975	1904–1920	2425	2000–2140	2600–2800	1385–1815
a	72–105	87	60–64	73	50	74–80	38–58
b	7–10	10	9–10	9	8	8	6–7
c	3–5	<3	<3	4	3	<3	3
c′	ND	55	39–45	ND	22	ND	21–26
ils and ols in single circle?	N	Y	Y	ND	Y	N	Y
ils	Papillose	Setose	Setose	Papillose	Setose	Setose	Papillose
ols length (µm)	5	5	5	ND	3–4	6	3
Ceph. setae length (µm)	7	5	3	ND	5	7	3–4
Amphid width (% cbd)	14	25	29–45	50	14	40	22–33
Amphid position	At posterior edge of buccal cavity, or slightly posterior to buccal cavity	At posterior edge of buccal cavity, or slightly posterior to buccal cavity	Posterior to buccal cavity	At anterior half of buccal cavity	At posterior edge of buccal cavity	Posterior to buccal cavity	Posterior to buccal cavity
# pharyngeal supplements	15	13–16	1	Absent	5–6	21	6–12 (when present)
# precloacal supplements	9	11+4+7–8	7	12	12	14	11–21
Spicule shape	Almost straight	Arcuate	Straight	Straight	Arcuate	Almost straight	Arcuate
Spicule length/cloacal body diameter	0.8	1.4	0.8	1.1–1.4	1.3	1.1	1.1–1.3
%V	31–43	16	20	ND	18–33	ND	31–34
Tail length (µm)	1200–1300 (males), 746–760 (females)	1270	806–858	657	610–942	1100	440–623

Material examined

Holotype

NEW ZEALAND • ♂; North Island, Firth of Thames, Kaharoa voyage KAH0310, site SD5, station 3400C, surface (0–5 cm) muddy sediments; 36.9133º S, 175.4983º E; depth 5 m; 13 Dec. 2003; D. Leduc leg.; NIWA 139240.

Paratypes

NEW ZEALAND • 2 ♂♂, 2 ♀♀; same collection data as for holotype; NIWA 139241. • 3 ♂♂, 2 ♀♀; same collection data as for preceding; NNCNZ 3325 to 3329.

Description

Male

Body cylindrical, slender, colourless except for presence in most specimens of numerous light refractive granules along lateral, ventral and dorsal chords, either along entire body or in pharyngeal and tail regions only. Metanemes not observed. Six minute inner labial papillae very close to the six outer labial setae; the latter each with single joint and narrow tip. Four cephalic setae, apparently without joints, slightly longer than the outer labial setae, 0.20–0.33 cbd long, situated near mid-level of stegostoma,

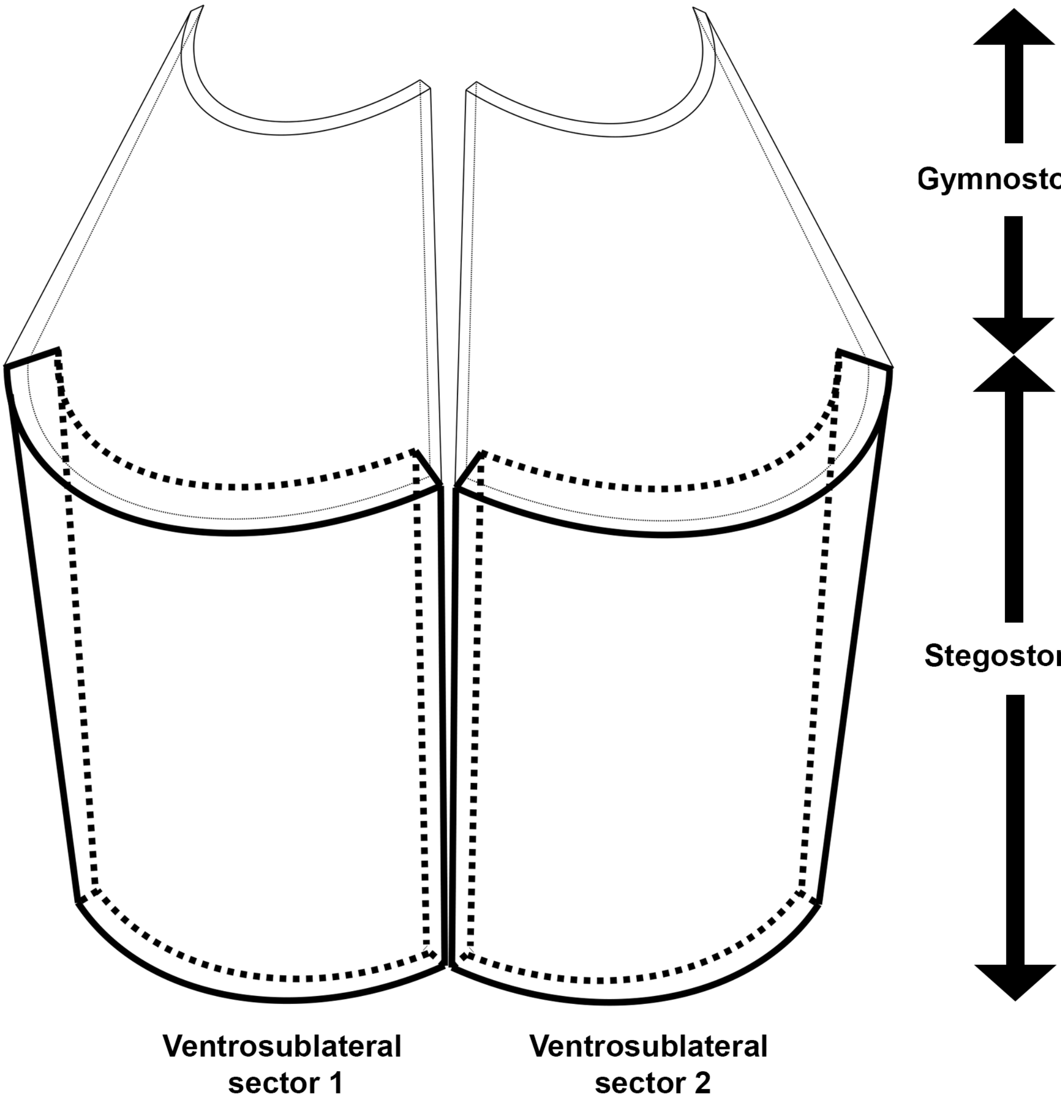

Fig. 5. Schematic representation of buccal cavity wall cuticularization in *Halanonchus* based on observations of *Halanonchus scintillatulus* Leduc sp. nov. and illustrations of other *Halanonchus* species. Only the two ventrosublateral sectors are shown (dorsal sector omitted for clarity). Thin lines show contours of gymnostoma, and thick lines show contours of stegostoma.

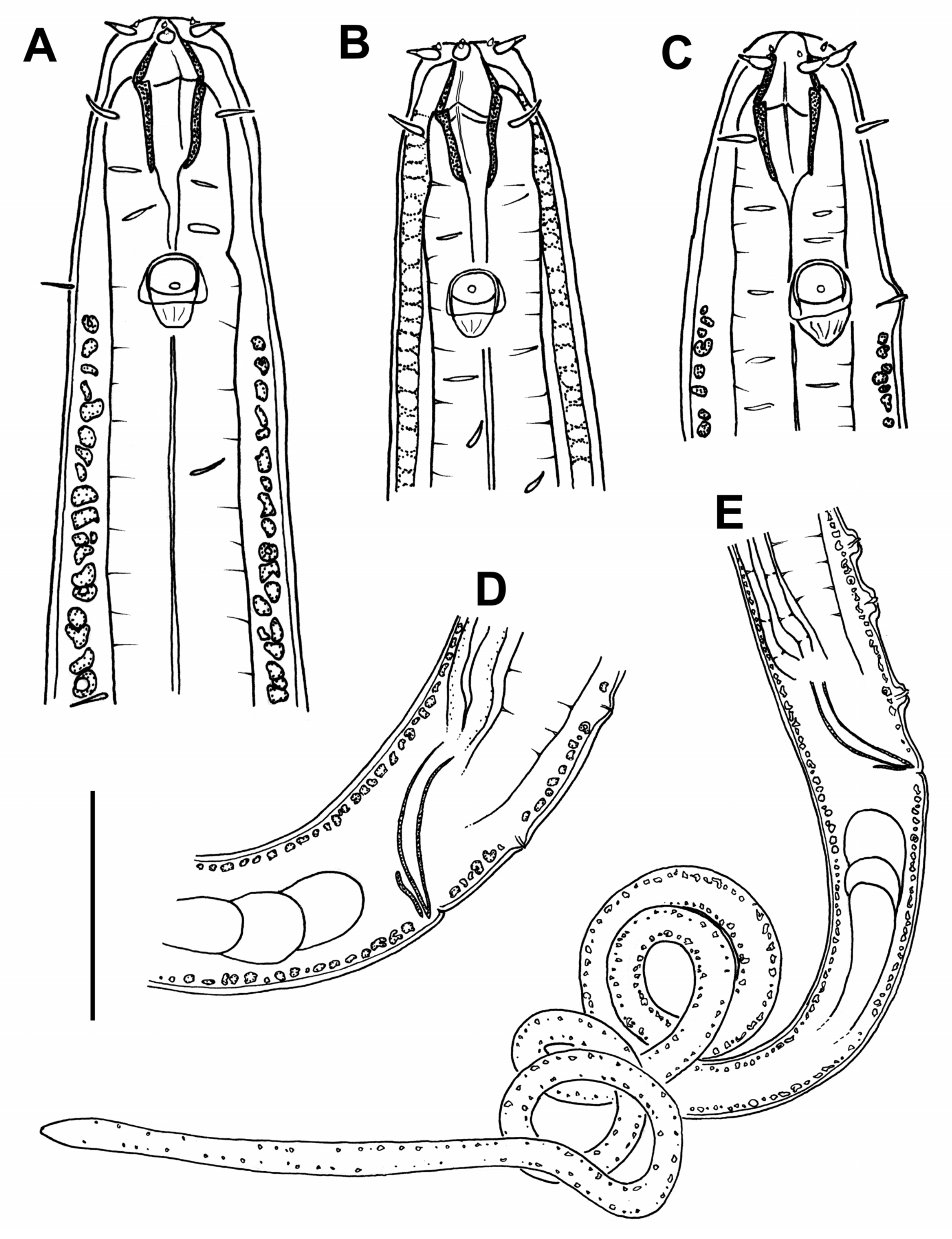

Fig. 6. *Halanonchus scintillatulus* Leduc sp. nov. **A**. ♀, cephalic region, paratype (NNCNZ3329). **B.** ♂, cephalic region, holotype (NIWA 139240). **C**. ♂, cephalic region, paratype (NIWA 139241). **D**. ♂, copulatory apparatus, holotype. **E**. ♂, posterior body region, holotype. Scale bars: A–C = 20 µm; D = 35 µm; E = 45 µm.

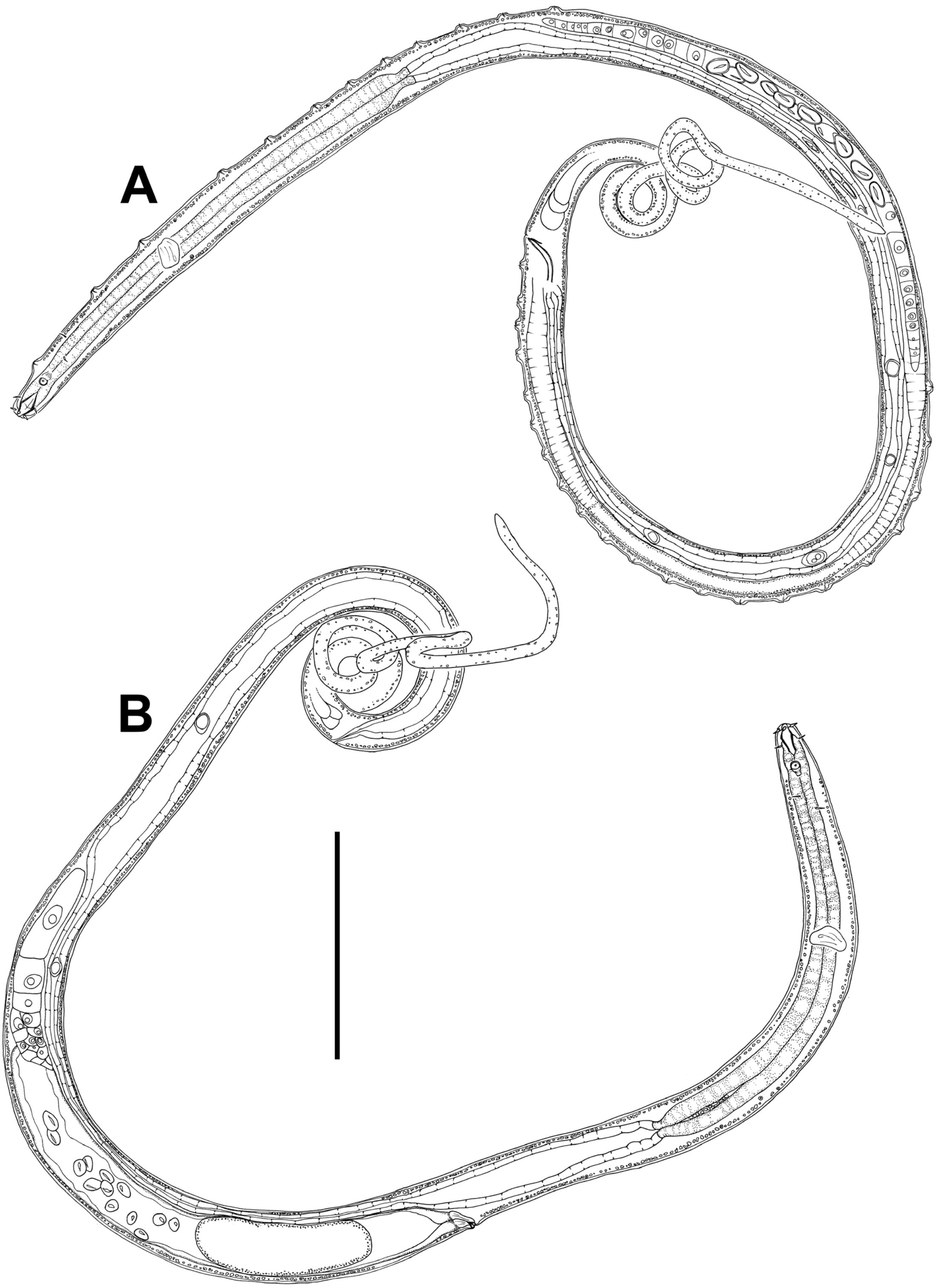

Fig. 7. *Halanonchus scintillatulus* Leduc sp. nov. **A**. Entire ♂, holotype (NIWA 139240). **B**. Entire ♀, paratype (NNCNZ3328). Scale bar = 125 µm.

ca 0.6 cbd from anterior extremity. Amphids located posteriorly to buccal cavity. Amphideal aperture oval; amphideal fovea larger, pocket-shaped or sometimes bean-shaped, amphideal duct with slightly cuticularized outline and duct pore visible near middle of amphideal aperture. One or two somatic setae present posterior to each amphid; sometimes one or two dorsal and/or ventral setae present at level of amphid. Somatic setae absent elsewhere. Midventral row of 6–12 pharyngeal supplements present in five out of six specimens, each consisting of a small papilla on a cuticular swelling with internal duct, located 22–69 µm apart, beginning 26–30 µm from anterior extremity to slightly posterior to pharynx. Buccal cavity barrel-shaped, with cuticularized walls, up to 5–6 µm wide and 13–16 µm deep. Discontinuity in cuticularization between gymnostoma and stegostoma walls, and at the junctions between dorsal and ventrosublateral sectors, may be interpreted as outline of "oval structures"; slight projection of stegostoma wall cuticularization into buccal cavity also gives appearance of small tooth-like structure in lateral cross-section. Pharynx cylindrical, muscular, widening slightly posteriorly but without forming true bulb; cardia short, not surrounded by pharyngeal tissue. Nerve ring situated at ca 40–45% of pharyngeal length from anterior end. Secretory-excretory system not observed. Reproductive system with two opposed outstretched testes, both located ventrally relative to intestine. Sperm cells relatively large, 13–17 µm long, 7–9 µm wide, ovoid or drop-shaped, with spindle-shaped nuclei. Spicules arcuate, 1.1–1.3 cbd long, lightly cuticularized; gubernaculum short, ca ⅓ of spicule length, flat or slightly curved. Eleven to twenty-one precloacal supplements present along midventral line, similar in structure and size to pharyngeal supplements; posterior-most supplement 12–15 µm from cloaca, remaining supplements 12–35 µm apart with anterior-most supplements tending to be slightly further apart than posterior-most supplements. Precloacal seta not observed. Tail filiform, very long, without setae; three caudal glands present.

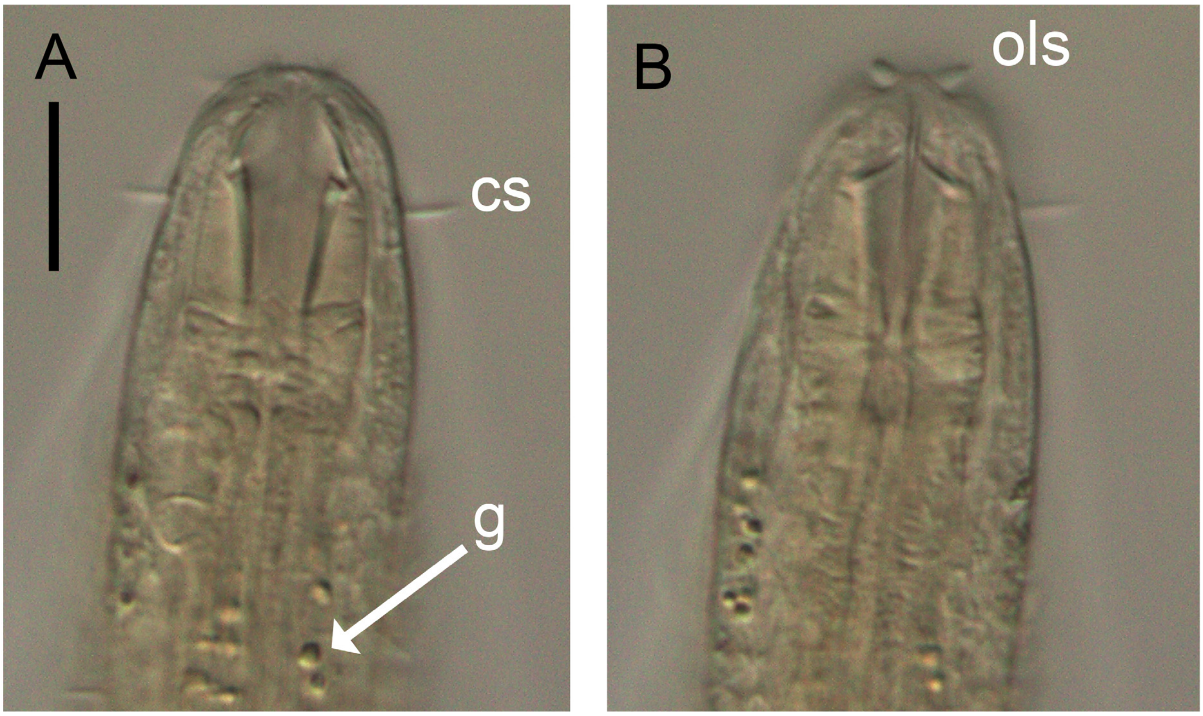

Fig. 8. *Halanonchus scintillatulus* Leduc sp. nov., light micrographs, ♀, paratype (NNCNZ3329). **A**. Sublateral optical cross section of buccal cavity showing teeth-like structures at anterior edge of stegostoma wall. **B**. Subsurface view of same specimen showing discontinuity in cuticularisation of buccal cavity wall between gymnostoma and stegostoma, and at the junctions between the dorsal and ventrosublateral sectors, which may be interpreted as forming "oval structures". Abbreviations see Material and methods. Scale bar = 10 µm.

Female

Similar to males, but with slightly lower values of a. Reproductive system monodelphic, with single reflexed posterior ovary situated ventrally relative to intestine. Mature eggs 95–103 µm long and 28–31 µm wide. Spermatheca not observed. Vulva at about one third of body length from anterior extremity. Cuticularized pars distalis vaginae, proximal portion of vagina uterina surrounded by constrictor muscle; vaginal glands not observed.

Key to the genera of suborder Trefusiina (*Simpliconema* not included due to lack of data on females, but see Fig. 9 for pictorial key of all genera)

1\. Female reproductive system monodelphic 2
– Female reproductive system didelphic 10

2\. Female reproductive system monodelphic with anterior ovary 3
– Female reproductive system monodelphic with posterior ovary 8

3\. Cephalic capsule present .... ***Xennella*** Cobb, 1920
– Cephalic capsule absent 4

4\. Buccal cavity with teeth 5
– Buccal cavity without teeth 6

5\. Cephalic setae and outer labial setae in single circle .... ***Tripylina*** Brzeski, 1963
– Cephalic setae and outer labial setae in separate circles .... ***Trischistoma*** Cobb, 1913

6\. Female gonad and intestine both ending in cloaca 7
– Vulva located very close to anus .... ***Lauratonematoides*** De Coninck, 1965

7\. Asymmetric male copulatory apparatus, gubernacular apophyses present .... ***Lauratonemella*** Tchesunov, 1984
– Symmetric male copulatory apparatus, gubernacular apophyses absent .... ***Lauratonema*** Gerlach, 1953

8\. Buccal cavity small, funnel-shaped .... ***Rhabdocoma*** Cobb, 1920
– Buccal cavity large, with cuticularized walls 9

9\. Buccal cavity uncompartmentalized, amphid elongate ...... ***Africanema*** Vincx & Furstenberg, 1988
– Buccal cavity with discontinuity between gymnostoma and stegostoma, amphid pocket-shaped with oval or circular opening .... ***Halanonchus*** Cobb, 1920

10\. Pairs of discoid supplements present in pre- and post-cloacal regions of male .... ***Cytolaimium*** Cobb, 1920
– Discoid supplements absent 11

11\. Cephalic setae and outer labial setae in single circle .... ***Trefusialaimus*** Riemann, 1974
– Cephalic setae and outer labial setae in separate circles 12

12\. Female reproductive system with two posterior ovaries, outer labial setae not jointed, secretory-excretory pore on setiform elevation .... ***Porocoma*** Cobb, 1920
– Female reproductive system with two opposed and reflexed ovaries, jointed outer labial setae, secretory-excretory pore not on setiform elevation .... ***Trefusia*** De Man, 1893

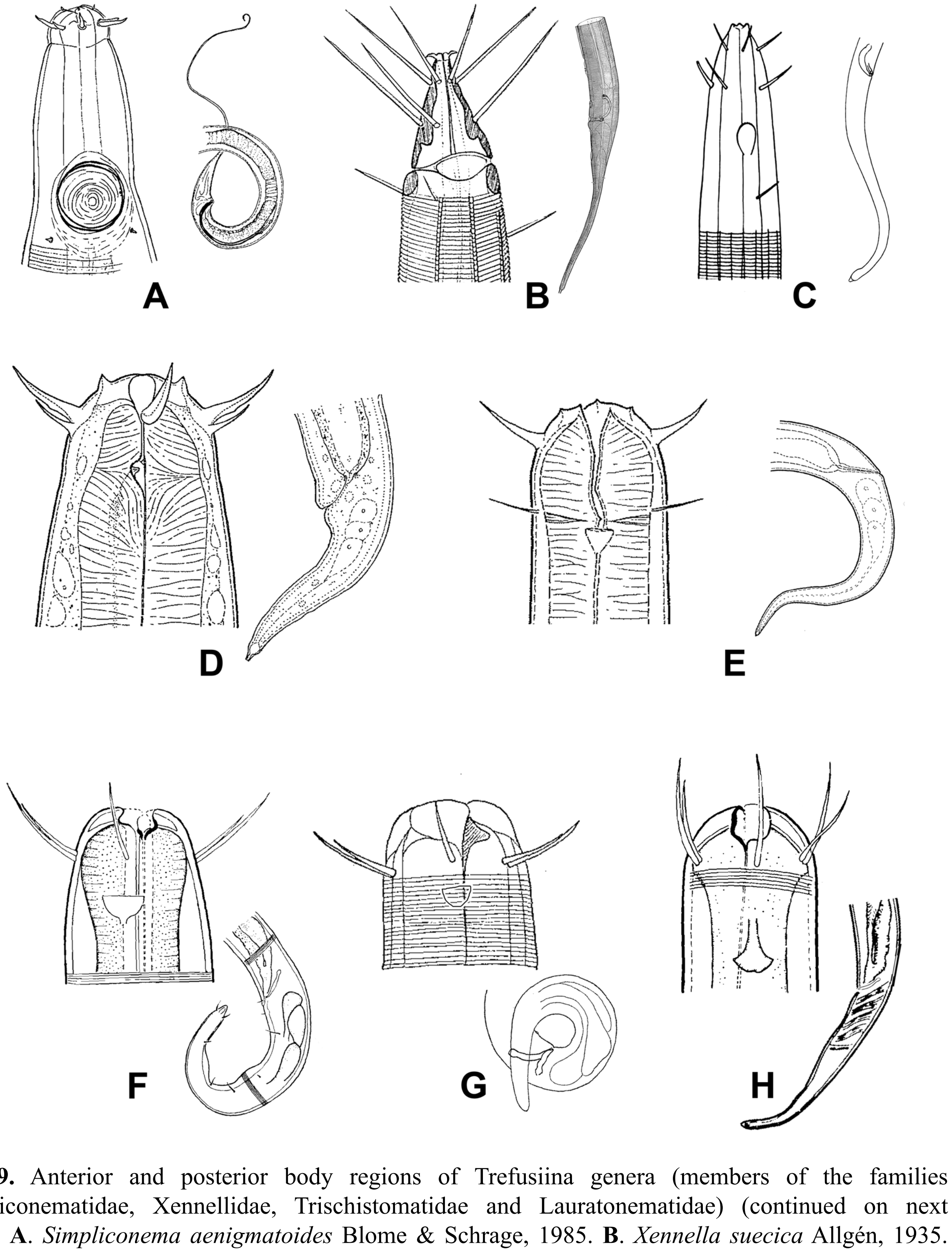

Fig. 9. Anterior and posterior body regions of Trefusiina genera (members of the families Simpliconematidae, Xennellidae, Trischistomatidae and Lauratonematidae) (continued on next page). **A**. *Simpliconema aenigmatoides* Blome & Schrage, 1985. **B**. *Xennella suecica* Allgén, 1935. **C**. *Porocoma striata* Cobb, 1920. **D**. *Tripylina arenicola* (de Man, 1880) Brzeski, 1963. **E**. *Trischistoma gracile* Andrássy, 1985. **F**. *Lauratonema juncta* Fadeeva, 1989. **G**. *Lauratonemella spiculifer* (Gerlach, 1959) Tchesunov, 1984. **H**. *Lauratonemoides minutus* (Platonova, 1971) Tchesunov, 1984. Drawings modified from De Coninck (1937), Gerlach (1959, 1962), Blome & Schrage (1985), Fadeeva (1989) and Andrassy (2007).

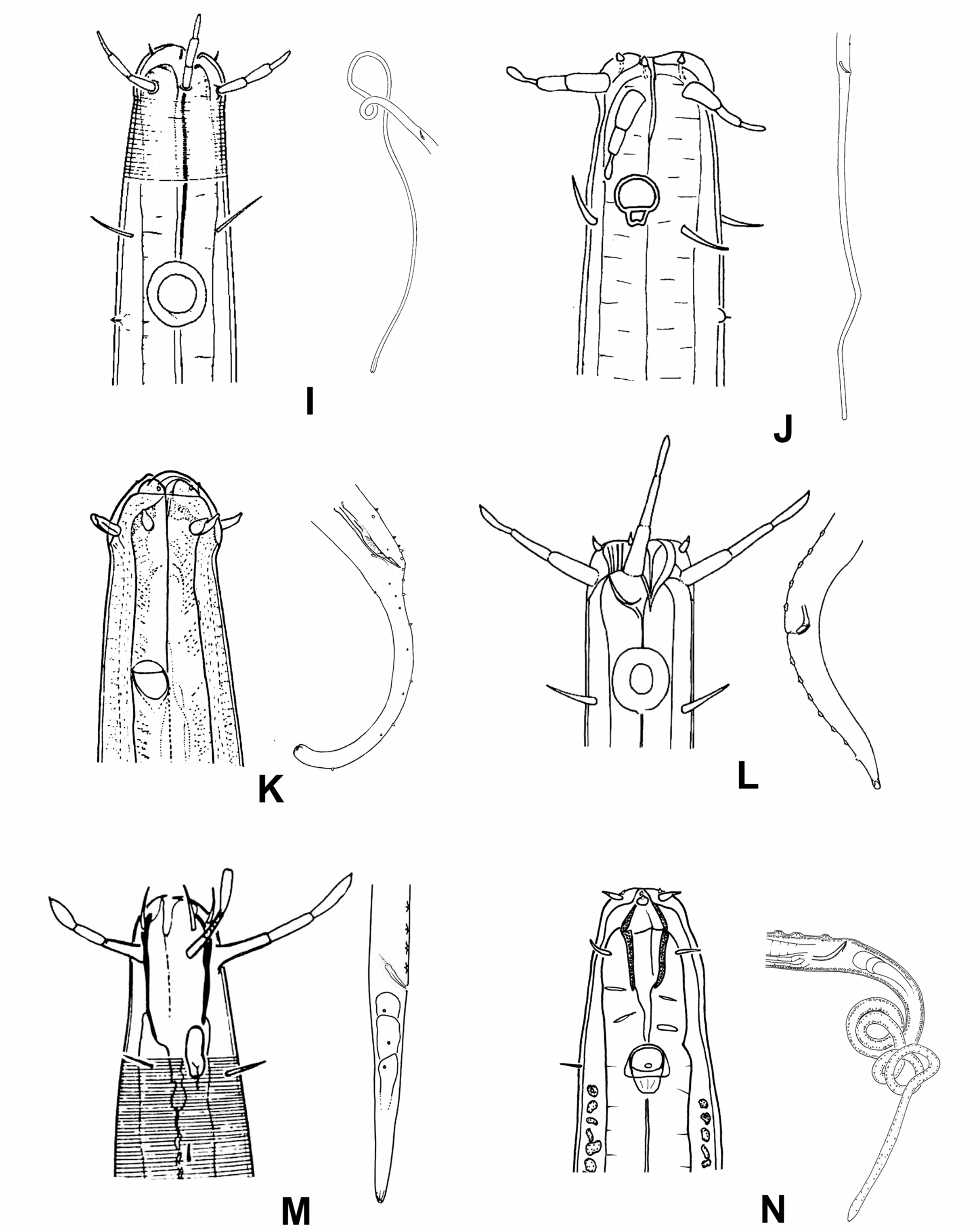

Fig. 9 (continued). Anterior and posterior body regions of Trefusiina genera (members of Trefusiidae). **I**. *Rhabdocoma americana* Cobb, 1920. **J**. *Trefusia spatula* Keppner, 1992. **K**. *Trefusialaimus monorchis* Riemann, 1974. **L**. *Cytolaimium gerlachi* Ott, 1977. **M**. *Africanema interstitialis* Vincx & Furstenberg, 1988. **N**. *Halanonchus scintillatulus* Leduc sp. nov. Drawings modified from Gerlach (1962), Riemann (1974), Vincx & Furstenberg (1988), Vincx & Vanreusel (1989) and Keppner (1992).

Discussion

In the present study, we provided the first overview of the Trefusiina since the work of Lorenzen (1981), which was later updated (Lorenzen 1994). The suborder now comprises five families, 14 genera and 92 valid species, most of which belong to either the Trischistomatidae (39 species) or the Trefusiidae (34 species). We provide the first record of the genus *Halanonchus* from the New Zealand region, describe the female reproductive system of *Trefusialaimus* for the first time and provide the first molecular sequences for this genus. Our SSU phylogenetic analyses confirm that the Trischistomatidae are closely related to the Trefusiidae and provide strong support for including this family within the Trefusiina. Our results, however, puts into question the placement of *Trefusialaimus* and *Lauratonema*, which appear to have affinities with taxa outside of the Trefusiina.

Trefusialaimus is characterized by some unusual morphological features within the Trefusiina, namely outer labial and cephalic setae in a single circle and female reproductive system with two outstretched ovaries. The former feature is also shared by the Lauratonematidae and *Tripylina*, but the latter feature is rare both within the Trefusiina (currently only known for *Cytolaimium exile*) and the Enoplida, where it is found in some Rhabdolaimidae, suborder Campydorina (Tahseen *et al.* 2012; Holovachov 2019). The SSU consensus tree indicates a possible relationship between *Trefusialaimus* and the Campydorina, although support for this placement was weak. The two taxa exhibit some similarities, including non-spiral amphids, monorchic male reproductive system, and outstretched ovaries (in some Campydorina taxa), but also show some dissimilarities in the structure of the cephalic sensilla (setose in *Trefusialaimus* vs usually papilliform in Campydorina), buccal cavity (without teeth in *Trefusialaimus* vs with teeth in Campydorina) and pharynx (no posterior bulb in *Trefusialaimus* vs bulb present in Campydorina). *Trefusialaimus* is also characterized by a somewhat unusual shape and structure of the sperm, which is elongated with a central rod and a cone-shaped nucleus at or near one end (Riemann 1974; this study). A similar sperm morphology has been observed in some species of *Syringolaimus*, order Campydorina, such as *S. loofi* Gourbault & Vincx, 1985 and *S. renaudae* Gourbault & Vincx, 1985, which also bears a flagella at the nucleated end of the sperm. We have observed numerous sperms in the pseudocoelom of both *Trefusialaimus idrisi* juveniles (Leduc 2013) and females (present study). While it is unclear how sperm reached the pseudocoelom in these non-males, their presence outside the male and female genital tracts indicate that they are highly mobile. Further work is required to understand our observations of sperm in the pseudocoelom of juveniles and females of *T. idrisi*, as well as the potential taxonomic significance of this unusual sperm morphology.

Our consensus SSU tree suggests that *Lauratonema* is closely related to *Anoplostoma*, family Anoplostomatidae Gerlach & Riemann, 1974, suborder Enoplina, with weak Maximum Likelihood support but strong posterior probability support. The Lauratonematidae and *Anoplostoma* share some morphological similarities, namely outer labial and cephalic setae in a single circle and pocket-shaped amphids with small opening. In addition, the buccal cavity of some *Lauratonema* species (i.e., *L. macrostoma*, *L. reniamphidum* and *L. mentulatum*) is spacious and heavily cuticularized as in *Anoplostoma*. The Lauratonematidae, however, are characterized by the absence of metanemes (present in *Anoplostoma*), and a different structure of the female reproductive system (monodelphic with vulva close to anus or forming a cloaca in Lauratonematidae vs didelphic in *Anoplostoma*) and of the male reproductive system (monorchic or diorchic with outstretched testes in Lauratonematidae vs diorchic with reflexed posterior testis in *Anoplostoma*). Nevertheless, our findings indicate that the placement of the Lauratonematidae may need to be changed in the future as more comprehensive molecular analyses are conducted.

Our SSU phylogenetic analysis supports the inclusion of the Trischistomatidae within the Trefusiina, as well as a close relationship between the Trefusiina and Tripyloidina, as indicated in previous SSU phylogenies (Holterman *et al.* 2006; Meldal *et al.* 2007; Zhao & Buckley 2009; van Megen *et al.* 2009;

Zhao *et al.* 2012). Because metanemes are present in most enoplids, and indeed both the Tripyloidina and Trischistomatidae, which occupy a basal position within enoplid clade 3, it appears likely that metanemes were lost in the ancestor of the Trefusiidae. Metanemes may in fact have been lost repeatedly if *Lauratonema* and *Trefusialaimus*, which both lack metanemes, are confirmed as belonging outside of the Trefusiina in future phylogenetic studies. Similarly, teeth are present in both the Tripyloidina and Trischistomatidae (but not the Trefusiidae), suggesting that they were lost in the ancestor of Trefusiidae. The inclusion of Trischistomatidae within the Trefusiina means that the suborder now includes terrestrial/freshwater taxa in addition to marine taxa, and that all of the enoplid suborders now include at least some terrestrial/freshwater representatives. This lack of separation between marine and terrestrial taxa reflects the multiple transitions that have occurred in both directions across the phylum between terrestrial and marine habitats (Holterman *et al.* 2019).

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