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History of tachinid classification (Diptera, Tachinidae)

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Abstract

The history of the classification of the Tachinidae (Diptera) is traced from Meigen to the present. The contributions of Robineau-Desvoidy, Townsend, Villeneuve, Mesnil, Herting, Wood and many others are discussed within a chronological, taxonomic, and geographic context. The gradual development of the Tachinidae into its modern concept as a family of the Oestroidea and the emergence of the classificatory scheme of tribes and subfamilies in use today are reviewed. Certain taxa that have in the past been difficult to place, or continue to be of uncertain affinity, are considered and some are given in a table to show their varied historical treatments. The more significant systematic works published on the Tachinidae in recent decades are enumerated chronologically.

Keywords

Tachinidae, Diptera, history, classification

Introduction

The Tachinidae are among the largest families of Diptera with about 8500 valid species[1]. One can only guess at the true diversity of the family but at least double the num-

[1] Pape et al. (2011) estimated the number of valid species of Tachinidae at slightly over 9600, but this number included *nomina dubia*. Most of the 1300+ *nomina dubia* in the Tachinidae are old names from the 1800s and many of them are likely senior or junior synonyms of other currently recognized species, at least within the well-known fauna of the Palaearctic Region. The number of valid species of Tachinidae without synonymous *nomina dubia* is here roughly estimated at 8500.

ber of valid species is a conservative estimate. What is not in doubt is the important ecological role these parasitoid flies play in the environment. It is desirable to organize these flies into a phylogenetically stable suprageneric classification as an aid to those who study them and to enable predictions to be made about the less studied species based on the known habits of related species.

The Tachinidae may not be the single largest family of flies on Earth but in terms of genera they tower over all of the other 140-odd families. The current number of valid genera is about 1520 (O'Hara 2012). The next largest family is Cecidomyiidae with about 760 genera and there are only two other families with more than 500 genera: Asilidae and Chironomidae (Pape et al. 2011). Taxonomically the Tachinidae are arguably the most difficult family of flies and perhaps because of this plus the size of the family and their high position on the evolutionary tree of Diptera they have received scant attention below the family level by those investigating dipteran relationships (e.g., Yeates et al. 2007, Kutty et al. 2010). There is currently an international effort aimed at addressing this imbalance by specifically targeting the Tachinidae for phylogenetic analysis using morphological and molecular data (Stireman et al. 2013).

It seems appropriate at this time to review the history of tachinid classification from its earliest beginnings, tracing how it has changed in response to discoveries of phylogenetically insightful characters and was affected by conflicting views on the nature of generic and suprageneric limits. The noticeable disharmony in the way tachinids were classified among the six biogeographic regions of the world is still in evidence today. The task that now awaits present and future tachinidologists is to determine to a better degree than in the past the evolutionary history of the Tachinidae and to classify the family in a manner than reflects its phylogeny and preserves the best elements of the most recent classifications.

The early years

The meagre number of tachinid species known in the early 1800s was placed in about a dozen genera with the majority of them in Meigen's (1803) broadly defined *Tachina*[2]. André-Jean-Baptiste Robineau-Desvoidy revolutionized tachinid classification with the publication of his *Essai sur les Myodaires* (Robineau-Desvoidy 1830), in which approximately 130 new genera now placed in Tachinidae were described (Evenhuis et al. 2010). Of this total, 73 genera are presently treated as valid (O'Hara 2012). Robineau-Desvoidy (1830) also proposed the name "Calypteratae" (Calyptratae) for a higher group within his Myodaria (essentially modern-day Schizophora), which with some modification in concept (most notably the addition of the Anthomyiidae) is now regarded as "one of the best established monophyletic subsections of the Schizophora" (McAlpine 1989: 1425). In this same work, Robineau-Desvoidy's six tribes of Ca-

[2] Early authors, beginning with Robineau-Desvoidy (1830), erred in using the name *Tachina* Meigen, 1803 for a genus that should have been called *Exorista* Meigen, 1803 (Sabrosky 1999).

lypteratae approximated some of the familial and subfamilial groupings in use today in this subsection. One of these, the Entomobiae (including most of the then-known taxa of the Tachinidae), included a small number of genera grouped under the Tachinariae. The priority of the name Tachinidae over other family-group names available for this family thus dates from Robineau-Desvoidy (1830).

The *Essai sur les Myodaires* was not without its faults and received mixed reviews from dipterists of the day. Robineau-Desvoidy's final contribution to dipterology, a massive two-volume work published in 1863 and six years after his death, *Histoire naturelle des diptères des environs de Paris*, has been justly criticized as an inferior work. In it were proposed about 160 new tachinid genera, only 25 of which are currently recognized as valid (O'Hara 2012). Similarly, a huge number of new species were described with many of them later becoming junior synonyms or *nomina dubia* (the latter resulting from the destruction of many of Robineau-Desvoidy's name-bearing types, Evenhuis et al. 2010: 233).

Contemporaneous with Robineau-Desvoidy were Meigen, Wiedemann, Macquart and Walker, each of whom contributed significantly to the description of species but not much to the higher classification of what are now the Tachinidae. Macquart (e.g., *Diptères exotiques nouveaux ou peu connus*, 1838–1855), like Robineau-Desvoidy, recognized the need for more genera to accommodate the emerging diversity and throughout his career described about 100 tachinid genera, of which 45 are currently valid (O'Hara 2012).

The study of Diptera during the mid to late 1800s continued to be led by Europeans. Among the more notable achievements during this time were the regional treatments on the Diptera of Scandinavia by Zetterstedt (1842–1860), on Italian Diptera by Rondani (1856–1880), and on Austrian Diptera by Schiner (1860–1864). A most ambitious and influential work on the Diptera of the Vienna Museum by Brauer and Bergenstamm (1889–1895) contributed greatly to the knowledge of world Tachinidae, but was marred by an unsatisfactory and artificial suprageneric classification (e.g., Coquillett 1897, Aldrich 1905, Villeneuve 1924, Wainwright 1928, Mesnil 1944). Brauer and Bergenstamm described over 250 genera and subgenera of Tachinidae, of which 99 are currently valid genera (O'Hara 2012).

New World tachinids came under increased attention near the turn of the century, first by van der Wulp (1888–1891) and then by Coquillett (1897). Commenting on the state of tachinid classification at the time, Coquillett (1897: 27) noted:

> "Probably no single family of Diptera has received greater consideration in Europe than the Tachinidae, and yet, strange as this may seem, no other family at the present time is in greater disorder. Several authors accord them only subfamily rank, but it appears desirable to consider them as a distinct family, although their relationship to the Dexidae and Sarcophagidae is a very intimate one."

Coquillett (1897) recognized five subfamilies of Tachinidae, four representing present-day Phasiinae and one (his Tachininae) representing modern Exoristinae +

Tachininae. No tribes were recognized. The "Dexidae" (Dexiidae) were regarded as a separate family and excluded.

Despite the chaotic state of tachinid classification in the late 1800s, an important methodological advance was made in the study of dipteran characters that would lead to a better understanding of natural groupings within the higher Diptera. Early authors like Meigen, Macquart and Robineau-Desvoidy had used certain large setae in their descriptions but it was Rondani (1845) who would apply the term macrochaetae (as "macrochetae") to them. Later Osten Sacken (1881, 1884) would formalize a nomenclature for such macrochaetae under the term chaetotaxy. With refinements of the system by Girschner (1893, 1896), the study of chaetotaxy began to revolutionize the study of the more setose Diptera. Osten Sacken (1884: 511) had observed that the "hypopleural" (meral) setae "occur only in some of the Diptera Calyptrata, which have a row or a tuft of them" and Girschner (1893) used this characteristic to define the Tachinidae in the broad sense of present-day Oestroidea. Girschner also recognized several subgroups within Tachinidae *s. lat*. based on other setal arrangements. The classification was not completely satisfactory and it was only later that the enlarged subscutellum would be used to delimit the Tachinidae in a more modern sense (see below). Frey (1921) built upon the work of Girschner to further advance the classification of this group of flies.

By the beginning of the 20th Century the taxonomic literature on Palaearctic Diptera was both voluminous and daunting, especially for new students of the group. The *Katalog der paläarktischen Dipteren* (1903–1907) was therefore of immense importance, bringing together under a single classification all the names of Palaearctic Diptera. The part by Bezzi and Stein (1907) on the Schizometopa relied heavily on the work of Girschner and proposed a higher classification of considerable merit for its day. The Schizometopa were split into two families, Tachinidae and "Anthomyidae" (Anthomyiidae) (present-day Muscoidea). Within Tachinidae, ten subfamilies were recognized and listed in the following order: Tachininae, Dexiinae, Rhinophorinae, Sarcophaginae, Calliphorinae, Phasiinae, Eginiinae, "Hypoderminae" (Hypodermatinae), Oestrinae and "Gastrophilinae" (Gasterophilinae). With the exception of the Eginiinae (now placed in Muscidae), the rest of the groups with some adjustment to relative ranking closely approximates the families now recognized in the Oestroidea.

Although the *Katalog der paläarktischen Dipteren* must have been a most welcome addition to the shelf of any dipterist of the day, Mesnil (1944: 2) later criticized the Bezzi and Stein (1907) portion on the grounds that it was "voll von Irrtümern und praktisch unverwendbar" ["full of mistakes and practically unusable"].

Classifying New World Tachinidae

North American Diptera were first catalogued by Osten Sacken (1858) and the few tachinid genera listed therein were included in the Muscidae. In the second edition of his catalogue, Osten Sacken (1878) revised the classification of Diptera and recognized

both the Tachinidae and "Dexidae" (Dexiidae) as families. The next catalogue was that of Aldrich (1905), and although the Tachinidae and Dexiidae were kept separate following Osten Sacken (1878) and Coquillett (1897), the suggestion was made that they might be better combined. Aldrich (1905) followed the order of genera of Tachinidae given by Coquillett (1897) and interpolated additional genera and species as necessary. Disparaging remarks were made about the monographic works of van der Wulp (1888–1891) and Brauer and Bergenstamm (1889–1895), and of the species descriptions of Bigot ("in every way objectionable, almost always referred to the wrong genus, and seldom containing the essential data", Aldrich 1905: 420).

Charles Henry Tyler Townsend, the most eccentric and prolific of all tachinidologists, published his first paper on tachinids in 1891 and his last in 1944, with almost 500 publications in total (the majority on tachinids) over this long period (Arnaud 1958). He took up the study of insects at the age of 10 and the study of flies at 25. He held a variety of jobs and professional appointments in the United States and later Peru before settling in Brazil for the last 25 years of his life (Townsend 1943). His most significant achievement was the *Manual of Myiology*, a 12-volume series on the "Oestromuscaria" published between 1934–1942 in which virtually every genus of these flies known at the time was placed in a suprageneric classification and given a detailed description.

Townsend was, by his own admission, a splitter of taxa. He was well versed in the works of others and offered this historical perspective on the struggle between "radicalism and conservatism" (Townsend 1935: 37):

> "History shows that the taxonomy of these flies has suffered much in the making, subjected as it has been for the past century to an alternation of radicalism and conservatism, commonly called splitting and lumping. … Desvoidy, the first radical, employed restricted genera and Macquart, the first conservative, lumped them; Róndani again restricted the genera and Schiner lumped them; Brauer & Bergenstamm split, while Coquillett and Aldrich lumped; Villeneuve and Townsend again split, while Curran and Malloch lumped."

The restricted genera of Townsend were based on the author's concept of a "physiological genus", defined as a "natural genus" comprising "all those species which can produce fertile crosses" (Townsend 1935: 38). As noted by van Emden (1945: 389–390), "the adoption of [this] principle implies the application of the generic unit to every unit considered to be a species in general zoological practice". One can learn, explained Townsend (1935: 56), "to make a complete description of a fly genus and its genotype [type species] in one hour for one sex and an hour and a half for both sexes". The ideal number of members within each of the categories of genus, tribe, family, suborder and order was set at five (Townsend 1935: 60–61). In practise Townsend rarely included more than one species per genus and throughout his career described 1491 genera and 1555 species (Arnaud 1958), with approximately 85% of the genera belonging to the Tachinidae. The number of valid tachinid genera attributed to Townsend currently

stands at 544 (O'Hara 2012), more than five times that of any other author. Second place is held by Robineau-Desvoidy with 104 valid genera (O'Hara 2012).

Townsend's methods and productivity are worth more than a cursory mention because this author has, in some ways, done more to retard tachinid taxonomy than advance it. The sheer volume of genera is one problem, and their assignment to suprageneric categories is another. Townsend knew that females of Tachinidae and related families possess a great diversity of reproductive systems that produce different kinds of eggs and larvae. After 25 years of dissecting specimens and studying the female reproductive system, he was able to recognize 36 distinct groups, most pertaining to present-day Tachinidae (Townsend 1934). Townsend (1935: 38) believed that tachinid relationships had proved to be a "Gordian knot" in the past and:

> "not until the wonderful diversity of female reproductive characters and early stages was demonstrated did any sword for the cutting of this knot appear. … We are now able to determine actual relationships with greater certainty, having found the key to affinities by correlating external anatomic characters with internal reproductive and early stage characters."

Thus armed with internal, external and larval characters, Townsend developed a unique classification that divided present-day Tachinidae among seven families (Gymnosomatidae, Oestridae, Prosenidae, Rutiliidae, Tachinidae, Dexiidae, Exoristidae) and about 90 tribes. Had this hierarchical system truly classified the Tachinidae along phylogenetic lines then it would have been the most significant advance in the history of tachinidology. However, it fell short of this goal and is now regarded as both unmanageable and artificial (e.g., Mesnil 1939, Wood 1985, 1987). Specialists also found the keys to tribes and genera in *Manual of Myiology* to be fraught with problems, thus hindering the recognition of Townsend's supraspecific taxa.

William Robin Thompson published a series of eight papers in the *Tachinids of Trinidad* (Thompson 1961–1968). He had difficulty interpreting the fauna of Trinidad according to the Townsend scheme and chose to avoid attempting to revise Townsend's genera:

> "[I have] decided also that in most cases an attempt to simplify the taxonomic problems by reducing Townsendian genera to the synonymy is impracticable because with the knowledge we now have it is impossible to know when to stop" (Thompson 1961: 22).

Thompson found the works of Mesnil and other Europeans (see below) more helpful than the works of Townsend for understanding the major groupings of Tachinidae. Although this led Thompson to classify the Tachinidae of Trinidad in a more natural way, he had a proclivity for describing unnecessary new genera.

The tribes, genera and species created by Townsend were described predominantly for New World taxa and by their sheer number continue to pose serious challenges

for taxonomists to this day. Sabrosky and Arnaud (1965), the first to catalogue the Tachinidae of America north of Mexico in the post-Townsend era, adopted a nearly modern concept of the family (differing only by the inclusion of Rhinophorinae) while otherwise retaining many of Townsend's tribes:

> "for present convenience, in the absence of any other published arrangement of the Nearctic genera, though with some combinations and generic transfers, notably where we agree with the recent work of Mesnil and coworkers in Europe. This is especially true in the Goniinae [= Exoristinae]" (p. 962).

The catalogue of the Tachinidae of America south of the United States by Guimarães (1971) followed shortly after Thompson's *Tachinids of Trinidad* and Sabrosky and Arnaud's catalogue. This author, faced with the huge number of tribes, genera and species described by Townsend and having to deal with other taxa inadequately described by earlier authors, could not revise the whole classification and mostly followed Townsend. This action, he admitted, resulted in a "catalogue arrangement [that] leaves much to be desired" (Guimarães 1971: 3). The rich fauna of the region was catalogued into 2864 species and (by Guimarães' own admission) an over-split 944 genera.

There have been to date only two major attempts to correct the generic imbalance that has impeded study of New World Tachinidae, both by Donald Montgomery Wood. The first was a conspectus of the Blondeliini of North and Central America and the West Indies (Wood 1985). Although this study excluded South American Blondeliini, it nevertheless reduced the number of valid genera from about 230 to 55. Many of the genera sunk into synonymy were Townsend's but there were also many described by Reinhard, Thompson, Curran and others. The second work to reduce the number of New World genera was Wood's (1987) Tachinidae chapter in *Manual of Nearctic Diptera*. The nomenclatural changes in this work, including almost 200 new generic synonyms, were later enumerated by O'Hara and Wood (1998).

Wood (1987) also successfully bridged the gap between the generic classifications of the Nearctic and Palaearctic regions created by Townsend some decades earlier. This was accomplished partly by reducing the number of genera but also by assessing genera from a Holarctic perspective. The catalogue by O'Hara and Wood (2004) further united the classifications of Nearctic and Palaearctic Tachinidae. The catalogue by Guimarães (1971) has not been updated and the 800+ genera currently recognized in America south of the United States will not be easily converted into a modern classification. A careful study of the name-bearing types of the type species of many of these genera will be necessary before a better classification can be constructed for Neotropical Tachinidae.

The European influence

The Europeans of the early 1900s continued to build on the discoveries of Girschner and others at the same time that Townsend in the New World was pursuing his own course

of investigations that would culminate in his *Manual of Myiology*. Joseph Villeneuve de Janti, a medical doctor by profession (like Robineau-Desvoidy), emerged as an early specialist on the Tachinidae and published actively on the family from 1900 until his death in 1944. He wrote an influential paper in 1924 reviewing earlier works on chaetotaxy and detailing his own views on characters useful for understanding the evolution of the "Myodaires supérieurs". This group comprised the "Tachinaires" (Tachinidae *sensu* present-day Oestroidea) and "Anthomyaires" (Anthomyiidae *sensu* present-day Muscoidea). Within Villeneuve's Tachinidae were Calliphorinae, Sarcophaginae, Dexiinae, Rhinophorinae, Phasiinae, and Tachininae. Particularly noteworthy and progressive was the division of the Tachininae into two groups, Eutachininae and Protachininae, of which the former was considered more evolved than the latter. As a rough approximation, the two correspond to present-day Exoristinae and Tachininae, respectively.

Villeneuve was well respected by contemporaries for his expertise in Tachinidae and willingly shared his knowledge with others. As noted by Wainwright (1928: 141), Villeneuve:

> "has contributed largely towards the reduction to something like order of our knowledge of these insects. Possibly the full value of his services to science may never be appreciated, because so many of the fruits of his labours have been given to the world by other workers, whom he has unselfishly and ungrudgingly assisted".

The discovery by Malloch (1923) that the Tachinidae and Dexiidae can be distinguished from Sarcophagidae, Calliphoridae and Muscidae by an enlarged "metanotum" (subscutellum) was a highly significant development in the classification and differentiation of these flies. It was likely this discovery that led Villeneuve (1933) to revise his earlier classification and divide the "Tachinaires" into three groups:

1) Tachinidae with Phasiinae, Dexiinae and Tachininae,
2) Sarcophagidae with Miltogramminae, Sarcophaginae and Calliphorinae, and
3) Rhinophoridae, a small group of isopod parasitoids.

Villeneuve (1933) treated the Eutachininae and Protachininae of the Tachininae at length.

Villeneuve was a mentor and friend of Louis Paul Mesnil, who was 36 years his junior (Mesnil 1950). It was originally Villeneuve who was invited by Lindner to author the Tachinidae volumes of his ambitious *Die Fliegen der palaearktischen Region* (hereafter *FPR*). However, as the project drew closer Villeneuve realized that the talented and younger Mesnil was a better choice to take on this demanding and long-term task (Herting 1987).

Mesnil was an avid student of Tachinidae. He demonstrated his enthusiasm and insight early by publishing, as one of his first works on the group, a lengthy treatise entitled *Essai sur les Tachinaires* (Mesnil 1939). He began the *Essai* by reviewing and

critiquing the classifications of his more illustrious predecessors: Robineau-Desvoidy, Macquart, Meigen, Rondani, Brauer and Bergenstamm, Pandellé, and Girschner. In proposing a new classification, Mesnil (1939) drew special inspiration from the works of Robineau-Desvoidy and Villeneuve, and like Brauer and Bergenstamm, started by grouping together related genera and building the classification "depuis la base vers le sommet" ["from the base to the summit"] (p. 20).

Mesnil (1939) restricted the term Tachinaires to the family Larvaevoridae[3] (i.e., Tachinidae). The main diagnostic feature of the family was the well-developed "postscutellum" (subscutellum), as previously implied in Villeneuve's (1933) classification and explicitly adopted by Curran (1934, as "metanotum"). Mesnil relegated the Rhinophorinae and Sarcophaginae to the Calliphoridae and subdivided the Larvaevoridae into six subfamilies: Salmaciinae[4], Phorocerinae, Larvaevorinae, Ameniinae, Dexiinae and Phasiinae (including Oestrini). These were keyed and characterized and most were further subdivided.

The Phorocerinae of Mesnil (1939) consisted of tachinids possessing a haired prosternum and a small "prealar" (postsutural supraalar) seta. Included within the Phorocerinae were three tribes: Phorocerini, Blondeliini, and Crocutini[5]. The Phorocerini, with vein M (as "4ᵉ") having an angular bend and a shadow fold, and the Blondeliini, with vein M having a rounded bend and no shadow fold, and both possessing divergent subapical scutellar setae (convergent in Crocutini), have continued to the present virtually unchanged in their characterization (Wood 1972, 1985). The Phorocerini have since become known as the Exoristini.

Mesnil began publishing *FPR* instalments a few years after his *Essai*. The goal was to treat all of the Palaearctic Tachinidae to species level but the task proved too great for him alone. After 35 years and some 1500 pages of text, the Larvaevorinae (present-day Exoristinae and Tachininae) were completed (Mesnil 1944–1975) along with one instalment on the Dexiinae (Mesnil 1980). Herting planned to publish on the remainder of the Dexiinae and all of the Phasiinae but only one instalment on the latter was published (Herting 1983).

Mesnil's (1944) first instalment for *FPR* began, as did his *Essai*, with general remarks about previous workers and their classifications. Mesnil (1944: 2) made these observations about the generic concepts of other workers:

> "Oft auch haben sie alte künstliche Gattungen aufrechterhalten, deren Umfang jedes Maß überschreitet und deren Heterogenität offenkundig ist; können sie doch sogar Arten verschiedener Tribus enthalten.

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[3] A long overlooked publication by Meigen (1800) gave *Larvaevora* as an earlier name for *Tachina*. For some years after this discovery family-group names based on *Larvaevora* commonly replaced those based on *Tachina*. The family-group name Larvaevoridae (-inae, -ini) was replaced by Tachinidae (-inae, -ini) when *Larvaevora* Meigen, 1800 was officially suppressed (ICZN 1963).

[4] When *Salmacia* Meigen, 1800 was suppressed (ICZN 1963), junior synonym *Gonia* Meigen, 1803 took its place. The next available family-group name Goniinae (-ini) replaced that of Salmaciinae (-ini).

[5] When *Crocuta* Meigen, 1800 was suppressed (ICZN 1963), junior synonym *Siphona* Meigen, 1803 took its place. The next available family-group name Siphonini replaced that of Crocutini.

> So lassen sich die meisten neuzeitlichen Dipterologen, da sie die wahren Merkmale der Tachinen zu wenig berücksichtigt haben, nach zwei Richtungen gruppieren: die einen unterteilen die Gattungen bis ins Unendliche und machen so fast alle monospezifisch (T. Townsend), die andern vereinigen zahlreiche Gattungen zu einem Ganzen und gelangen so zu monströsen Zusammenfassungen (Curran)."
>
> ["Often, they have maintained old artificial genera whose scope exceeds all bounds and whose heterogeneity is obvious; even though they may contain species of different tribes.
>
> Since most modern dipterists have not taken the true characteristics of tachinids into account, they can be grouped in two directions: some subdivide the genera into infinity and thus make almost all of them monospecific (T. Townsend), others unite numerous genera into a whole and arrive at monstrous compilations (Curran)".]

Lindner (1933) established the classification of the Diptera that would be followed in *FPR* six years before Mesnil's (1939) *Essai*. This constrained Mesnil (1944) into keeping Larvaevoridae in the older and broader sense of present-day Oestroidea instead of in the restricted sense of present-day Tachinidae. Recognized within Larvaevoridae were subfamilies Larvaevorinae (with tribes Salmaciini, Phorocerini and Larvaevorini), Dexiinae and Phasiinae. Mesnil's (1939) Oestrini (then placed in Phasiinae) became the "Gastrophilinae" (Gasterophilinae), Oestrinae and "Hypoderminae" (Hypodermatinae) of Lindner (1933). It is clear that this higher classification did not appeal to Mesnil. To him, the true definition of the Larvaevoridae was undeniable ("unbestreitbare") and based on the enlarged subscutellum and parasitic habits of the family (Mesnil 1944). His only recourse was to chart the classification he would have followed had he been permitted to do so (numbers in parentheses refer to Lindner's numbering system for families) (Mesnil 1944: 20):

I Haplostomata Frey
II Thecostomata Frey
- A Muscidae (63)
- B Calliphoridae
  - a Calliphorinae (64i)
  - b Hypoderminae (64b)
  - c Sarcophaginae (64h)
  - d Rhinophorinae (64e)
- C Larvaevoridae
  - a Phasiinae (incl. Oestrini) (64c)
  - b Dexiinae, Ameniinae (64f)
  - c Larvaevorinae (64g)

The Lindner series was published in small instalments ("Lieferungen"), the length of each being determined by the number of printed signatures used per instalment.

Frequently an instalment would end in the middle of a description or in the middle of a key. This may have been cost-effective for the publisher but created havoc nomenclaturally. New generic names, for example, were often *nomina nuda* in one instalment and not made available until years later in another instalment. A great number of such nomenclatural issues as they pertain to the Tachinidae were dealt with by O'Hara (1996), Evenhuis and O'Hara (2008), and Evenhuis et al. (2008).

Mesnil's *FPR* instalments by definition dealt primarily with the Palaearctic fauna but incorporated information on the taxa of other regions, except for the nearly impenetrable taxa of Neotropical Tachinidae. The result, in concert with a great many papers published by Mesnil outside *FPR*, was a classification for the bulk of the Tachinidae that could be hailed by contemporaries as a leap forward in the quest for a scheme reflecting the true relationships of the family. The suprageneric classification of Townsend (1934–1942) was largely ignored by Europeans who were making progress through their own investigations.

The first of Mesnil's (1944) instalments in *FPR* gave only a glimpse of the classification that would follow. The Ameniinae were transferred to the Calliphoridae and kept as a subfamily, although the family itself is not currently considered monophyletic (e.g., Rognes 1997, Kutty et al. 2010). Mesnil's (1944) three tribes of Larvaevorinae were split over the duration of *FPR* into a number of subtribes: nine in Salmaciini, six in Phorocerini and over 40 in Larvaevorini. The Larvaevorini were revisited by Mesnil (1966) and reclassified as Tachinini *s. str*. and Voriini. All of the subtribes of Mesnil (1944–1975) are now generally tribes and tribe Larvaevorini is present-day Tachininae. Many of the tribes continue to this day virtually unchanged whereas a few have undergone dramatic restructuring in the light of subsequent discoveries. The most significant changes resulted from research on the female postabdomen by Herting (1957) and male genitalia by Verbeke (1962a).

Benno Herting began his career on Tachinidae much the same way as did Mesnil (and even Robineau-Desvoidy) with an early publication based on original and extensive research (Herting 1957). It was a study of the female postabdomen and was based on the examination of about 500 species of calyptrate flies. Information about eggs and first instar larvae were taken into account but unlike Townsend's studies the focus was more on the morphology of the terminal segments of the postabdomen than on the internal reproductive system. Herting (1957) used his findings to characterize the structural features of the female postabdomen throughout the families, subfamilies and lower groups of the Calyptratae. He tried to interpret these findings in a phylogenetic context and to adjust the classification accordingly.

Five subfamilies of the Tachinidae were recognized by Herting (1957): Echinomyiinae[6], Dexiinae, Phasiinae, Ocypterinae[7], and Eutachininae. At a gross level, Echinomyiinae corresponded to the Protachininae of Villeneuve (1924, 1933) and to the Lar-

[6] Founded on *Echinomya* Latreille, 1804. This name is currently recognized as a junior synonym of *Tachina* Meigen, 1803. The family-group name Tachininae (-ini) has priority over Echinomyiinae (-ini).

[7] Founded on *Ocyptera* Latreille, 1804. This name is currently recognized as a junior synonym of *Cylindromyia* Meigen, 1803. The family-group name Cylindromyinae (-ini) has priority over Ocypterinae (-ini).

vaevorinae of Mesnil (1939; and later, Larvaevorini of Mesnil 1966–1975); Ocypterinae was formerly treated within Phasiinae by both Villeneuve (1924, 1933) and Mesnil (1939); and Eutachininae was proposed by Villeneuve (1924) and corresponded to the Salmaciinae (-ini) and Phorocerinae (-ini) of Mesnil (1939, 1944). Herting (1957) treated the Oestridae as a separate family.

Herting (1957) followed Villeneuve (1924, 1933) in using the subfamily name Eutachininae in his classification. He subdivided this subfamily into the Goniini and Eutachinini. He could not find reliable characters in the female postabdomen to separate these tribes and therefore chose to organize his discussion according to the reproductive habits of the species. Oviparous species were placed in the Eutachinini and distributed mostly between the *Winthemia* Robineau-Desvoidy group and *Eutachina*[8] Brauer and Bergenstamm group. These were essentially the Winthemiina and Phorocerina that Mesnil (1944) had placed in tribes Salmaciini and Phorocerini, respectively. Ovolarviparous species grouped by Mesnil (1944) in the Blondeliina (tribe Phorocerini) were also assigned to the Eutachinini. The ovolarviparous *Siphona* Meigen group (Siphonina, tribe Phorocerini, of Mesnil 1944) was more clearly defined but its placement in Eutachinini or Goniini was not discussed. Similarly, the "*Ethylla*" (*Ethilla*) Robineau-Desvoidy group was included in Eutachininae but its further placement was not discussed. No members of the Acemyina (tribe Phorocerini) of Mesnil (1944) were studied by Herting (1957).

The composition of Herting's (1957) Goniini consisted of species with two reproductive modes. One is quite specialized and involves the production of tiny (microtype) eggs that females oviposit on the food plants of hosts. These eggs hatch only after ingestion by a potential host. This sort of egg and the biology associated with it were already well known as a result of earlier studies (e.g., Sasaki 1887, Townsend 1908, 1911, Pantel 1910[9]). The rest of Herting's (1957) Goniini were mostly ovolarviparous species with a few oviparous species. This broad concept of the Goniini was essentially the Salmaciinae (-ini) of Mesnil (1939, 1944) without Ethyllina and Winthemiina.

Herting (1957) introduced an important change to the placement of the Voriini. The members of this tribe had been included in the Protachininae of Villeneuve (1924, 1933) and the nearly equivalent Larvaevorinae of Mesnil (1939). Herting (1957) placed the tribe in the Dexiinae, bringing to three the number of Palaearctic tribes recognized in the subfamily: Dexiini, Voriini and Dufouriini. This move was supported by female postabdominal characters and by features of the male genitalia communicated to Herting by Verbeke (see below).

Mesnil (1956–1965) published on the Phorocerini in *FPR* over a ten-year period. He subdivided the tribe into subtribes Phorocerina, Blondeliina, Atylomyina, Neominthoina, Acemyina, and Siphonina, describing all the Palaearctic species and working in the same meticulous way that he had earlier for the Salmaciini (Mesnil 1944–1956). He had already revised the Old World Phorocerina (as Phorocerini) in a

[8] *Eutachina* Brauer and Bergenstamm, 1889 is currently a junior synonym of *Exorista* Meigen, 1803.

[9] It was Pantel (1910) who coined the term "microtype" for these tiny ingestible eggs of goniines.

separate publication (Mesnil 1946) that he had probably begun before starting *FPR*. Mesnil (1956–1965) was halfway through the Phorocerini when Herting published his next great work on the Tachinidae, a monograph on the biology of the West Palaearctic species (Herting 1960). This work had a different focus from his earlier study but included a hierarchical arrangement of taxa that the former work had lacked. A clear classification was in evidence and although it was congruent in many respects with Mesnil's it differed from it in some significant ways. Herting (1960) proposed a major restructuring of Mesnil's Salmaciini (Mesnil 1944–1956) and Phorocerini (Mesnil 1956–1965). Both were united to form the Exoristinae[10], consisting of a broadly defined Goniini (see above), Ethillini (Mesnil's Ethyllina and Atylomyina), and the following tribes that corresponded to Mesnil's remaining subtribes (except for the mixed and non-Palaearctic Neominthoina): Winthemiini, Exoristini (Mesnil's Phorocerina), Blondeliini, Acemyiini, and Siphonini.

Herting's (1960) Echinomyiinae included just three tribes: Echinomyiini, Leskiini and Microphthalmini. This work was published after Mesnil (1939) but before the *FPR* instalments on the same group (Mesnil 1966–1975, as "Larvaevorini oder Tachinini"). Mesnil (1939) had treated this group as the Larvaevorinae and noted that it was very close to Villeneuve's (1933) Protachininae except for the exclusion of section *Winthemia* (placed by Mesnil in Salmaciinae [= Villeneuve's Eutachininae], as Winthemiini). Mesnil's (1939) Larvaevorinae had consisted of eight tribes[11]: Campylochaetini, Athryciini, Larvaevorini, Rhamphinini, Leskiini, Minthoini, Thelairini, and Macquartiini. This heterogeneous assemblage was considerably altered by Herting (1960): Larvaevorini and part of Macquartiini were placed in Echinomyiini; Campylochaetini, Athryciini, Thelairini and part of Macquartiini (i.e., the Phyllomyina) were moved to Voriini in the Dexiinae; Minthoini were included in Leskiini; and Rhamphinini were not treated but were later placed in Voriini by Herting (1984). The Microphthalmini of Herting (1960) were moved to the Tachininae from Mesnil's (1939) section Dexiosomina (Dexiini, Dexiinae).

At the same time that Mesnil (1956–1965) was working through the Phorocerini using external characters and Herting (1957, 1960) was studying the female postabdomen, Jean Verbeke (1962a, 1962b[12], 1963) was investigating tachinid male genitalia. Verbeke was communicating some of his findings to Herting before publishing them himself, thus contributing at least to Herting's concept of the Dexiinae (see above). Verbeke (op. cit.) recognized within the complexity of the male genitalia a few general "types" associated with three structures. Firstly, the connection between the basiphallus and distiphallus is either "direct and non-mobile" (type I) or "indirect and

[10] Mesnil (1956–1965) had called this tribe "Phorocerini oder Exoristini". Nomenclaturally, Herting's (1960) use of the name Exoristinae was simply an elevation of Mesnil's Phorocerini to a subfamily under an alternate name.

[11] Mesnil (1939) referred to names ending in -inae as tribes and names ending in -ini as subtribes. To avoid confusion within this paper such names are called subfamilies and tribes, respectively.

[12] Verbeke (1962b) provided a similar discussion of male genitalia as Verbeke (1962a). The latter is more often cited for information that appears in both publications and this convention has been followed here.

mobile" (type II). Secondly, the distiphallus either lacks (POS [= *Phasia*, *Ocyptera*, *Strongygaster*] type) or possesses (DEG [= *Dexia*, *Echinomyia*, *Gonia*] type) longitudinal ventral microstructures. Thirdly, the "posterior paramere" (pregonite) has three types: type A, lobe-like and sensorial; type B, intermediate; and type C, strap-like and connective. These structural types do not form unique combinations and Verbeke (1963: 4) understood that "this repeated appearance of similar structures in different groups implicates a parallelism between the male genitalia of these groups". Verbeke (1962a) concluded that the Tachinidae were best divided into six subfamilies: Phasiinae were characterized on the basis of a POS type distiphallus, whereas other Tachinidae have a DEG type distiphallus; Echinomyiinae (i.e., Tachininae) and Eutachininae (i.e., Exoristinae) have a type I connection between basiphallus and distiphallus; Dexiinae and Voriinae have a type II connection between basiphallus and distiphallus; and Dufouriinae with tribes Macquartiini and Dufourini, the former with a type I connection between the basiphallus and distiphallus and the latter with a type II connection but both tribes having a pregonite of type B. The subfamily Dufouriinae was clearly one of convenience and was not thought to be monophyletic. Verbeke (1963: 3) noted:

> "Many other characters prove the intermediate situation of both tribes [intermediate between Dexiinae-Voriinae and Echinomyiinae-Eutachininae, see illustration in Verbeke (1962a: 147)] and for this reason we fused them into a new subfamily".

Herting (1957, 1960) was aware of Verbeke's studies on the male genitalia in advance of the publications on this subject (Verbeke 1962a, 1963) and was also familiar with the pioneering work on male genitalia by Rubtzov (1951). Herting (1957) discovered that features in the female postabdomen—and corroborated by evidence from the male postabdomen—supported a new concept of the Dexiinae. The Dexiini, Voriini and Dufouriini were brought together to form the Dexiinae. Although this classification differed from the one proposed later by Verbeke (1962a, 1963), it can be seen that Verbeke's type II phallus and type C pregonite accurately defines Herting's (1957, 1960) Dexiinae. This understanding of the subfamily continues to this day (e.g., Herting 1984, Tschorsnig 1985, Wood 1987, Tschorsnig and Richter 1998, O'Hara and Wood 2004, Cerretti 2010). Verbeke's Macquartini, the other half of his Dufouriinae, was placed by Herting (op. cit.) in the Echinomyiinae but not retained as a tribe.

Mesnil (1966–1975) next published a series of instalments in *FPR* on the Larvaevorini, or Tachinini *s. lat.* In the first instalment, Mesnil (1966) introduced some changes to his earlier classification of the Larvaevorinae (i.e., Tachinidae). The classification proposed consisted of six tribes (equivalent to subfamilies of other authors): Phasiini, Exoristini, Goniini, Dexiini, Voriini, and Tachinini *s. str.* (see chart, Mesnil 1966: 882). The first three were characterized as producing planoconvex eggs and the last three as producing membranous eggs. Herting (1966) also noted this distinction in egg type between what he considered the two lineages of Tachinidae. Mesnil (1966)

recognized the Phasiini as distinct based on the POS-type distiphallus of Verbeke (1962a) and the characteristic female postabdomen of Herting (1957). An unusual group that defies easy placement to this day, the Eutherina, were placed in the Voriinae by Verbeke (1962a) (based on male genitalia) and in the Phasiinae (-ini) by both Herting (1966) and Mesnil (1966) (based on egg type).

Mesnil (1966) was further influenced by Herting (1957) and Verbeke (1962a) to remove the voriines from the Larvaevorinae (-ini) of Mesnil (1939, 1944) and place them next to the dexiines. He kept the groups separate as Voriini and Dexiini rather than place them in the Dexiinae as did Herting (1957). The Dufouriinae of Verbeke (1962a) were split along similar lines to Herting (1957, 1960) with the Dufourini moved to Voriini as Dufouriina and Macquartiini kept in Tachinini *s. str.* (as Macquartiina) following Mesnil (1939). The original Dufouriina of Mesnil (1939) was a mixed group placed in Phasiini of Phasiinae and included such aberrant genera as *Graphogaster* Rondani and *Rondaniooestrus* Villeneuve in addition to *Dufouria* Robineau-Desvoidy and other typical dufouriines. Mesnil (1966) treated a more restricted Dufouriina in Voriini, placed *Graphogaster* in the small subtribe Graphogastrina in Tachinini *s. str.*, and recognized *Rondaniooestrus* as sole member of Rondaniooestrina in Phasiini.

The Tachinini *s. str.* of Mesnil (1966) were split among about 30 subtribes. This tribe was equivalent to Mesnil's (1939) Larvaevorinae and its eight tribes except for the removal of the voriines. In revising the earlier classification of Mesnil (1939) for *FPR*, Mesnil (1966) reduced his former tribes to subtribes and raised some former sections to tribes (especially among the Larvaevorini and Macquartiini of Mesnil 1939). This classification bears some resemblance to the groupings of Brauer and Bergenstamm (1889–1895) and Townsend (1934–1942) and reflected the uncertainty inherent in attempting to classify this heterogeneous and likely polyphyletic assemblage.

The Dexiosomina, treated in Dexiini of Dexiinae by Mesnil (1939), became part of Mesnil's (1966–1975) Microphthalmina in Tachinini *s. str.*

Over 30 years elapsed between Mesnil's (1944–1975) first and last *FPR* instalments on the Larvaevorinae. Mesnil (1975a, 1975b) included an Addenda and Corrigenda at the end of the Larvaevorini section in which he made corrections to earlier mistakes, added notes, and revised certain groups. His most significant change concerned the Goniinae (Salmaciini of Mesnil 1944–1956; i.e., present-day Exoristinae). This group had been based on external characters and needed revision to conform to the reproductive types discussed by Herting (1957, 1960). Mesnil (1975a: 1374) concluded:

> "Nach Untersuchungen, die besonders durch B. Herting 1957 … über die Anatomie des Postabdomens der mikrooviparen Weibchen durchgeführt wurden, ist es möglich, die Gattungen der Goniinae in 2 Triben zu ordnen: die Goniini Rob.-Desv. (1830) mit mikrotypen Eiern und die Eryciini Rob.-Desv. (1830), die ovolarvipar oder ovipar sind." ["According to studies that have been carried out especially by B. Herting 1957 ... on the anatomy of the postabdomen of microoviparous females, it is possible to arrange the genera of Goniinae into two tribes: the

Goniini Rob.-Desv. (1830) with microtype eggs and Eryciini Rob.-Desv. (1830), which are ovolarviparous or oviparous."]

Goniini (*s. str.*) + Eryciini of Mesnil (1975a, 1975b) corresponded to Goniini (*s. lat.*) + Winthemiina + Ethillina of Herting (1960). Mesnil's restriction of the Goniini to microovolarviparous tachinids was a key development in the classification of the Exoristinae. Herting (1984) would later remove the Winthemiina and Ethillina from Eryciini and treat them as tribes of Exoristinae, thereby creating a concept of Goniini *s. str.* + Eryciini equaling that of Herting's (1960) Goniini.

The microovolarviparous tachinids had been recognized informally as a natural group within a broader Goniini since Herting's (1957) study of the female postabdomen. A few years later Herting (1960) again grouped these tachinids as the "Mikroovipare Arten" within his broadly defined Goniini. Herting was known to be in favour of classifying the Goniini in a more restricted sense even before this was proposed by Mesnil (1975a). Very likely the idea was more his than Mesnil's, although the two colleagues surely discussed the issue and may have influenced each other in how best to classify these tachinids. What is known is that Herting corresponded with others about his thoughts on this suprageneric complex prior to Mesnil (1975a) publishing on it. This is evident in Crosskey's (1973b: 77) comments on the tribal classification he was adopting for Australian Goniinae (i.e., Exoristinae):

> "Herting (personal communication) considers that the multifarious genera of the Goniini-Carceliini-Sturmiini-Eryciini complex should be aggregated into two tribes (for which the names Eryciini and Goniini would be nomenclaturally correct) according to whether they have an ovolarviparous or a microoviparous reproductive habit. Such a course has much to commend it insofar as it would probably reflect the real phylogeny more accurately than the present tribal system. But it is impossible to adopt such a system as yet for the Australian fauna, in which the reproductive habit of most of the genera remains unstudied."

Thompson (1963), based on his own study of innumerable dissections, also recognized the microovolarviparous tachinids as a distinct group and devoted a separate part of *Tachinids of Trinidad* to the "goniines with microtype eggs". Thompson (1963: 258) noted: "In the classification of Townsend, species producing microtype eggs are scattered through at least 14 tribes: Eriothrixini, Compsilurini, Phoroceratini, Phorinini, Actiini, Hyperecteinini, Frontinini, Goniini, Belvosiini, Harrisiini, Sturmiini, Lydellini, Phrynoini and Trypherini."

Sabrosky and Arnaud (1965) (see also above) were caught between the Townsend legacy of New World tachinid taxonomy and the rapidly evolving views on tachinid relationships and classification of the European specialists Mesnil, Herting and Verbeke. Sabrosky and Arnaud (1965) recognized both the Goniini and Eryciini but neither tribe corresponded very closely to the Goniini and Eryciini later defined by Mesnil (1975a, 1975b).

There was no Palaearctic catalogue of Tachinidae published between those of Bezzi and Stein (1907) and Herting (1984). Authors in the Old World wishing to treat regional faunas during this period were given overviews of emerging classifications first by Villeneuve (1924, 1933) and then by Mesnil (1939, 1944–1975), with contributions in particular from Herting (1957, 1960) and Verbeke (1962a). Villeneuve was acknowledged as a significant influence in the regional treatments of Stein (1924), Lundbeck (1927) and Wainwright (1928). As noted above in a quote from Wainwright (1928), Villeneuve's personal assistance to contemporary dipterists was as valuable a contribution to science as were his publications.

Before the Second World War, tachinid specimens from Africa were routinely sent to the Imperial (later Commonwealth) Institute of Entomology in London for identification, but in practise they were identified by Villeneuve in France. This changed when the war severed relations with Villeneuve and the task of identifying Tachinidae fell to the recently hired dipterist, Fritz Isidor van Emden. Thus began van Emden's foray into the Tachinidae that resulted in his valuable contributions on the faunas of the Afrotropical (as "Ethiopian") Region (van Emden 1945, 1947, 1960 [the last posthumously]) and British Isles (van Emden 1954). In choosing a classification to follow, van Emden (1954: 7) noted:

> "a sound classification has only recently been suggested by Villeneuve (1924, 1933) and worked out by Mesnil (1939, 1944). Being of such recent date, this ingenious classification has not so far been checked and applied to the whole of the family."

Van Emden was slightly too early to take advantage of the progress to come during the 1960s through the efforts of Mesnil, Herting and Verbeke. Van Emden had planned to prepare keys to the whole of the Afrotropical Tachinidae but died before the third part was published (van Emden 1960) and before the last and largest part (on Exoristinae, as "Goniinae") could be started.

Dugdale (1969) was more fortunate in being able to consider the works of Herting (1957, 1960), Verbeke (1962a), and Dupuis (1963) along with the recently revised classification of Mesnil (1966) in his treatment of New Zealand Tachinidae. Dupuis (1963) had concerned himself exclusively with the Phasiinae and his classification of the subfamily differed from that of Verbeke's principally in the exclusion of the Strongygasterini and Rondaniooestrini. Despite Dugdale's (1969) detailed review of recent advances, the New Zealand fauna is a small and isolated one and the affinities of some of its taxa were not resolved by Dugdale and remain uncertain to this day.

Roger Ward Crosskey became the next dipterist with the Commonwealth Institute of Entomology after the death of van Emden. His would be a remarkable tenure, singlehandedly producing a revision of the Rutiliini (a tribe of Dexiinae confined to the Oriental and Australasian regions, Crosskey 1973a), conspecti on the Tachinidae of Australia (Crosskey 1973b) and the Oriental Region (Crosskey 1976), a catalogue of the Afrotropi-

cal[13] Tachinidae (Crosskey 1980b), and keys to the tachinid genera of tropical and southern Africa (Crosskey 1984). Additionally, Crosskey later assisted with the preparation of a catalogue of the Tachinidae of the Australasian and Oceanian regions (Cantrell and Crosskey 1989). These resources offered a wealth of information on the names, classification, identification and hosts of Old World non-Palaearctic Tachinidae. The function of these works, however, was not to investigate and further illuminate the phylogenetic relationships of the Tachinidae. Perhaps for this reason and for the sake of consistency, the classificatory scheme adopted for the earliest conspectus was carried through with little change to the final catalogue, despite advances in tachinid systematics in the interim.

The classifications of Crosskey (1973b, 1976, 1980b) and Cantrell and Crosskey (1989) are very nearly the same and are best compared to the overview of tachinid classification given by Mesnil (1966) and, with respect to the Goniini–Eryciini, Mesnil (1975a). The classification in these works differed from that of Mesnil most significantly in the following respects[14]:

1) Tachininae included, in addition to the Tachininae *sensu* Herting (1984), most of Mesnil's (1966) Voriini as tribes Campylochetini, Parerigonini, Phyllomyini, Thelairini, Voriini, and Wagneriini. Mesnil's (1966) voriine subtribe Dexiomimopsina was included in Leskiini (later, *Dexiomimops* Townsend was treated in Voriini of Dexiinae by Herting 1984 and Shima 1987).
2) The "Goniini-Carceliini-Sturmiini-Eryciini complex" of Goniinae (i.e., Exoristinae) was not divided into Goniini and Eryciini according to egg type as advocated by Herting (see quote above from Crosskey 1973b) and Mesnil (1975a). Crosskey (1973b) gave two practical reasons for this: the reproductive habits of most of the genera involved were unknown and separating the redefined Goniini and Eryciini in a key on the basis of external morphology would not be possible even if egg type of each genus was known.
3) Dufouriinae were recognized as a subfamily with tribes Dufouriini and Imitomyiini; Mesnil (1966) had treated the former as a subtribe of Voriini and the latter as a subtribe of Phasiini.
4) Doleschallini were recognized as a tribe of Dexiinae; Mesnil (1966) had treated the single Oriental/Australasian genus *Doleschalla* Walker in the Doleschallina of Voriini[15].
5) Oxyphyllomyiini were recognized as a tribe of Tachininae; Mesnil (1966) had treated the single Oriental genus *Oxyphyllomyia* Villeneuve in the Oxyphyllomyiina of Voriini. Later, Shima (1983) transferred *Oxyphyllomyia* to Leskiini.

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[13] The term Afrotropical was proposed by Crosskey and White (1977) to replace Ethiopian for this biogeographic region. Crosskey (1980a) was chief editor of the voluminous *Catalogue of the Diptera of the Afrotropical Region*.

[14] Bear in mind that Mesnil (1966) treated all Tachinidae at one rank lower than Crosskey, placing them all in subfamily Tachininae (or Larvaevorinae) with tribes Dexiini, Voriini, Tachinini, Exoristini, Goniini and Phasiini.

[15] According to Crosskey (1973b: 74), Verbeke (1962a) misidentified *Doleschalla* as *Torocca* Walker; the true *Torocca* was not studied.

6) Thelairini of Tachininae included Mesnil's (1966) Zambesina of Exoristini (see discussion, Crosskey 1973b: 75).
7) Palpostomatini and Glaurocarini were recognized as tribes of Tachininae; Mesnil (1966) had treated both as subtribes of Exoristini.
8) Neaerini and Siphonini were recognized as tribes of Exoristinae; Mesnil (1966) had treated both as subtribes of Tachinini.
9) Rondaniooestrini were placed in Tachininae; Mesnil (1966) had treated the Rondaniooestrina as a subtribe of Phasiini.

The Modern Era

Nearly 25 years after writing about the biology of the West Palaearctic Tachinidae (Herting 1960) and over 75 years after the Palaearctic Tachinidae were last catalogued (Bezzi and Stein 1907), Herting (1984) published a long-awaited *Catalogue of Palearctic Tachinidae*. Much had changed since the former catalogue, both in terms of the suprageneric classification and number of genera and species. The tachinid fauna of the Palaearctic Region was the most intensively studied of all regional faunas and an up-to-date catalogue was an invaluable resource. Mesnil's classification had evolved significantly over the years since publication of *Essai sur les Tachinaires* in 1939 but the changes had taken place in stages and must not have been easy for a non-specialist to follow. Herting had introduced changes too, some accepted by Mesnil and others not. Coincidently, Herting's (1984) catalogue came out at the end of Mesnil's long career and there have not been any sweeping changes to tachinid classification since. What has changed will be discussed further on. Herting (1984: 2) compared his classification to that of Herting (1960):

> "The subdivision into four subfamilies is the same, only the name Echinomyiinae had to be changed into Tachininae. Some alterations have been made on the tribal level: The tribe Goniini is now restricted to the microoviparous forms, whereas the oviparous and ovolarviparous genera are assembled in a separate tribe, Eryciini. In the subfamily Tachininae, the number of tribes has been moderately increased, but not all the divisions made by Mesnil (1966b) in Lindner 64g: 885–896, have been accepted. The Siphonini are transferred from the Exoristinae to the Tachininae, where they are certainly better placed."

The classification of Herting (1984) differed from that of Mesnil (1966, 1975a) primarily in the following respects:

1) Winthemiini and Ethillini were recognized as tribes of Exoristinae; Mesnil (1975a) had included them in Eryciini, the former as Winthemiina and the latter as the three subtribes Ethillina, Phorocerosomina, and Atylomyina.

2) Dufouriini were recognized as a tribe of Dexiinae; Mesnil (1966) had treated Dufouriina as a subtribe of Voriini.
3) Voriini were recognized, without subtribes, alongside Dexiini and Dufouriini as one of three Palaearctic tribes in Dexiinae. Mesnil (1966) had treated his Voriini on the same level as the Voriinae of Verbeke (1962a) with 17 subtribes (see above for the treatment of Mesnil's Voriini in Tachininae by Crosskey).
4) Tribes of Tachininae were significantly reduced from the subtribes of Tachinini of Mesnil (1966), although there was a sizable increase over the three tribes formerly recognized by Herting (1960). This increase over Herting (1960) was due primarily to a finer splitting of Echinomyiini and the separation of Minthoini from Leskiini.

Following closely after Herting's (1984) catalogue was a comprehensive and insightful study of the male postabdomen by Tschorsnig (1985), Herting's student and later his successor in Stuttgart. Tschorsnig took a comparative approach, describing the structures comprising the male postabdomen, detailing variation throughout the family, and discussing at the end of each taxonomic group the evidence regarding affinities. The work was less focused on the phallus and the pre- and postgonites than that of Verbeke (1962a) and arrived at some different conclusions. For example, the Phasiinae were considered monophyletic based on the structure of the hypandrium rather than on Verbeke's POS type distiphallus, and the Dexiinae of Herting (1984) and not Verbeke (1962a) were considered monophyletic based on Verbeke's type II phallus and type C pregonite. Although Tschorsnig's study was phylogenetic in nature it did not include a cladogram of inferred relationships. The author may have considered the subject too complex and uncertain to condense into a single cladogram and may have preferred instead to present information about possible relationships in a narrative format.

Cantrell (1988) also conducted a comparative study, this one on the postabdomen of both sexes of Australian Tachinidae with descriptions of first instars and puparia. It was based on a thesis that was presumably completed prior to the publication of Tschorsnig (1985) because this work was not cited. The study provided a good overview as well as notes about each tribe of Australian Tachinidae.

Herting's (1984) catalogue has been particularly influential to modern tachinidology because it summarized the current state of knowledge after a long period of change and has been followed subsequently by a period of relative stability. There have been highly significant works on Tachinidae published since 1984 but no revolutionary ideas have emerged about higher level relationships and classification. This is not to say that Herting's classification is a true reflection of tachinid phylogeny, but rather it has changed little because the large groups that are least likely to be monophyletic (e.g., Eryciini, Tachininae, Voriini) have remained too little understood to permit their reclassification along phylogenetic lines.

Some major regional treatments and larger taxonomic works since Herting (1984) are reviewed below. There is still uncertainty about the proper placement of certain

Table 1. The varied taxonomic placements of certain taxa of the Tachinidae by different authors are shown. Square brackets are used to indicate that a family-group name based on the taxon in question is given in the work but the taxon itself is not named in the work.

Taxon/ Authors	***Acemya* Rob.-Des.**	***Campylocheta* Rondani**	***Dufouria* Rob.-Des.**	***Euthera* Loew**	***Imitomyia* Townsend**	***Microphthalma* Macquart**	***Oxyphyllomyia* Villeneuve**	***Palpostoma* Rob.-Des.**
Herting (1960)	Exoristinae, Acemyiini	Dexiinae, Voriini	Dexiinae, Dufouriini	—	—	Tachininae, Microphthalmini	—	—
Verbeke (1962a, 1963)	Eutachininae[1], Acemyini	Voriinae, *Campylocheta* group	Dufouriinae, Dufouriini	Voriinae, *Euthera* group	Dufouriinae, Macquartiini[2]	Echinomyiinae[1], *Microphthalma* group	—	Dufouriinae, Macquartiini[2]
Sabrosky & Arnaud (1965)	Goniinae[3], Acemyini	Tachininae, Campylochetini	—	Phasiinae, Eutherini	Phasiinae, Imitomyiini	Proseninae, Dexillini	—	[Phasiinae, Palpostomatini]
Mesnil (1966)[4]	Exoristini, Acemyina	Voriini, Campylochetina	Voriini, Dufouriina	Phasiini, Eutherina	Phasiini, Imitomyina	Tachinini, Microphthalmina	Voriini, Oxyphyllomyina	Exoristini, Palpostomatina
Guimarães (1971)	[Goniinae, Acemyini]	Tachininae, Campylochetini	—	Phasiinae, Eutherini	—	Proseninae, Dexillini	—	—
Crosskey (1973, 1976, 1980b, 1984)	Goniinae, Acemyini	Tachininae, Campylochetini	[Dufouriinae, Dufouriini]	Phasiinae, Eutherini	Dufouriinae, Imitomyiini	Tachininae, Microphthalmini	Tachininae, Oxyphyllomyiini	Tachininae, Palpostomatini
Herting (1984), Herting & Dely-Draskovits (1993)	Exoristinae, Acemyini	Dexiinae, Voriini	Dexiinae, Dufouriini	Phasiinae, Eutherini	—	Tachininae, Microphthalmini	—	—
Cantrell & Crosskey (1989)	[Goniinae, Acemyini]	Tachininae, Campylochetini	—[5]	Phasiinae, Eutherini	—	Tachininae, Microphthalmini	—	Tachininae, Palpostomatini
Shima (1989)	—	—	—	Dexiinae	—	—	—	—
Ziegler (1998)	Exoristinae, Acemyini	Dexiinae, Voriini	Dexiinae, Voriini	Phasiinae, Eutherini	—	Tachininae, Microphthalmini	—	Tachininae, Palpostomatini
Richter (2004)	Exoristinae, Acemyini	Dexiinae, Voriini	Dexiinae, Dufouriini	Phasiinae, Eutherini	Phasiinae Imitomyiini	Tachininae, Microphthalmini	—	—
O'Hara & Wood (2004)	Tachininae, Acemyini	Dexiinae, Campylochetini	Dexiinae, Dufouriini	Dexiinae, Eutherini	Dexiinae, Imitomyiini	Tachininae, Megaprosopini	—	Dexiinae, Palpostomatini
O'Hara et al. (2009)	Tachininae, Acemyini	Dexiinae, Campylochetini	Dexiinae, Dufouriini	Dexiinae, Eutherini	Dexiinae, Imitomyiini	[Tachininae, Megaprosopini]	Tachininae, Leskiini	Tachininae, Palpostomatini
Cerretti (2010)	Exoristinae, Acemyini	Dexiinae, Voriini	Dexiinae, Dufouriini	Dexiinae, Eutherini	[Dexiinae, Imitomyiini]	Tachininae, Megaprosopini	—	—

Table 1. Continued.

Taxon/ Authors	*Rondaniooestrus* Villeneuve	*Strongygaster* Macquart	*Thelaira* Rob.-Des.	Goniini	Neaerini	Siphonini	Voriini
Herting (1960)	—	Phasiinae, Strongygastrini	Dexiinae, Voriini	Goniini *s. lat.*[6]	Echinomyiinae, Echinomyiini	Exoristinae, Siphonini	Dexiinae, Voriini
Verbeke (1962a, 1963)	Phasiinae, Strongygastrini[7]	Phasiinae, Strongygastrini[7]	Dexiinae, Thelairini	Goniini *s. lat.*[6]	Echinomyiinae[1], *Gymnocheta* group	—	Voriinae
Sabrosky & Arnaud (1965)	—	Phasiinae, Strongygastrini	Dexiinae, Thelairini	Goniini (restricted)[6]	Goniinae, Siphonini, Neaerina	Goniinae, Siphonini, Siphonina	Tachininae, Voriini
Mesnil (1966)[4]	Phasiini, Rondaniooestrina	Phasiini, Strongygastrina	Voriini, Thelairina	Goniini *s. lat.*[6]	Tachinini, Neaerina	Tachinini, Siphonina	Voriini *s. lat.*
Guimarães (1971)	—	Phasiinae, Strongygastrini	Dexiinae, Thelairini	Goniini (restricted)[6]	[Goniinae, included in Siphonini]	Goniinae, Siphonini	Tachininae, Voriini
Crosskey (1973, 1976, 1980, 1984)	Tachininae, Rondaniooestrini	—	Tachininae, Thelairini	Goniini (restricted)[6]	Goniinae, Neaerini	Goniinae, Siphonini	Tachininae, Voriini
Herting (1984), Herting & Dely-Draskovits (1993)	—	Phasiinae, Strongygastrini	Dexiinae, Voriini	microtype Goniini	Tachininae, Neaerini	Tachininae, Siphonini	Dexiinae, Voriini
Cantrell & Crosskey (1989)	—	Phasiinae, Strongygastrini	Dexiinae, Voriini	Goniini (restricted)[6]	Tachininae, Neaerini	Tachininae, Siphonini	Dexiinae, Voriini
Shima (1989)	—	Phasiinae, Strongygastrini	—	microtype Goniini	—	—	Dexiinae, Voriini
Ziegler (1998)	—	Phasiinae, Strongygastrini	Dexiinae, Voriini	microtype Goniini	Tachininae, Neaerini	Tachininae, Siphonini	Dexiinae, Voriini
Richter (2004)		Phasiinae, Strongygastrini	Dexiinae, Voriini	microtype Goniini	Tachininae, Neaerini	Tachininae, Siphonini	Dexiinae, Voriini
O'Hara & Wood (2004)	—	Phasiinae, Strongygastrini	Dexiinae, Voriini	microtype Goniini	Tachininae, Neaerini	Tachininae, Siphonini	Dexiinae, Voriini
O'Hara et al. (2009)	—	Tachininae, Strongygastrini	Dexiinae, Thelairini	microtype Goniini	Tachininae, Neaerini	Tachininae, Siphonini	Dexiinae, Voriini

Taxon/ Authors	*Rondaniooestrus* Villeneuve	*Strongygaster* Macquart	*Thelaira* Rob.-Des.	Goniini	Neaerini	Siphonini	Voriini
Cerretti (2010)	—	Tachininae, Strongygastrini	Dexiinae, Voriini	microtype Goniini	Tachininae, Neaerini	Tachininae, Siphonini	Dexiinae, Voriini

[1] Eutachininae and Echinomyiinae were used in the sense of Exoristinae and Tachininae (or Larvaevorinae), respectively.

[2] Verbeke (1962a) was often unclear when applying his findings to a classification. With respect to *Imitomyia* and *Palpostoma*, these were part of Macquartiini (also as 'Macquartiines') in Part 1 but were treated as Imitomyiini and Palpostomatini in Part 2, although in the latter they were presumably still in a subordinate relationship with Macquartiini of Part 1.

[3] The name Goniinae was changed to Exoristinae when the latter was determined to have priority.

[4] Mesnil (1966: 882) treated all Tachinidae as Tachininae and recognized six tribes, the equivalent of other author's subfamilies.

[5] Cantrell and Burwell (2010) recognized the Dufouriini as a tribe of Dexiinae.

[6] "Goniini *s. lat.*" comprises both microtype and non-microtype taxa and "Goniini (restricted)" comprises only a portion of the microtype taxa.

[7] Verbeke (1963) was not certain about the phylogenetic position of *Strongygaster* and *Rondaniooestrus* and suggested they might be "intermediaries" between Phasiinae and Dufouriinae.

taxa among some of these works and in comparison with the major works during Mesnil's era. These differences mostly concern smaller taxonomic units, often genera, and rather than discuss them below they are listed in Table 1.

Among the larger regional treatments of the 1980s were Cantrell's (1984) study of Australian Phasiinae and Wood's (1985) conspectus of the Blondeliini of North and Central America and the West Indies (the latter discussed above). The first modern key to the genera of Nearctic Tachinidae was published by Wood (1987) in *Manual of Nearctic Diptera*. The Siphonini of the world were revised at the generic level by O'Hara (1989). The Tachinidae of the Australasian and Oceanian regions were catalogued by Cantrell and Crosskey (1989), not only bringing Crosskey's (1973b) conspectus of Australian Tachinidae up-to-date but cataloguing for the first time the non-Australian tachinids of the Australasian and Oceanian regions. Shima (1989) published a general paper on tachinids aimed at a Japanese audience; this work, unpretentious in nature, was remarkably detailed and presented the first cladogram of inferred relationships among the major (and controversial) tachinid lineages.

Other than the detailed study of the systematics of Australasian Dexiini by Barraclough (1992), the 1990s were dominated by European authors. Pape (1992) published on the phylogeny of the Tachinidae family-group, wherein the Tachinidae were inferred to form a monophyletic group (see also analysis by Pape and Arnaud 2001). Belshaw (1993) produced a handbook to the tachinids of the British Isles, replacing the earlier handbook by van Emden (1954). A new Palaearctic catalogue of the Tachinidae was published by Herting and Dely-Draskovits (1993) in the series *Catalogue of Palaearctic Diptera*, essentially reproducing the catalogue of Herting (1984) with corrected spellings to conform with nomenclatural rules and including long lists of *nomina dubia* not given in the earlier catalogue. Tschorsnig and Herting (1994) produced a valuable work on the identification, distribution and ecology of the tachinids of Central Europe. Mihályi (1986) published a comprehensive identification guide to tachinid genera and species of Hungary. The Siphonini of Europe were revised by Andersen (1996). The Tachinidae chapter of *Manual of Palaearctic Diptera* was authored by Tschorsnig and Richter (1998), the Palaearctic equivalent of Wood's (1987) chapter in *Manual of Nearctic Diptera*. Chao et al. (1998) reviewed the Tachinidae of China in *Flies of China*, with keys to species and numerous illustrations of external features and male genitalia. The first-ever detailed study of the puparia and larval cephalopharyngeal skeletons of Tachinidae was published by Ziegler (1998). Ziegler, in his phylogenetic conclusions (pp. 192–194), proposed placing Glaurocarini within Ormiini *s. lat.* and placing *Dufouria* Robineau-Desvoidy (type genus of Dufouriini) and *Rondania* Robineau-Desvoidy within Voriini *s. lat.* The decade closed with Sabrosky's (1999) posthumously published volume on family-group names in Diptera. This work was about 50 years in the making and will be an indispensable reference for decades to come. The Tachinidae with 429 entries dwarfs all other dipteran families.

Traditional taxonomic works of the 21st Century began with a revision of the Polideini of America north of Mexico by O'Hara (2002). There followed a large and well-illustrated work on the identification of Tachinidae of the Russian Far East by

Richter (2004). That same year, O'Hara and Wood (2004) published a catalogue of the Tachinidae of America north of Mexico (discussed above). In this work the previous classification of Sabrosky and Arnaud (1965) was revised to conform more closely to the European model of Herting (1984). An interactive online resource to the Tachinidae of Europe was produced by Tschorsnig et al. (2004) as part of the Fauna Europaea project and continues to provide easy access to names and distributions. A catalogue of the Tachinidae of China by O'Hara et al. (2009) provided information on the names, types, distributions, and references of the approximately 1100 species known from this country. The *Manual of Central American Diptera* included a chapter on the Tachinidae by Wood and Zumbado (2010) in which 232 genera were reviewed, keyed, and illustrated (mostly with figures from Wood 1987), thereby forming a fine companion to Wood (1987). A Ph.D. thesis formed the nucleus of Cerretti's (2010) two-volume work on the Tachinidae of Italy. This treatise provided a wealth of general information on tachinids in addition to generic descriptions and keys to species of Italian Tachinidae. Also included was an interactive key to the tachinid genera of the West Palaearctic Region using the program MOSCH, developed primarily by Cerretti. An online MOSCH key to the tachinid genera of the Palaearctic Region was made available recently by Cerretti et al. (2012a).

The first molecular studies devoted to the Tachinidae made their appearance early in the 21st Century. The Exoristinae were the subject of Stireman's (2002) molecular study of genes 28S rRNA and EF-1α. The results were only partly congruent with evidence derived from morphology, most notably in not supporting the monophyly of the Goniini. A reappraisal of the same data using a Bayesian analysis (Stireman 2005) did not produce a convincing consensus tree, suggesting that the chosen genes may not be good for inferring tribal relationships within Tachinidae. In a more recent study of the Exoristinae by Tachi and Shima (2010), four genes (white, 18S, 28S and 16S rDNA) were studied. The results were similar in most respects to those of Stireman (2002, 2005), although monophyly of the Goniini was supported. Kutty et al. (2010) examined nine gene regions to infer relationships within the Calyptratae and especially the Oestroidea. In this study their Tachinidae were either monophyletic or not, depending upon the type of analysis performed. In general, these early molecular studies have shown promise and more sophisticated approaches in the future using combined morphological and molecular data sets are expected to yield more convincing results.

Conclusion

It has been written that to understand the future one must know the past. This is as true of tachinid classification as anything else. The path from Meigen has diverged, joined and meandered to where we are today. Along the way evolutionary thought changed our view of the natural world and the quest to organize animal life then took on new meaning. Chaetotaxy was revealed as an indicator of descent, as were structures

of the male and female genitalia. Homoplasy was and continues to bedevil the proper interpretation of tachinid evolution and is the reason why tachinid classification remains unstable. Nevertheless, a great amount of progress has been made in the last 200 years and new technologies are expected to bring about a better understanding of tachinid phylogeny and with it a more stable and predictive classification.

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RESEARCH ARTICLE

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Description of *Caurinus tlagu*, new species, from Prince of Wales Island, Alaska (Mecoptera, Boreidae, Caurininae)

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Abstract

A new species of the cryptic, minute, wingless, and enigmatic taxon *Caurinus*, and the second for the subfamily Caurininae, is described from Prince of Wales Island in the Alexander Archipelago, Alaska. It is distinguished from its only congener, *Caurinus dectes* Russell, 1979b, which occurs 1,059 km southeast in Oregon and Washington, based on external morphology and sequences of the mitochondrial gene cytochrome oxidase II. These two species are probably evolutionary relicts – the only known members of a clade dating to the Late Jurassic or older.

Keywords

Caurinus, Boreidae, Mecoptera, taxonomy, Prince of Wales Island, refugium

Introduction

Russell (1979a,b, 1982) described the monotypic subfamily Caurininae, genus and species *Caurinus dectes,* known only from Oregon and Washington, and later described by Beutel et al. (2008) as "arguably one of the most bizarre and cryptic species of Mecoptera and endopterygote insects." Indeed, members of the genus do not key to any order in most keys to insect orders because they lack a produced rostrum, typical of the order Mecoptera, and lack the diagnostic traits that would place them within *any*

insect order containing flightless adults with rudimentary or vestigial wings. However, they do share with members of the family Boreidae a very distinctive wing morphology and sexual dimorphism in which the adult females are nearly wingless while the males bear shortened scissor-like wings, useless for flight, that bear spines for grasping females during mating. The placement of *Caurinus* within the Mecopteran family Boreidae as the sister taxon to the Boreinae (*Boreus* 26 spp., *Hesperoboreus* 2 spp. [Penny 2013]), is apparently well established based on morphological study (Russell 1979a, b, Beutel et al. 2008, Friedrich et al. 2013) and molecular phylogenetics (Whiting 2002). However, despite recent efforts, the genus remains enigmatic due to its preponderance of plesiomorphic and autapomorphic traits (Beutel et al. 2008). The close relationship of the Mecoptera with the fleas, order Siphonaptera, is of particular evolutionary interest (Grimaldi and Engel 2005, Whiting 2002, Trautwein et al. 2012).

It was therefore with some excitement that we began accumulating *Caurinus* specimens from a large sampling project on the northern end of Prince of Wales Island, Alaska, some 1,059 km from the known range of *Caurinus dectes* Russell. Herein we describe this new species.

Materials and methods

Collections. Specimens will be deposited in the following collections:

CAS California Academy of Sciences, San Francisco, California, USA. (Norm Penny)

MTEC Montana Entomology Collection, Bozeman, Montana, USA. (Michael Ivie)

OSAC Oregon State Arthropod Collection, Oregon State University, Corvallis, Oregon, USA. (David R. Maddison)

PMJ Phyletisches Museum, Jena, Germany (Rolf G. Beutel)

SEMC Snow Entomological Museum, University of Kansas, Lawrence, Kansas, USA. (George Byers)

UAM University of Alaska Museum Insect Collection, University of Alaska, Fairbanks, Alaska, USA. (Derek S. Sikes)

USNM National Museum of Natural History, Washington D.C., USA. (Ollie Flint)

Morphological methods. Images of *Caurinus tlagu* were captured using a Leica DFC425 camera mounted on a Leica MZ16 stereomicroscope in combination with Leica Application Suite © software v.3.8.0. Images were edited using Adobe Photoshop v.7 to remove the background and lighten the images. Observations were made with a Leica MZ16 stereomicroscope (7.1×–115× magnification, 1x planapochromatic objective/10× eyepieces, max resolution 420 Lp/mm, Leica Microsystems (Switzerland) Ltd.). Measurements were made using an ocular micrometer in the MZ16 scope at 50×. Five *C. tlagu* specimens were prepared for scanning electron microscopy (SEM) using a Tousimis Samdri-790 Critical Point Dryer and sputter (gold) coating with a

Ladd coating unit. The scanning electron micrographs were taken with a ISI-SR-50 SEM and the digital imaging program Iridium Digital Imaging System. In addition to the images included herein, many more SEMs and habitat photos are associated with their specimen records via our online database Arctos (http://arctos.database.museum/saved/Caurinus-spp).

Taxon sampling. Two Mecoptera COII sequences from GenBank were used as outgroups: *Boreus westwoodi* Hagen (EU335963.1) and *Boreus hyemalis* (L.) (AF423998.1). *Boreus* species were chosen because they share the family assignment of Boreidae with *Caurinus* and therefore should be more closely related to *Caurinus* than any other genus in GenBank. The single *Caurinus dectes* COII sequence on GenBank (AF424001.1) was initially included (and its existence drove our desire to sequence COII rather than the more common gene COI), but later excluded due to it being suspected of errors (see below). One of the five Alaskan *Caurinus* specimens had ambiguous reads in both directions for its COII sequence, possibly due to co-amplification of a nuclear copy. We excluded this sequence from analysis.

Caurinus dectes specimens were provided by L. Russell. Seven specimens from Lewis County, Washington, collected in 1978 were provided for morphological study and 12 larval and 11 adult specimens from 2012 collections made in Benton and Tillamook Counties, Oregon, for DNA analysis (Table 1). Our collecting efforts on Prince of Wales Island have yielded 37 specimens (18 males, 19 females) of *Caurinus tlagu* (see Collecting methods below, Table 1). Additional, non-type specimens are likely to be found as sampling progresses. These specimens will be archived in UAM and recorded in our online database, Arctos.

DNA sequencing. Adult specimens and larvae designated for DNA extraction were stored at -70°F in cryovials containing 100% EtOH. Specimen data are presented in Table 1. DNA was extracted from whole bodies of five adult specimens from the Alaskan population and from seven whole bodies of the Oregon larvae. During the extraction process, specimens were opened with a pin prick to allow full extraction of DNA from soft tissues. After extraction was complete, specimens were soaked overnight in 70% EtOH to stop further deterioration of specimen exoskeletons in order to preserve them for future morphological study. Extractions were performed using a Qiagen DNeasy© blood and tissue extraction kit which was used according to the spin-column protocol for animal tissues. To amplify the COII gene, the following primer pair was used: forward COII-2a (ATAGAKCWTCYCCHTTAATAGAACA) and reverse COII-9b (GTACTTGCTTTCAGTCATCTWATG) taken from Whiting (2002).

Upon completion, extraction success was tested using a nano-drop spectrophotometer. DNA concentrations were (0.5–4.0 ng/μL). Primers were diluted at a relatively high concentration of 10μM in accordance with Whiting (2002). PCR was performed using the following 25μl PCR-mix: 12.5μl GoTaq DNA polymerase, 1μl each of the forward and reverse primers, 1μl Mg+, 9.75μL DNA-grade distilled water and 1μL template DNA. The following cycling regime was applied: (1) 1 min at 95°C, followed by (2) 35 cycles of 1 min at 95°C, 1 min at 59°C, and 1 min at 72°C, and (3) a final extension of 7 min at 72°C. Amplification success and correct band length was

Table 1. Specimen data (n=50 lots). Also available online at http://arctos.database.museum/saved/Caurinus-spp via Arctos. Geocoordinates are in WGS84 datum. PoW = Prince of Wales Island. * = holotype male *Caurinus tlagu*, with genitalia everted and COII gene sequenced. All other *C. tlagu* specimens are paratypes. W-screen = wet screen, Hab. = habitat. Habitat type codes: T2 = thinned secondary growth, 2= young secondary growth (unthinned), 2o = old (80yr) secondary growth, CC = clearcut, CCe = clearcut / forest ecotone, OG = old growth, AH = alpine heath. Date1 and Date2 = start and stop dates for trap samples.

Catalog Number	Species	State	Locality	Hab.	Method	Date1	Date2	Latitude	Longitude	+/- (m)	sex / stage
UAM:Ento:121022	*C. tlagu*	Alaska	PoW Is. Coffman Cv	T2	pitfall	4/27/10	5/15/10	55.9795	-132.86256	101	male
UAM:Ento:121023	*C. tlagu*	Alaska	PoW Is. Coffman Cv	T2	Berlese	5/13/10		55.9795	-132.86256	101	female
UAM:Ento:135818	*C. tlagu*	Alaska	PoW Is. Coffman Cv	T2	pitfall 4	5/14/10	5/28/10	55.9795	-132.86256	101	male
UAM:Ento:159146	*C. tlagu*	Alaska	PoW Is. Coffman Cv	T2	pitfall 2	7/14/10	7/26/10	55.9795	-132.86256	101	male
UAM:Ento:202339	*C. tlagu*	Alaska	PoW Is. Coffman Cv	T2	pitfall 4	5/18/11	5/31/11	55.9795	-132.86256	101	female , male
UAM:Ento:204005	*C. tlagu*	Alaska	PoW Is. Coffman Cv	T2	Berlese 2	6/14/11		55.9795	-132.86256	101	female , male
UAM:Ento:229946	*C. tlagu*	Alaska	PoW Is. Coffman Cv	T2	pitfall 4	7/27/11	8/7/11	55.9795	-132.86256	101	female
UAM:Ento:229944	*C. tlagu*	Alaska	PoW Is. Hatchery Ck.1	OG	Berlese	8/9/11		55.92444	-132.93938	4	female
UAM:Ento:142985	*C. tlagu*	Alaska	PoW Is. Hatchery Ck.4	OG	pitfall 2	5/14/10	5/30/10	55.88602	-132.8607	11	female
UAM:Ento:142986 *	*C. tlagu*	Alaska	PoW Is. Hatchery Ck.4	T2	pitfall 3	5/30/10	6/14/10	55.88433	-132.89734	26	male
UAM:Ento:204239	*C. tlagu*	Alaska	PoW Is. Hatchery Ck.4	OG	pitfall 2	5/31/11	6/14/11	55.88602	-132.8607	11	male
UAM:Ento:217990	*C. tlagu*	Alaska	PoW Is. Hatchery Ck.4	OG	pitfall 3	6/28/11	7/12/11	55.88602	-132.8607	11	male
UAM:Ento:221708	*C. tlagu*	Alaska	PoW Is. Hatchery Ck.4	2	Berlese 5	7/27/11		55.88285	-132.89795	27	female
UAM:Ento:203237	*C. tlagu*	Alaska	PoW Is. Luck Lk. 1 Rd.	OG	pitfall 4	5/24/11	6/5/11	55.97805	-132.75456	10	female
UAM:Ento:216180	*C. tlagu*	Alaska	PoW Is. Luck Lk. 1 Rd.	OG	pitfall 4	6/21/11	7/6/11	55.97805	-132.75456	10	male
UAM:Ento:154335	*C. tlagu*	Alaska	PoW Is. Luck Lk. 2 Rd.	OG	pitfall 1	7/8/10	7/30/10	55.96855	-132.75615	10	female
UAM:Ento:203238	*C. tlagu*	Alaska	PoW Is. Luck Lk. 2 Rd.	OG	pitfall 3	5/24/11	6/5/11	55.96855	-132.75615	10	male
UAM:Ento:159119	*C. tlagu*	Alaska	PoW Is. Luck Lk. 3 Rd.	OG	Berlese 4	7/29/10		55.95347	-132.7708	5	female
UAM:Ento:203239	*C. tlagu*	Alaska	PoW Is. Luck Lk. 3 Rd.	OG	Berlese 1	6/5/11		55.95347	-132.7708	5	female
UAM:Ento:133943	*C. tlagu*	Alaska	PoW Is. Luck Point	CC	Berlese 2	5/21/10		55.98497	-132.787	25	male
UAM:Ento:159120	*C. tlagu*	Alaska	PoW Is. Luck Point	CC	pitfall 1	7/9/10	8/1/10	55.97953	-132.77156	24	female
UAM:Ento:167053	*C. tlagu*	Alaska	PoW Is. Luck Point	CC	pitfall 1	8/1/10	8/11/10	55.97953	-132.77156	24	male
UAM:Ento:203011	*C. tlagu*	Alaska	PoW Is. Luck Point	T2	pitfall 1	5/23/11	6/5/11	55.98261	-132.77986	6	female
UAM:Ento:229942	*C. tlagu*	Alaska	PoW Is. Luck Point	CC	pitfall 1	8/2/11	8/9/11	55.97953	-132.77156	24	female
UAM:Ento:229943	*C. tlagu*	Alaska	PoW Is. Luck Point	CC	Lindgren	8/2/11	8/9/11	55.97939	-132.77216	25	male

Catalog Number	Species	State	Locality	Hab.	Method	Date1	Date2	Latitude	Longitude	+/- (m)	sex / stage
UAM:Ento:121024	*C. tlagu*	Alaska	PoW Is. Staney Ck.	CCe	pitfall	4/27/10	5/15/10	55.87126	-133.06697	5	male
UAM:Ento:202344	*C. tlagu*	Alaska	PoW Is. Staney Ck.	CC	pitfall 3	5/16/11	5/31/11	55.872	-133.06523	26	male
UAM:Ento:229945	*C. tlagu*	Alaska	PoW Is. Staney Ck.	OG	pitfall 4	7/12/11	7/27/11	55.79901	-133.11782	20	male
UAM:Ento:230091	*C. tlagu*	Alaska	PoW Is. Staney Ck.	OG	pitfall 2	5/14/12	5/28/12	55.79901	-133.11782	20	female
UAM:Ento:231726	*C. tlagu*	Alaska	PoW Is. nr Black Lk	AH	pitfall	7/9/11	7/10/11	55.58818	-132.88881	2	male
UAM:Ento:231727	*C. tlagu*	Alaska	PoW Is. nr Black Lk	AH	pitfall	7/9/11	7/10/11	55.58818	-132.88881	2	female
UAM:Ento:235023	*C. tlagu*	Alaska	PoW Is. Hatchery Ck.4	OG	pitfall	5/15/12	5/28/12	55.88602	-132.8607	11	female
UAM:Ento:235024	*C. tlagu*	Alaska	PoW Is. Luck Point	CC	Berlese	5/31/12		55.98497	-132.787	25	female
UAM:Ento:235025	*C. tlagu*	Alaska	PoW Is. Luck Lk. 1 Rd.	OG	pitfall	5/16/12	5/31/12	55.97805	-132.75456	10	female
UAM:Ento:235026	*C. tlagu*	Alaska	PoW Is. Luck Lk. 3 Rd.	OG	Berlese	5/22/12		55.95347	-132.7708	5	male
UAM:Ento:230088	*C. dectes*	Oregon	Mary's Peak	2o	w-screen Berlese	10/30/12		44.50413	-123.55125	5000	female, male
UAM:Ento:228446	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228447	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228448	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228449	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228450	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228451	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228452	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228453	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228454	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228455	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228456	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228457	*C. dectes*	Oregon	Marys Peak	2o	w-screen	5/11/12		44.50413	-123.55125	5000	larva
UAM:Ento:228458	*C. dectes*	WA	Lewis Co.	2o	Berlese	5/6/78		46.62848	-122.27701	5000	male, female
UAMObs:Ento:228643	*C. dectes*	Oregon	Cape Lookout	2o	w-screen	11/6/12		45.33954	-123.99289	5000	male
UAM:Ento:234931	*C. dectes*	Oregon	Cape Lookout	2o	w-screen	11/6/12		45.33954	-123.99289	5000	male

confirmed visually on an agarose gel stained with ethidium bromide. Bi-directional sequencing was performed at the University of Washington's High Throughput Genomics Unit.

Alignment. Sequences were aligned using CodonCode Aligner v4.0.4 (http://www.codoncode.com/aligner/) and proofread by eye with reference to codon position and the inferred amino acid sequence based on Liu and Beckenbach (1992). Alignment was without difficulty due to the absence of indels within the protein-coding sequence. MacClade was used to produce a consensus of forward and reverse reads (Maddison and Maddison 2005).

Model Selection. JModelTest v2.1.3 (Darriba et al. 2012, Guindon and Gascuel 2003) was used to determine the best fitting model among 88 available for testing. The AIC, BIC and DT all chose the model HKY+G as the best fit for the data.

Analysis. Bayesian analyses were conducted using MrBayes v3.2 (Ronquist and Huelsenbeck 2003) under the HKY+G model with default priors. Two simultaneous MCMC runs with four chains each (3 hot and 1 cold) were performed for 10 million generations and sampled every 1,000 steps discarding a burnin of 25%. To evaluate whether the MCMC analysis had reached stationarity, trace files were examined in Tracer v1.5 (Rambaut and Drummond 2003). These showed signs of good mixing and had plateaued at equal values. The average standard deviation of split frequencies between the two runs had dropped below 0.01 by 12% of the 10M step run, also indicating both runs had converged. Maximum Likelihood analyses were conducted using Garli v.2.0.1019 (Zwickl 2006) under the HKY+G model with 1000 non-parametric bootstrap search replicates in addition to a non-bootstrap analysis of 100 search replicates from random starting trees.

Collecting methods and results. Specimens of this new species were collected primarily using pitfall traps and Berlese funnels (Table 1) as part of our four year, ongoing project investigating forestry practices in the Tongass National Forest (Fig. 1). Two specimens were caught in a very different habitat in pitfall traps set on a transect of 20 traps spaced 100m apart in a treeless alpine zone (917m elevation) near Black lake, Prince of Wales Isl., with tundra-alpine-heath vegetation (e.g. *Harrimanella stelleriana*, *Luetkea pectinata, Rhytidiadelphus loreus*). This collection was part of a rapid biotic assessment of Southeast Alaska alpine zones (Fig. 1A) and was located 45 km southwest of the Coffman Cove collection sites. Pitfall traps consisted of paired (Coffman Cove) or single (alpine) plastic cups 8.3 cm in diameter and 7.5 cm deep filled 1/2–2/3 with non-toxic propylene glycol based antifreeze, Sierra © brand (Coffman Cove), or soapy water (alpine) with rain-roofs ~3 cm from the ground above the traps. Traps were emptied once every two weeks (Coffman Cove) or daily (alpine zone). Paired traps were 30cm apart with a plastic ruler embedded in the ground between them to act as a barrier to divert arthropods into the traps. As part of the Tongass sampling, BioQuip © collapsible Berlese funnels were used with ~ 1m^2 of leaf/moss litter sifted prior to running under 40 watt bulbs for 48h. These methods resulted in 37 specimens collected. However, incredible effort was involved. A total of 1,136 pitfall trap and 284 Berlese samples were processed from 2010 and 2011 that have generated 10,218 beetle speci-

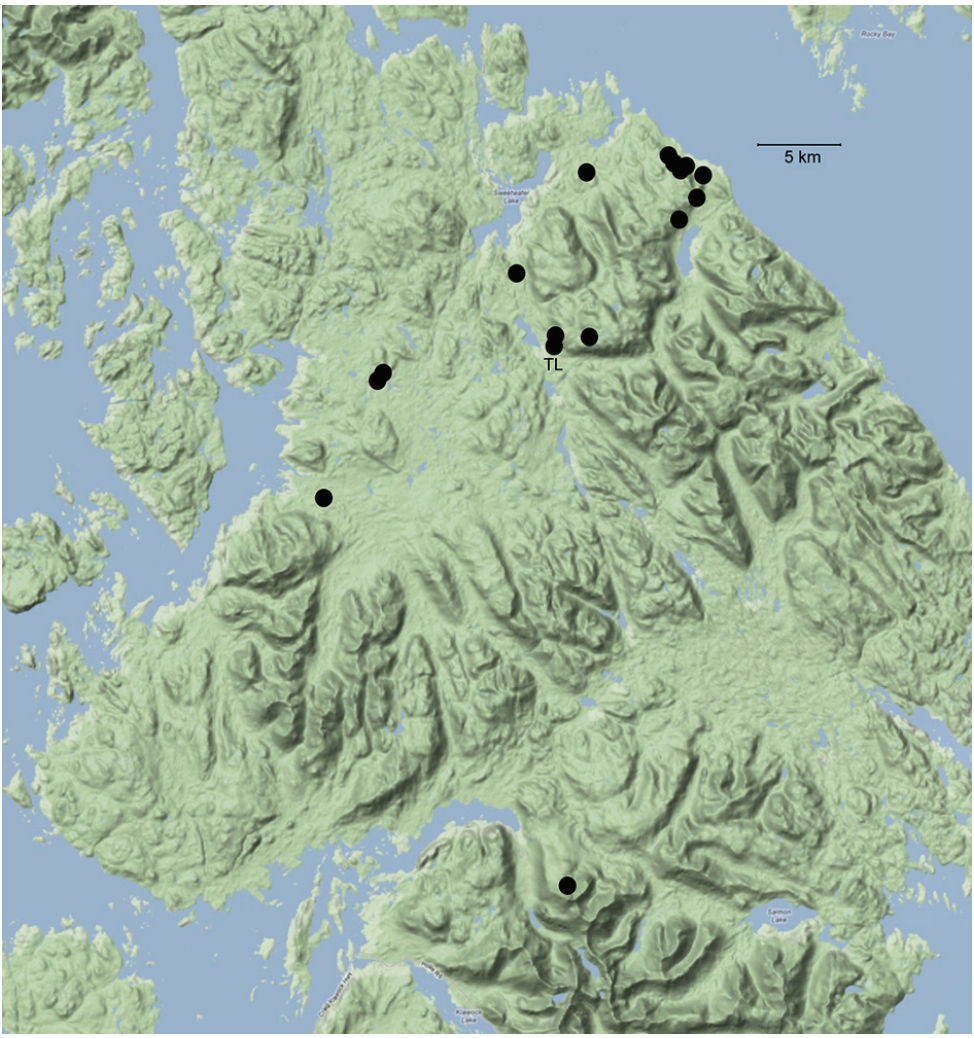

Figure 1. Sixteen sites at which *Caurinus tlagu* specimens were found, north end of Prince of Wales Island, Alaska. Table 1 lists site and specimen data, also available online at http://arctos.database.museum/saved/Caurinus-AK. TL = type locality.

mens to date. The alpine sampling involved 83 pitfall trap samples, which yielded two *Caurinus* specimens. Twenty-six *Caurinus* specimens were captured in pitfall traps, ten in Berlese funnels, and one, surprisingly, in a Lindgren funnel. Great care was taken to ensure pitfall trap rims were at or below the level of the ground – certainly an important factor when trapping an animal ~ 2 mm in size.

The majority of specimens (35/37) were collected in perhumid rainforest dominated by Sitka spruce (*Picea sitchensis*), western hemlock (*Tsuga heterophylla*), lodgepole pine (*Pinus contorta* var. *contorta*), Alaska yellow cedar (*Chamaecyparis nootkatensis*), red cedar (*Thuja plicata*), and red alder (*Alnus rubra*) (Fig. 2). Of 24 sites sampled in

Figure 2. Habitats of *Caurinus tlagu* **A** Habtiat of type locality, thinned secondary growth with 18 ft. spacing between trees, 55.88433, -132.89734 **B** example of old growth habitat in which specimen UAM:Ento:204239 was found, 55.88602,-132.8607 **C** example of clearcut, a habitat type in which seven specimens were found, 55.872, -133.06523 **D** example of treeless, alpine heath – tundra in which two specimens were found, 55.58818, -132.88881.

the Tongass National Forest project, *Caurinus* was found in 14 sites. Fifteen specimens were found in six of six sampled old growth sites, eleven in three of six sampled thinned secondary growth sites, seven in four of six sampled clear cuts, and one in one of six sampled unthinned secondary growth sites. One additional specimen was found in an ecotone next to a clear cut that was not part of the 24 structured sampling sites. The null hypothesis of *Caurinus* being equally trappable in all four habitat types: old growth, thinned secondary growth, unthinned secondary growth, and clear cuts, (ignoring the ecotone), is rejected ($Chi^2 = 12.59$, df=3, P=0.0056). These animals are less trappable in

unthinned secondary growth sites than expected under the null, and more trappable in old growth and thinned secondary growth sites than expected under the null.

Although boreids are considered winter active insects, our projects were restricted to the summer months. We caught *Caurinus* more or less evenly throughout the period of sampling (mid May – mid August) (Table 1).

Results from molecular analyses

DNA sequence characteristics. The final alignment of the DNA sequences (11 *Caurinus* sequences, 2 outgroup *Boreus* sequences) was 639 base pairs long with 491 constant sites, 21 variable but parsimony-uniformative sites, and 127 parsimony informative sites. Among the *Caurinus* sequences there were 604 constant sites and 35 parsimony informative sites. Of these 35 variable sites between the *Caurinus* species, 34 were binary with all specimens of each species sharing the same base differing from the other species. As expected, most (29) of these variable sites were third codon positions, with six variable first codon position sites, and zero variable second codon position sites. The null hypothesis of homogeneity of base frequencies across taxa was not rejected by a Chi-square test performed in PAUP*4.0b10 (Chi^2=27.5, df=36, P=0.85) (Swofford 2003). These sequences are available from Genbank (accession numbers KF282717 through KF282727) and the aligned NEXUS and tree files are available from TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S14415) under study Accession number 14415.

The *Caurinus* species are 98.5% identical in their inferred COII amino acid sequences (209 of 212 amino acids are identical). The three amino acid replacements are as follows: The 113th site of the amino acid translation is an Alanine (nonpolar) shared by all seven *Caurinus dectes* specimens but is a Threonine (polar) in all five *Caurinus tlagu* specimens; at the 114th site an Aspartic acid (acid polar) shared by all seven *Caurinus dectes* specimens is a Asparagine (polar) in all five *Caurinus tlagu* specimens; and at the 148th site an Isoleucine (nonpolar) shared by all seven *Caurinus dectes* specimens is a Valine (nonpolar) in all five *Caurinus tlagu* specimens.

All seven *C. dectes* share identical COII nucleotide sequences whereas only three of the *C. tlagu* share identical sequences, the fourth *C. tlagu* differs at one site (0.156% divergent) from the other three *C. tlagu*. The two *Caurinus* species are 5.44% divergent from each other (uncorrected "p" distance). The two outgroup species are 3.9% divergent from each other, and 21% (*B. hyemalis*) to 20% (*B. westwoodi*) divergent from *Caurinus*. The COII GenBank record of *C. dectes* (AF424001.1) is 21.7% divergent from the seven *C. dectes* we sequenced. Using the parameter values from the Garli analysis (see below) to set the HKY+G model in PAUP*4.0b10 allowed the estimation of distances corrected for multiple hits: the two *Caurinus* species are 7.17% divergent from each other. The two outgroup species are 5.6% divergent from each other, and 106.7% (*B. hyemalis*) to 103.5% (*B. westwoodi*) divergent from *Caurinus*.

Bayesian Analysis. Tracer reported auto-correlation times of 1027 and 1015 for the two runs with Effective Sample Sizes for all parameters of each run above 7000 (with samples from both runs combined, the ESS of each parameter was above 15,000). Parameter estimates of both runs combined were as follows: the harmonic mean of the estimated marginal likelihood was –1515.7, tree length 0.692, the transition/transversion rate ratio (kappa) 6.59, pi(A) 0.356, pi(C) 0.151, pi(G) 0.102, and pi(T) 0.391 with the alpha shape parameter at 0.258.

Garli Analysis. The 1000 bootstrap replicate analysis resulted in similarly strong branch support values as the Bayesian analysis (Fig. 3). One hundred non-bootstrap replicates were completed, the best tree of which was found in 96 of the searches and was identical in topology to the Bayesian tree (Fig. 3) with a -lnL of 1476.75, tree length of 0.858, and parameter values of: K parameter 8.789, ti/tv 3.321, pi(A) 0.3596, pi(C) 0.1481, pi(G) 0.0991, and pi(T) 0.3933 with the alpha shape parameter at 0.1733.

Both the Bayesian and maximum likelihood analyses found strong support for reciprocal monophyly of both *Caurinus* species (Fig. 3).

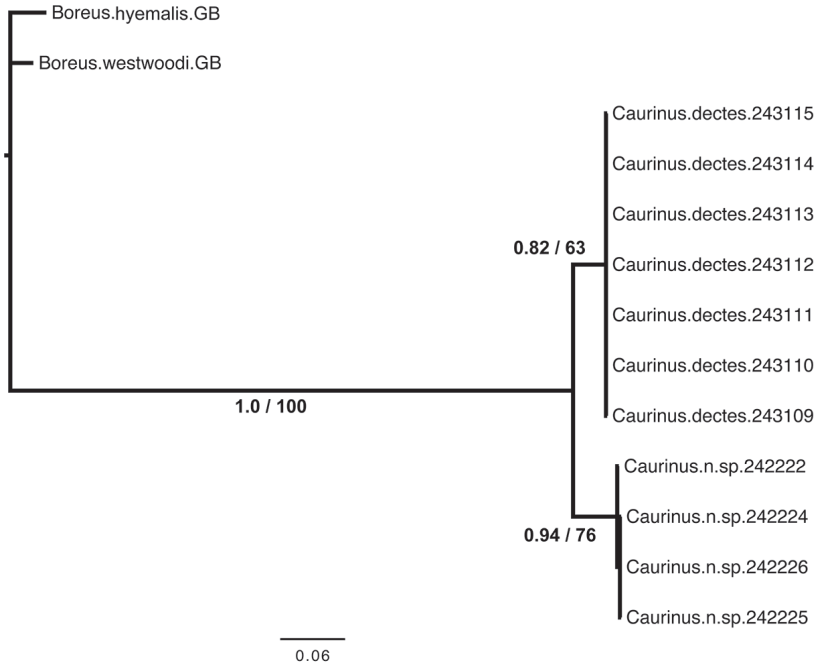

Figure 3. Inferred phylogeny from Bayesian analysis. Each terminal is a single specimen with the UAM cryovial barcode of its DNA extraction indicated by a six digit number. Branch support is indicated as estimated posterior probability from the Bayesian analysis first and maximum-likelihood bootstrap percentages second. Branch lengths are proportional to the number of substitutions per site as reconstructed by MrBayes 3.2. Specimen 242224 is the holotype of *Caurinus tlagu* http://arctos.database.museum/guid/UAM:Ento:142986. The remaining three *C. tlagu* specimens correspond to the following paratypes in Table 1: 242222 (UAM:Ento:135818), 242225 (UAM:Ento:159119), and 242226 (UAM:Ento:154335).

Systematics

***Caurinus tlagu* Sikes & Stockbridge, sp. n.**
urn:lsid:zoobank.org:act:BFFF780A-737D-4187-8539-32270D80D4C5
http://species-id.net/wiki/Caurinus_tlagu

Holotype. Male (in UAM), here designated, labeled "USA: Alaska, Prince of Wales Is. Hatchery Ck.4, 30 May-14 June 2010, 55.88433°N 132.89734°W ± 26m, 82m elev., thinned secondary growth with 18 ft. spacing between trees, pitfall 3, J. Stockbridge, C. Bickford" , / "HOLOTYPE *Caurinus tlagu* Sikes & Stockbridge 2013 UAM:Ento:142986" [red paper]. doi: 10.7299/X7GH9J4M

Paratypes. 36 Specimens (Table 1). The following 17 paratypes will be deposited in the collections indicated: male UAM:Ento:159146, female UAM:Ento:142985, female UAM:Ento:235025 (CAS); male UAM:Ento:229945, female UAM:Ento:235024, female UAM:Ento:229942 (OSAC); male UAM:Ento:235026, female UAM:Ento:203239, female UAM:Ento:203011 (PMJ); male UAM:Ento:167053, female UAM:Ento:229944, female UAM:Ento:235023 (SEMC); male UAM:Ento:217990, female UAM:Ento:221708, female UAM:Ento:159120 (USNM); male UAM:Ento:229943, female UAM:Ento:230091 (MTEC), and the 19 remaining in UAM.

Type Locality. USA: Alaska, Prince of Wales Is. Hatchery Ck, 55.88433°N 132.89734°W ± 26m, 82m elev. (Fig. 1, 2A).

Measurements. Restricted to specimens with retracted genitalia (3 males, 10 females), length, min. – max., mean ± SD : male 1.58–2.02, 1.74 ± 0.24 mm, female 1.64 – 2.00, 1.79 ± 0.13 mm.

Diagnosis. Circumference of eye of males comprises 31-35 (n=3) ommatidia (*C. dectes* males have 38–39, n=3). Scanning electron microscope-level resolution is required to obtain reliable counts (Fig. 4). Female 8th sterna without a median notch (n=10), or with a shallow median notch (n=5) (Fig. 5A,C, 6C,D). *Caurinus dectes* females have a shallow median notch or a pronounced median notch (Fig. 5B, see also Russell [1979b] fig. 10). This is visible at 40× and higher magnification.

Description. Body length 1.5–2.3 mm, flea-like in lateral view, color reddish brown, sparsely pubescent, strongly sclerotized (Fig. 6). Rostrum absent or reduced. Clypeolabral suture present. Clypeus divided into post and anteclypeus. Penultimate maxillary palpomere enlarged and club shaped. Antennal insertion lateral, widely separated. Ocelli absent. Antennae with sixteen antennomeres and a single countersunk sensilla on antennomeres 4, 5, and 6 (Fig. 7). Mandible with two subapical teeth (Fig. 6B). Male forewings extend to end of first abdominal segment, with six bristles (Fig. 8A), hindwings absent. Female forewings pad-like, hindwings absent. Tarsi five segmented, tarsal claws present. Pilosity absent. Abdomen widest at segments 4 and 5, segments 2-6 fused, annular. Male 8th tergum and sternum not fused. Male 9th tergum and sternum not fused. Genitalia normally concealed in both sexes. Male gonostyles flattened, deeply incised (Fig. 8B).

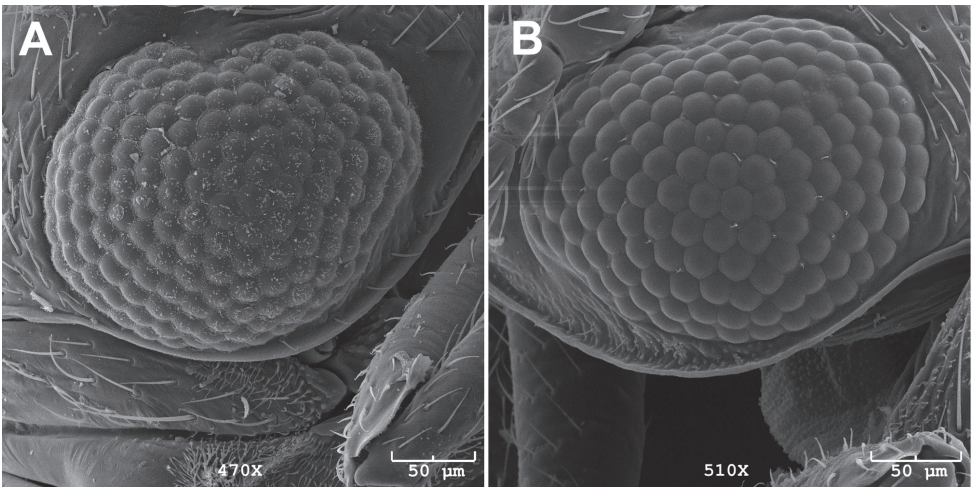

Figure 4. Eye of **A** male *Caurinus dectes* (UAM:Ento:230088) showing 38 ommatidia around circumference of right eye, dorsal is to the left, and **B** male *Caurnius tlagu* (UAM:Ento:202344) showing 35 ommatidia around circumference of left eye, dorsal is to the right. Scale bar = 50 µm.

Figure 5. A ventral view of female *Caurinus tlagu* (UAM:Ento:203239) showing 8th sternum with shallow median emargination / notch, scale bar = 500 µm **B** ventral view of abdomen of female *Caurinus dectes* (UAM:Ento:228458) showing 8th sternum with a pronounced notch, scale bar = 200 µm **C** ventral view of abdomen of female *Caurinus tlagu* (UAM:Ento:203011) showing 8th sternum with shallow emargination / notch, scale bar = 200 µm.

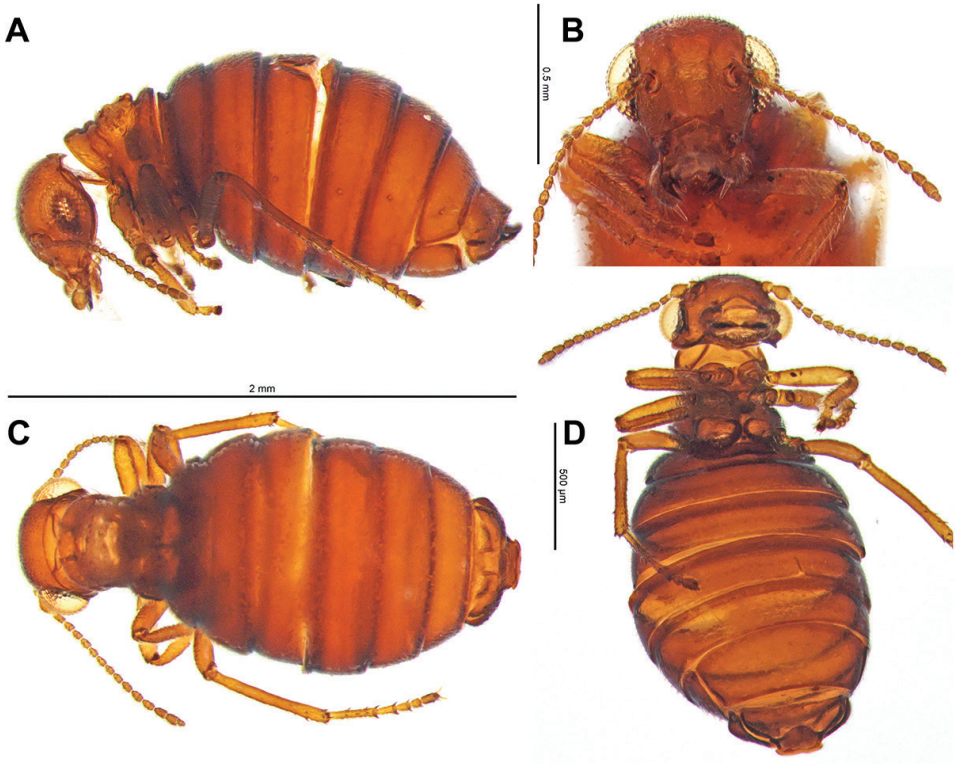

Figure 6. Female *Caurinus tlagu* (UAM:Ento:159119) that had been cleared in KOH. **A** lateral view (broken abdomen), scale bar = 2 mm **B** face, scale bar = 0.5 mm **C** dorsal view, scale bar = 2 mm **D** ventral view, scale bar = 0.5 mm.

Variation. One male (UAM:Ento:231726) has 7 bristles on its right wing, as a result of a very small extra basal bristle, and six on its left.

Geographic Distribution and Habitat. This species is only known from the northern half of Prince of Wales Island within a region about 45 km in size (Fig. 1). It was collected in forest habitat of various stages: old growth, secondary growth (thinned and unthinned), and young clear cuts; in addition to two specimens caught in alpine heath habitat and one in an ecotone of clearcut / secondary forest. The species is not restricted to lowland forests, nor to old growth forests.

Etymology. "*Tlagu,*" pronounced "tlu-gu," is derived from the Alaska Native tribal language Tlingit meaning "ancient, forever" (Crippen 2013) or "old, from the past" (Edwards 2009). Bierhorst (1985) provided this elaboration: "Among the Tlingit, for example, there are two kinds of stories, *tlagu* (of the long ago) and *ch'kalnik* (it really happened)." We name this species in honor of the place it occurs, its people, and history, in addition to the apparent great age of the genus *Caurinus*.

Discussion

Diagnostic characters were not easily found. These species are very similar phenotypically. The use of ommatidia counts around the circumference of the eyes of males (females we examined overlapped in these counts) is certainly not an ideal character because it is limited to one sex and requires SEM imaging to obtain accurate counts. In part because of this difficulty, and the rarity of specimens, our sample sizes for the assessment of this character are suboptimal. Despite these small sample sizes (n=3 for each species) the means differ significantly based on an unpaired, two-tailed student's t-test ($p = 0.0142$). We hope that ongoing morphological study of the Mecoptera by Rolf Beutel and others (e.g. Beutel et al. 2008) will better document variation between and within these *Caurinus* species.

During our examination of characters we compared both species for the paired cupuliform and countersunk antennal sensilla described by Beutel et al. (2008, fig. 3D) as occurring on the distal part of antennomeres 3 and 4. We found these on antennomeres 4, 5, and 6 (Fig. 7) but could not find them on antennomere 3 of either species. Also, we found the countersunk sensillum but not the cupuliform sensillum. We studied 5 specimens of *C. dectes* and 5 of *C. tlagu*, 3 males and 2 females of each, and were able to see sensilla on 2 female *C. dectes* and 1 male and 2 female *C. tlagu* but on no others. A shorter type of setae with a thicker apex is present near the countersunk sensilla (Fig. 7) which were also visible on those specimens on which we did not find sensilla. This lack of confirmation is likely due to the fixed positioning of the specimens for SEM imaging hiding the sensilla from view, although infraspecific variation and absence cannot yet be eliminated as explanations. The lack of sensilla on antennomere 3 of *C. dectes* raises the possibility that there are multiple species under the name *C. dectes*.

We examined the gonostyles of the males (Fig. 8B) for diagnostic characters. These complex structures may still hold diagnostic potential. In particular, the apex of the

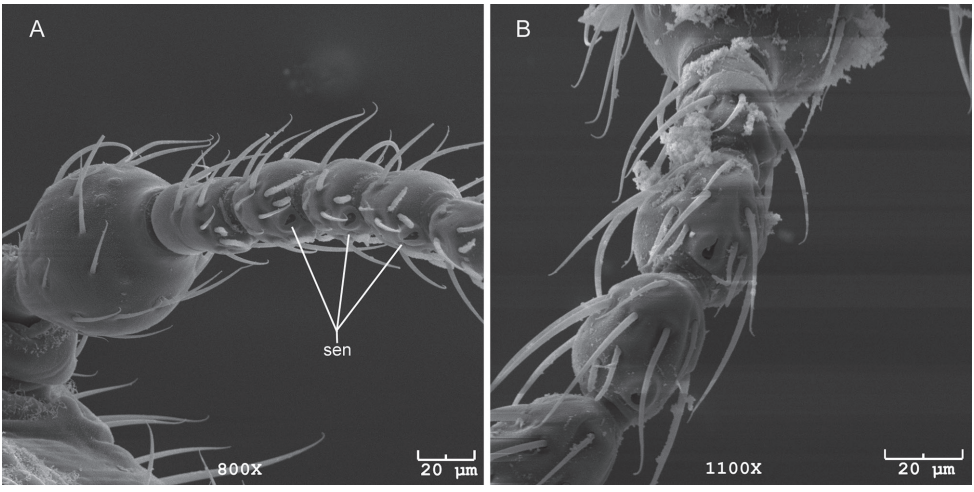

Figure 7. Base of *Caurinus* antenna showing sensilla on antenomeres 4, 5, and 6. **A** female *Caurinus dectes* (UAM:Ento:230088), **B** female *C. tlagu* (UAM:Ento:203237); sen = sensilla, scale bars = 20 µm.

gonostyle's setose basal tooth appeared tapered in *C. tlagu* and truncate in *C. dectes*. However, we were not able to confirm this state was constant in each species. The shape of the upper blade and the pattern of scale-like ridges on the upper blade also appeared to differ. Further study indicated these differences were probably due to differences in the available angles of viewing within the SEM.

We do not know the explanation for the very large COII difference (21.7%) seen between the GenBank *C. dectes* record and our own sequences of seven *C. dectes* specimens. Both samples were made by the same collector, and author of the species, L. Russell, from the type locality. The GenBank record for the *C. dectes* COII is 4.5% different from that of the GenBank record for *Panorpa debilis* (AF424023.1) from the same study (Whiting 2002) which suggests possible contamination or data mixup. Given the ambiguity of the GenBank record's accuracy we decided to exclude it from our analyses.

The two specimens recovered from the treeless alpine tundra site appear to violate characterizations of *Caurinus* being a forest associated lineage. However, *C. dectes* is often recovered from forested and open rocky sites with the common moss *Rhytidiadelphus loreus*, which represented 20% of the total vegetation at the alpine site (K. LaBounty pers. com.). That *C. tlagu* occurs in clear-cuts and secondary growth sites suggests it is not a habitat specialist. However, within the secondary growth sites in which *C. tlagu* was found, it was significantly more common in thinned sites (n= 11) than in unthinned (n=1). The former have been opened by the Forest Service program TWYGS (Tongass Wide Young Growth Studies) in which the trees have been thinned to encourage old-growth conditions whereas the latter habitats are closed-canopy and dark due to the overcrowding of even-aged trees. This does raise questions about the feeding and breeding ecology of *C. tlagu*. Russell (1979b, 1982) documented *C. dectes* as a specialist on epiphytic and terrestrial leafy liverworts (Jungermanniales). We lack

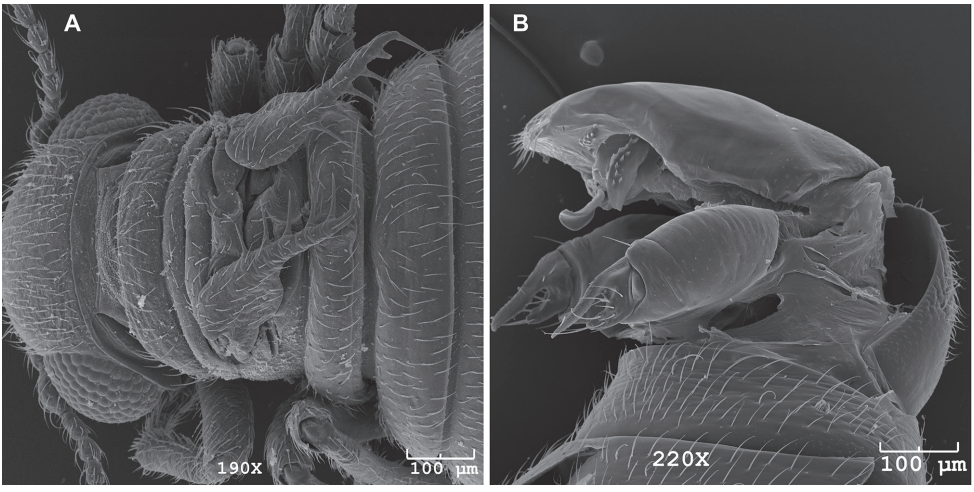

Figure 8. SEM images of male *Caurinus tlagu* (UAM:Ento:204239), scale bars = 100 µm **A** dorsal view showing wings **B** everted genitalia showing paired gonostyles, oblique lateral view.

adequate data on the bryophyte communities of the lowland forested sites to assess whether *C. tlagu* shows the same bryophyte associations as *C. dectes.* In particular, seven specimens (19% of our total catch) were found in recently deforested clear cuts, which are likely to have highly disturbed bryophyte communities.

Another notable difference between these *Caurinus* species may be their phenology. Russell (1982) describes adult *C. dectes* as primarily active during the winter (October – April), but reappearing in unseasonably wet, cool weather during the summer. This contrasts with our findings of summer presence of adult *C. tlagu*. Of course, *C. tlagu* could also be active year-round but our sampling regime would fail to detect anything but summer activity.

Various plausible scenarios exist to explain the 1,059 km range disjunction and presumed allopatric speciation within this genus of wingless mecopterans. Either or both populations could be the result of ancient (paleoendemism) or recent (neoendemism) dispersal from the other population or elsewhere (now extinct, or as yet unfound). Such dispersal could be as simple as the ancient transport of *Caurinus*-laden bryophytes by a bird. Given the genetic divergence between the populations, human transport is unlikely because it would be too recent. Alternatively, and we think more likely, both populations may be relicts of an ancient, and much larger population, with subsequent intervening extinction (paleoendemism). A multi-locus population genetics analysis with incorporation of data regarding the region's geological history would be needed to test these alternatives. Finally, these animals are not easily found and undetected populations may occur in intervening British Columbia.

Prince of Wales Island was mostly buried under an ice sheet during the maximum of the late Wisconsin glaciation 26,000 to 13,000 ^{14}C years BP (Carrara et al. 2007) and had been repeatedly buried by ice during the Pleistocene. However, considerable biological and geological evidence suggests that ice-free refugia may have existed during this time, allowing many diverse taxa to continue to evolve in relative isolation, and re-seed the region after deglaciation (Carrara et al. 2007). Of 108 mammal species or subspecies occurring in southeastern Alaska, 27 are endemic to the area (Cook et al. 2001). The known locations of *C. tlagu* are in regions that were reconstructed as under ice by Carrara et al. (2007, fig. 3). Post deglaciation dispersal to these sites from ice-free refugia is the most likely explanation. This suggests, and it would be likely regardless, that *C. tlagu* is more widely distributed than we have documented.

Despite their strong phenotypic similarity, the weight of the evidence supports the conclusion that these separate populations are not conspecific. Their mtDNA sequences being 7.17% divergent (corrected for multiple hits) suggests they have been isolated for probably less than 10 million years (Klicka and Zink 1997, Papadopoulou et al. 2010). Regardless, they have probably been isolated for longer than *Boreus westwoodi* and *Boreus hyemalis* have been isolated from each other. This degree of separation eliminates a late Pleistocene (100,000–250,000 YBP) speciation event hypothesis. The corrected genetic distances between *Boreus* and *Caurinus* (over 103%), indicate the COII gene is fully saturated with multiple hits at this level of comparison, and support the hypothesis of Russell (1979b) that *Caurinus* is a lineage of great age and not

an example of relatively recent evolutionary reversal that would make the Boreinae paraphyletic.

This suggests the split between the genus *Caurinus* and the remaining boreids likely predates the oldest confirmed boreid fossil, *Palaeoboreus zherichini* Sukatsheva & Rasnitsyn, of the Late Jurassic (Grimaldi and Engel 2005) which appears to be a boreine due to its size and external ovipositor, although it lacks the produced rostrum typical of extant species (Russell pers. com.). If confirmed, such a great age (>145 Ma) for a genus of two extant species would make the lineage an evolutionary relict and its species certainly deserving of conservation attention (Habel and Assmann 2010, Naskrecki 2011).

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Thank you to Loren Russell, Rolf Beutel, Frank Friedrich, and Michael Ivie for providing comments on an earlier version of the manuscript. We thank L. Russell for collection and donation of *C. dectes* specimens. We thank Michael Ivie, who first identified our specimens to genus (while we and various other entomologists remained unsure of its ordinal placement!). We are grateful for our field and lab technicians Casey Bickford, Ian MacDougall, Sarah Meierotto, Sayde Ridling, and Bennett Wong. We thank Kitty LaBounty who identified the plants of, and provided the habitat photo of, the site near Black lake. We thank the Oregon State Arthropod Collection for a loan of *C. dectes* specimens. Funding for this project came from two grants to the first author from the Alaska Department of Fish and Game.

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Addendum

During 16–17 May 2013, Loren Russell, the author of *C. dectes* and authority on the ecology of the genus, joined us on Prince of Wales to collect and study *C. tlagu*, and show us how to target its host bryophyte. It took us two years (2010 and 2011) to collect 37 *C. tlagu* specimens using three structured sampling methods at 24 sites. In a few hours of collecting, L. Russell was able to collect over a dozen *C. tlagu* and taught us how to brush them from one of their preferred hosts (*Scapania bolanderi*). A video of L. Russell showing this method is available at https://vimeo.com/68819818 and a second video showing *C. tlagu* hopping is available at https://vimeo.com/68819819. Russell also alerted us to an earlier, ecological study that documented *Caurinus* from the Maybeso Experimental Forest on Prince of Wales Island (LeSage et al. 2005). We were able to confirm that voucher specimens of *Caurinus* from this 2005 study are deposited in the Michigan State University collection.

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RESEARCH ARTICLE

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A tropical Atlantic species of *Melibe* Rang, 1829 (Mollusca, Nudibranchia, Tethyiidae)

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Abstract

A new species of *Melibe* is described based on two specimens collected in Florida. This new species is well differentiated morphologically and genetically from other species of *Melibe* studied to date. The four residue deletions in the cytochrome c oxidase subunit 1 protein found in all previously sequenced tropical species of *Melibe* sequenced (and *Melibe rosea*) are also present in this new species. These deletions do not appear to affect important structural components of this protein but might have fitness implications. This paper provides the first confirmed record of *Melibe* in the tropical western Atlantic Ocean.

Keywords

new species, molecular taxonomy, anatomy, Opistobranchia, western Atlantic

Introduction

Melibe Rang, 1829 (family Tethyiidae Rafinesque, 1815) is an unusual genus of cladobranch nudibranchs that feed by expanding a large oral hood fringed with sensory tentacles to capture small crustaceans. The digestive morphology of this group is largely modified, all species lack a radula and have a circularized stomach (Gosliner and Smith 2003).

Gosliner and Smith (2003) reviewed the systematics of *Melibe* and concluded that there are 14 valid species and three uncertain species. According to Gosliner and Smith (2003) *Melibe* includes a mixture of temperate and tropical species. Temperate species include *M. australis* (Angas, 1864) and *M. maugeana* Burn, 1960 from southern Australia, *M. leonina* (Gould, 1852) from California, and *M. rosea* Rang, 1929 and *M. liltvedi* Gosliner, 1987 from South Africa. Most tropical species have widespread ranges in the tropical Indo-Pacific including *M. viridis* (Kelaart, 1858), *M. pilosa* Pease, 1860, *M. papillosa* (de Filippi, 1867), *M. bucephala* Bergh, 1902, *M. engeli* Risbec, 1937, *M. megaceras* Gosliner, 1987, and *M. digitata* Gosliner & Smith, 2003. Only *M. minuta* Gosliner & Smith, 2003 and *M. tuberculata* Gosliner & Smith, 2003 appear to have restricted ranges (in Okinawa and the Philippines, respectively). In a recent paper Gosliner and Pola (2012) described two additional new species, *M. coralophilia* Gosliner & Pola, 2012 and *M. colemani* Gosliner & Pola, 2012, both from the tropical Indo-Pacific and provided the first molecular phylogeny for this group conforming the sister-group relationship of *Melibe* with *Tethys* Linnaeus, 1767, and the monophyly of both *Melibe* and Tethyiidae.

Melibe is also unusual biogeographically as it appears to be completely absent from the tropical Eastern Pacific and is poorly represented in the Atlantic Ocean. The only two confirmed records from the Atlantic are the two South African species *M. rosea* and *M. liltvedi*, which are found on the Atlantic side of the Cape Peninsula. *Melibe viridis* has been reported from the Mediterranean – originally as *M. fimbriata* (Alder & Hancock, 1864), but is considered a non-native species (Thompson and Crampton 1984). The only record of *Melibe* in the tropical Atlantic Ocean is a photograph of a potentially undescribed species found in Guanaja, Honduras, Caribbean Sea (Valdés et al. 2006), but this record has never been confirmed with the examination of specimens.

In this paper we describe the first species of *Melibe* from the tropical Atlantic Ocean based on two specimens recently collected in Florida. Molecular and morphological data obtained from the two specimens are compared with other congeners.

Methods

Source of specimens and morphological examination

Two specimens were collected by SCUBA diving in Lake Worth Lagoon Florida, photographed alive, and preserved in pharmacy grade ethyl alcohol. Once in the laboratory they were transferred to ethanol 95%. All the specimens examined are deposited at the Natural History Museum of Los Angeles County, USA (abbreviated LACM).

The anterior portion of the digestive system and the reproductive system were dissected and drawn under a dissecting microscope with a camera lucida attachment. The stomach was also dissected to expose the stomach plates. The buccal mass was dissolved in a NaOH 10% solution to isolate the jaws. The jaws and the stomach were rinsed in distilled water, dried, mounted on a stub, and sputter coated for examination under a scanning electron microscope (SEM) Hitachi S-3000N.

DNA extraction, PCR amplification and sequencing

DNA extraction was performed using a hot Chelex® protocol. Approximately 1-3 mg of the foot was cut into fine pieces for extraction. For the Chelex® extraction, the foot tissue was rinsed and rehydrated using 1.0 mL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 20 minutes. A 10% (w/v) Chelex® 100 (100-200 mesh, sodium form, Bio-Rad) solution was prepared using TE buffer. After rehydration, the tissue mixture was then centrifuged, 975.00 µL of the supernatant was removed, and 175.00 µL of the Chelex® solution was added. Samples were then heated in a 56°C water bath for 20 minutes, heated in a 100°C heating block for 8 minutes, and the supernatant was used for PCR.

Histone-3 universal primers (H3 AF 5'-ATGGCTCGTACCAAGCAGACG-GC-3', H3 AR 5'-ATATCCTTGGGCATGATGGTGAC-3' developed by Colgan et al. 1998), 16S rRNA universal primers (16S ar-L 5'-CGCCTGTTTAT-CAAAAACAT-3', 16S br-H 5'- CCGGTCTGAACTCAGATCACGT-3' developed by Palumbi 1996), and CO1 universal primers (LCO1490 5'-GGTCAACAAATCAT-AAAGATATTGG-3', HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' developed by Folmer et al. 1994) were used to amplify the regions of interest for all specimens.

The master mix was prepared using 34.75 µL H2O, 5.00 µL Buffer B (ExACT-Gene, Fisher Scientific), 5.00 µL 25 mM MgCl2, 1.00 µL 40mM dNTPs, 1.00 µL 10mM primer 1, 1.00 µL primer 2, 0.25 µL 5 mg/mL Taq, and 2.00 µL of extracted DNA. Reaction conditions for H3 (universal) and 16S (universal) were as follows: lid heated to 105°C and initial denaturation of 94°C for 2 min, 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, followed by a final elongation of 72°C for 7 min. Reaction conditions for CO1 (universal) were as follows: lid heated to 105°C and initial denaturation of 95°C for 3 min, 35 cycles of 94°C for 45 s, 45°C for 45 s, and 72°C for 2 min, followed by a final elongation step of 72°C for 10 min. PCR products yielding bands of appropriate size were purified using the Montage PCR Cleanup Kit (Millipore). Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Each primer was diluted to 4.0 pmol/µL to send out for sequencing with the PCR products. PCR products were diluted to 6.0, 7.5, and 11.5ng/µL for H3, 16S, and CO1 respectively. Samples were sequenced at Eton Bioscience, Inc. (San Diego, CA).

Sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) with the accession numbers KC992314 for CO1, KC992313 for 16S, and KC992315 for H3. Sequences of other species of *Melibe* and *Tethys fimbria* Linnaeus, 1767 were downloaded from GenBank (Table 1) and included in the phylogenetic analysis. Sequences for each gene were assembled and edited using GENEIOUS Pro 4.7.4 (Drummond et al. 2010). GENEIOUS Pro 4.7.4 was also used to extract the consensus sequence between the primer regions, construct the alignment for each gene using the default parameters, concatenate the alignments, translate the CO1 sequences intro protein sequences and align the protein sequences. The sequences were not trimmed

Table 1. Specimens sequenced, including locality information, collection voucher numbers and GenBank accession numbers.

Species	Voucher	Locality	GenBank accession numbers		
			CO1	16S	H3
T. fimbria	-	-	AY345035	AY345035	EF133468
M. leonina	LACM174849	California, USA	GQ292059	GU339202	-
M. digitata	CASIZ175724	Philippines	JX306069	JX306061	JX306076
M. digitata	CASIZ177478	Philippines	HM162699	HM162617	HM162523
M. viridis	CASIZ176981	Mozambique	JX306075	JX306068	JX306083
M. viridis	CASIZ177524	Philippines	HM162700	HM162618	HM162524
M. rosea	CASIZ175734	South Africa	JX306070	JX306063	JX306078
M. rosea	CASIZ176355	South Africa	JX306071	JX306064	JX306079
M. rosea	CASIZ176367	South Africa	JX306073	JX306066	JX306081
M. rosea	CASIZ176356	South Africa	JX306072	JX306065	JX306080
M. rosea	CASIZ176392	South Africa	HM162701	HM162620	HM162526
M. engeli	CASIZ177625	Philippines	-	HM162619	HM162525
M. engeli	CASIZ177757	Philippines	-	JX306062	JX306077
M. arianeae	LACM3259	Florida, USA	KC992314	KC992313	KC992315

after alignment. A total of 328 bp for H3, 453 bp for 16S, and 657 bp for CO1 including gaps were used for the phylogenetic analyses.

Phylogenetic analyses

To assess whether H3, 16S, and CO1 have significantly conflicting signals the incongruence length difference (ILD) test (Mickevich and Farris 1981, Farris et al. 1994), implemented in PAUP*4.0 as the partition homogeneity test (Swofford 2002), was conducted for all genes combined. The levels of saturation for each gene and for the first and second versus third codon positions of CO1 and H3 were investigated using the substitution saturation test developed by Xia et al. (2003) and Xia and Lemey (2009) implemented in the program DAMBE 4.0 (Xia and Xie 2001).

The Akaike information criterion (Akaike 1974) was executed in MRMODELTEST 2.3 (Nylander 2004) to determine the best-fit model of evolution. Bayesian analyses were executed in MRBAYES 3.2.1 (Huelsenbeck and Ronquist 2001), partitioned by gene (unlinked), with *Tethys fimbria* as the outgroup. Outgroup selection was based on the sister relationship between *Tethys* and *Melibe* (Gosliner and Pola 2012). The Markov chain Monte Carlo analysis was run with two runs of six chains for ten million generations, with sampling every 100 generations. Effective sample sizes and convergence of runs were assessed using TRACER 1.4.1 (Rambaut and Drummond 2007). The default 25% burn-in was applied before constructing majority-rule consensus tree/s.

Results

The saturation analysis showed insignificant levels of saturation for all three genes, CO1: Iss (0.4398) < Iss.c (0.7384), $P = 0.000$; 16S: Iss (0.6502) < Iss.c (0.7087), $P = 0.007$; H3: Iss (0.5591) < Iss.c (0.7193), $P = 0.000$. The ILD test showed NS conflicting signals between the genes combined: CO1 vs. H3 ($P = 0.99$), and 16S vs. H3 ($P = 0.08$), except CO1 vs. 16S ($P = 0.001$). MRMODELTEST 2.3 selected the models GTR+I+G for CO1 and 16S and GTR+I for H3 (CO1 γ shape = 0.34, proportion of invariant sites = 0.26; 16S γ shape = 0.84, proportion of invariant sites = 0.24; H3 proportion of invariant sites = 0.81).

The combined analysis of the three genes (H3, 16S, and CO1) produced a consensus Bayesian tree in which the monophyly of *Melibe* is well supported, posterior probability (pp) = 1 (Fig. 1). Within *Melibe*, the temperate species *M. leonina* is the most basal, however this result must be taken cautiously as several other species were not included in the analysis. For the rest of the species analyzed, *M. arianeae* sp. n. is sister to the tropical Indo-Pacific and South African taxa (pp = 1), which includes the Mediterranean non-native *M. viridis* as well as *M. engeli*, the species morphologically more similar to *M. arianeae*. All the species with more than one specimen included in

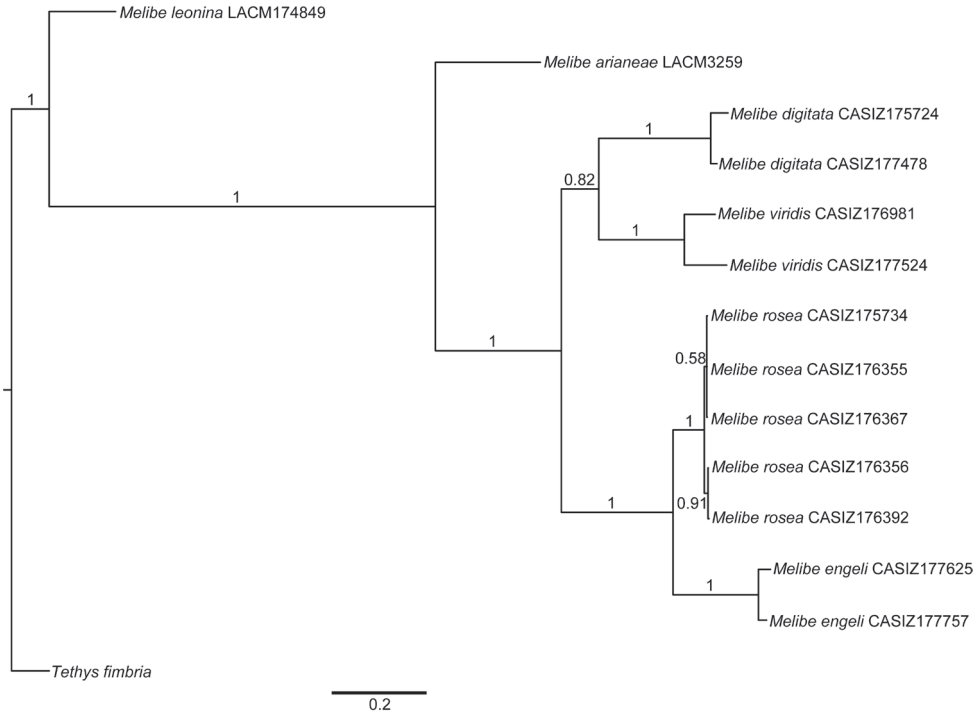

Figure 1. Bayesian consensus tree of *Melibe* including museum voucher numbers and posterior probabilities. Abbreviations: CASIZ, California Academy of Sciences, Invertebrate Zoology; LACM, Natural History Museum of Los Angeles County.

the analysis (*M. digitata*, *M. viridis*, *M. rosea* and *M. engeli*) are monophyletic and well supported (pp = 1).

When aligned with other species of opisthobranchs including *Tethys*, the CO1 sequence of *M. arianeae* as well as those of other tropical species of *Melibe* available in GenBank show 4 codon deletions. These codons are in positions 87–89, 352–354, 470–472, and 473–475 (2) of the partial sequence alignment.

Systematics

***Melibe arianeae* sp. n.**
urn:lsid:zoobank.org:act:B9B242B1-9440-4AC3-88D9-A7260986172E
http://species-id.net/wiki/Melibe_arianeae
Figs 2–4

Melibe sp. Valdés et al., 2006: 234–235.

Type-locality. Lake Worth Lagoon, Palm Beach County, Florida (26.782781, -80.04468), 3 m depth, 6 mm preserved length, April 6, 2013, A. Dimitris leg.

Type-specimens. Holotype preserved in ethanol 95%, dissected but no organs removed (LACM 3258). Paratype preserved in ethanol 95%, dissected, reproductive system in the same vial, stomach on a SEM stub (LACM 3259).

Description. The living animals are nearly transparent, with numerous orange flecks and opaque white blotches all over its surface, and orange-brownish colored internal organs (Fig. 2). The body is limaciform and elongate, somewhat compressed anterolaterally, tapering posteriorly into a long, conical posterior end of the foot. The entire body surface, including cerata and rhinophoral sheaths are covered by numerous minute, opaque white tubercles. In the center of the dorsum of the holotype there are several (8) transparent tentacular papillae of different sizes, also covered with small white tubercles and having opaque white apices. The foot sole is wider anteriorly, it is covered with orange flecks and opaque white blotches as the dorsal surface, but it also has a faint white rim. The circular oral hood is small compared to the rest of the body. The margin of the hood is entire (with no indentations) and bears two rows of elongate papillae. There are papillae on the dorsal surface of the hood, generally resembling those on the body surface, and more concentrated towards the anterior margin. The rhinophores emerge from the posterior end of the oral hood. The rhinophores have 3–4 perfoliations. The rhinophoral sheaths are somewhat inflated and cylindrical, lacking a leaf-like posterior process. The sheaths have 2–3 posterior papillae. The cerata are inflated, oval, completely covered with small tubercles that give it a broadly warty look. Their distal ends of the cerata are either simple, bifurcate or trifurcate, independently of their size. The cerata are transparent, and the branches of the digestive gland within them are visible as a brownish axis. There are seven cerata alternating on each side of the dorsal midline of the holotype. The anus is located dorsol-lateraly, midway

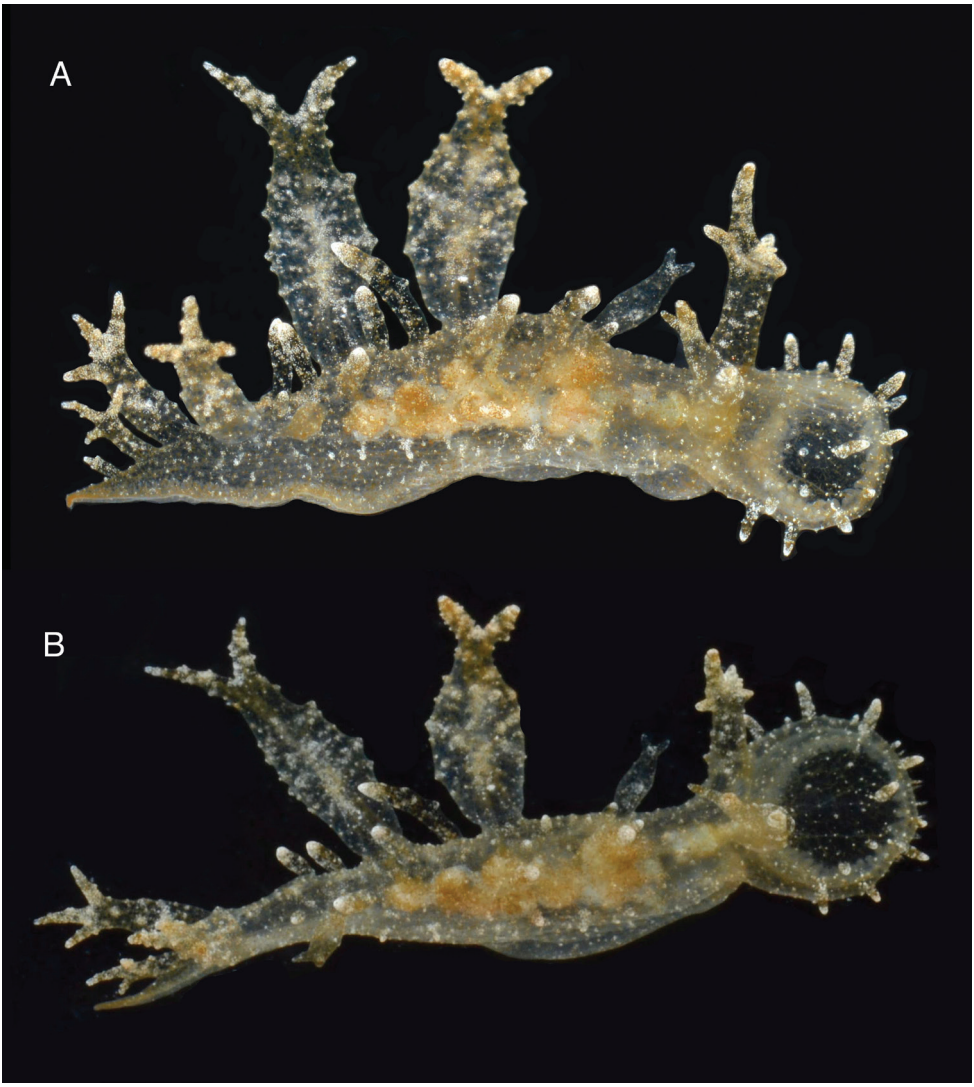

Figure 2. Two views of the holotype of *Melibe arianeae* sp. n. (LACM 3258). A. Dorsolateral view showing the right side of the animal. B. Dorsal view showing the oral hood border through the semi-transparent skin.

between the first and second anterior cerata. The position of the nephroproct could not be determined. The gonopore is lateral, anterior to the anteriormost right ceras. There are no papillae associated with the gonopore.

The buccal mass is devoid of a radula but contains a pair of simple, chitinous jaws. The jaws (not illustrated) have smooth borders and lack denticles on the masticatory border. The short esophagus emerges from the posterior end of the buccal mass and expands into a muscular stomach (Fig. 3B). Two small salivary glands are located laterally, one on each side of the buccal mass. The posterior portion of the stomach contains

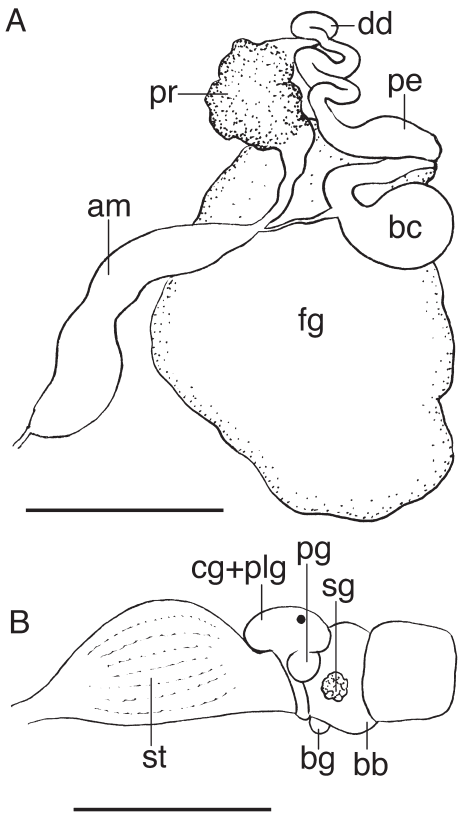

Figure 3. A Reproductive system of the paratype of *M. arianeae* sp. n. (LACM 3259) **B** Anterior portion of the digestive system of the holotype of *M. arianeae* sp. n. (LACM 3258). Scale bars = 1mm. Abbreviations: am, ampulla; bb, buccal bulb; bc, bursa copulatrix; bg, buccal ganglion; cg, cerebral ganglion; dd, deferent duct; fg, female gland complex; pe, penis; plg, pleural ganglion; pg, pedal ganglion; pr, prostate; sg, salivary gland; st, stomach.

18 elongate, thick and robust chitinous plates of various sizes (Fig. 4). The reproductive system is triaulic and contains a series of four spherical, well-separated ovotestis bodies, connected to a large ampulla. The ampulla connects into the female gland complex (Fig. 3A) near the point where the prostate emerges. The prostate is a short and wide glandular structure connected to a long, and convoluted deferent duct that expands distally into the penial sac. The vagina is short and wide and connects directly into a large bursa copulatrix. The narrow and straight uterine duct connects to the female gland complex. A serial seminal receptacle (present in other species of *Melibe*) was not observed. The central nervous system (Fig. 3B) is located above the esophagus and contains a fused pair of cerebral and pleural ganglia, as well as a pair of pedal ganglia. The buccal ganglia are located at the proximal end of the buccal mass.

Etymology. This species is named for Ariane Dimitris, amateur naturalist and passionate sea slug enthusiast, who collected the specimens here examined.

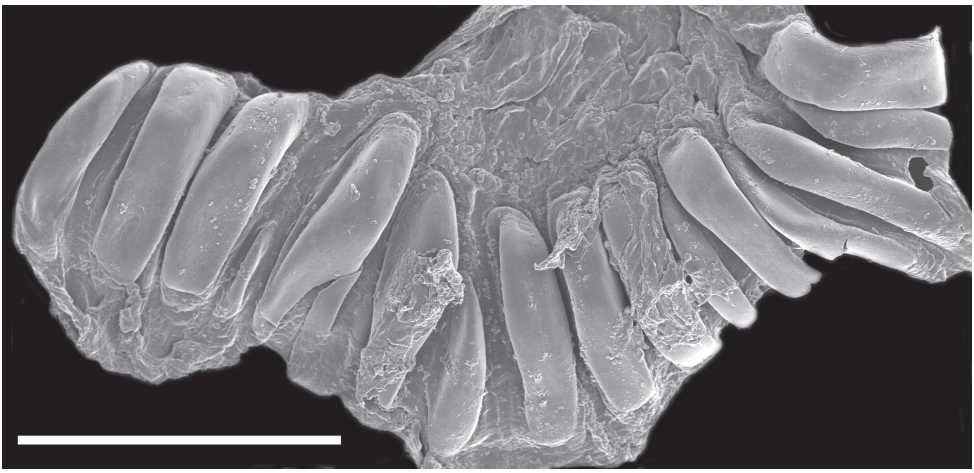

Figure 4. Scanning electron micrograph of the dissected stomach of the paratype of *M. arianeae* sp. n. (LACM 3259) showing the stomach plates. Scale bar = 500 μm.

Discussion

Melibe arianeae is well differentiated morphologically relative to other congeners (discussed below). Molecular evidence is provided to support the placement of this new species in *Melibe* and to compare it to other species for which sequence data are available. There are large gaps in the molecular coverage of *Melibe*, thus comparison to all other species is not possible at this point. However, the phylogenetic tree here presented shows that *M. arianeae* is sister to a well-supported clade containing some of the most morphologically similar species to *M. arianeae*.

Melibe arianeae is externally most similar to *M. engeli*, originally described from New Caledonia and recently reported from Japan, the Hawaiian Islands and the Philippines (Gosliner and Smith 2003). Both species have a nearly transparent body with large cerata covered with conical tubercles and having bifurcated or trifurcated tips, as well as a large oral hood with two rows of papillae. Distinguishable characteristics include the absence of leaf-like posterior processes on the rhinophoral sheaths of *M. arianeae* and the lack of a vestibular gland in this species, both present in *M. engeli* (Gosliner and Smith 2003). The body papillae of *M. engeli* are more conical than those of *M. arianeae* and the cerata of *M. engeli* are covered with large papillae giving them a serrate appearance (Gosliner et al. 2008); these are absent in *M. arianeae*. Additionally, *M. engeli* is much larger, reaching up to 45 mm in length (Gosliner et al. 2008), whereas all specimens of *M. arianeae* are smaller than 15 mm. Additionally, *M. engeli* and *M. arianeae* are genetically distinct, with a pairwise identity of 71.4% in 16S and 96% in H3 (there are no CO1 sequences available for *M. engeli*). For comparison, within species pairwise identity values in *Melibe* range between 96.2–99.3% for 16S and between 99.3–100% for H3.

All other species of *Melibe* examined and reviewed by Gosliner and Smith (2003) and Gosliner and Pola (2012) are externally easily distinguishable from *M. arianeae*. The California species *M. leonina* has flat, smooth, leaf-like cerata and a large oral

hood with comparatively small rhinophores. The Australian species *M. australis* and *M. maugeana* are also easily distinguishable; *M. australis* has short, densely papillate flask-like cerata, and a round oral hood with a thick margin, whereas *M. maugeana* has a very large oral hood and few, long and cylindrical or fusiform cerata. The other two species found in the Atlantic, the South African *M. rosea* and *M. liltvedi*, are also clearly different; *M. rosea* has a pinkish general color, a large oral hood, and irregular and small club-shaped certata, and *M. liltvedi* has a comparatively small hood and very characteristic club-shaped, very large cerata. The tropical Indo-Pacific species exhibit a remarkable morphological diversity that makes most species easily identifiable. All the species are illustrated in Gosliner et al. (2008) based on live animals and here compared to *M. arianeae*. *Melibe megaceras* is a very distinctive species with very elongate and bifurcate cerata and a relatively small oral hood. *Melibe digitata* has long cerata with the apices densely covered with long papillae. *Melibe minuta* has long, highly ramified cerata ending in multifid acutely tapering apices. *Melibe tuberculata* is easily identifiable because of the presence of large, stalked tubercles on the cerata. *Melibe papillosa* and *M. pilosa* are very similar externally, both have a large oral hood and apically flattened cerata with a regular wedge shaped margin, bearing a few thin papillae. *Melibe bucephala* is a large species with an incised oral hood, the body covered with papillae and the cerata with large apical digitations. *Melibe coralophilia* resembles a live coral head, and it is densely covered with tubercles that form a mid-dorsal crest and cover the surface of the cerata. *Melibe colemani* is a transparent species with a series of white interconnecting digestive gland ducts visible throughout the body. *Melibe viridis*, which has been reported from the Mediterranean Sea, is distinguishable from *M. arianeae* by having a large oral hood and flattened, saccate, oval to cylindrical cerata with tubercular and papillate surfaces.

The specimen of *Melibe* sp. illustrated by Valdés et al. (2006) from Honduras probably is *M. arianeae* as it shares a similar external morphology, but this needs to be confirmed with examination of specimens. If this record is confirmed the known range of *M. arianeae* includes Florida and Honduras.

One of the most intriguing aspects of the genetics of *Melibe* is the presence of four deletions in the sequence of the cytochrome c oxidase subunit 1 protein in tropical congeners and *M. rosea* from South Africa. A protein alignment revealed that these deletions resulted in the loss of 4 residues in the cytochrome c oxidase subunit 1 protein. The structure of the cytochrome c oxidase of *Paracoccus denitrificans* was reconstructed by Iwata et al. (1995) who found that the subunit 1 contains 12 membrane-spanning, primarily helical segments and binds to haem *a* and the haem a_3-copper B binuclear center where molecular oxygen is reduced. The alignment of the *Melibe arianeae* cytochrome c oxidase subunit 1 sequence with the annotated sequence of *P. denitrificans* based on the structural data collected by Iwata et al. (1995), shows that the residue deletions are located at the very beginning of helix II and in between helical segments III–IV and IV–V. These deletions do not seem to be affecting the shape of important structural elements, thus their functional implications might be limited. However, the fact that they are only present in tropical species of *Melibe* and the South African species *M. rosea* among all nudibranchs sequenced to date (including the temperate spe-

cies *Melibe leonina* and the closely related Mediterranean species *Tethys fimbria*), and that some of them are located in highly conserved regions, suggest that they could have important fitness effects for the respiratory electron transport chain of mitochondria.

Conclusion

Molecular and morphological evidence confirmed that the specimens from Florida here examined belong to *Melibe* and therefore this paper is the first confirmed record of this group in the tropical western Atlantic Ocean. Morphological examinations also confirmed that these specimens constitute an undescribed species, which is morphologically distinct from other species of *Melibe* described to date. Additionally, these specimens are genetically distinct from other species of *Melibe* so far sequenced. Our knowledge of the phylogeny of *Melibe* is spotty, as many species have not been sequenced yet, thus few conclusions can be drawn from the Bayesian consensus tree. However, it clear that the tropical Indo-Pacific species studied so far form a monophyletic group. The presence of four deletions in the sequence of the cytochrome c oxidase subunit 1 protein in some *Melibe* could have important implications to understand protein function and selection on mitochondrial genes.

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RESEARCH ARTICLE

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Where's *Waldo*? A new commensal species, *Waldo arthuri* (Mollusca, Bivalvia, Galeommatidae), from the Northeastern Pacific Ocean

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Abstract

A galeommatid bivalve mollusk, representing a new species, is described from off the coasts of California and Vancouver Island, British Columbia. The new bivalve has a commensal relationship with the heart urchin, *Brisaster latifrons*. It has been observed crawling between the oral spines of this urchin, frequently near the peristome. The bivalve has been recorded from 80 (Vancouver Island) to 444 (southern California) meters depth, in muddy sediments.

In common with other galeommatoideans, the new species broods its young; however it differs from the large majority of commensal members in lacking planktotrophic larval development.

Waldo arthuri, new species, has multiple morphological, ecological and developmental similarities to other members of the genus *Waldo* Nicol, 1966, from the southern Atlantic and Antarctic Oceans. This is most pronounced for the Argentine species, *Waldo paucitentaculatus* Zelaya & Ituarte, 2013, *W. arthuri*'s sister species in nuclear and mitochondrial gene trees. Despite this close relationship, *W. arthuri* is phylogentically distinct and possesses several hinge, shell sculpture, foot, and mantle tentacle characteristics that merit its description as new.

Keywords

Commensal relationships, Bivalvia, Galeommatidae, *Waldo*, Echinodea, urchin, taxonomy

Introduction

The unusual lifestyles of galeommatoidean bivalve mollusks have been extensively studied for over 185 years (Turton 1825). They are found in all oceans, occupy benthic habitats from the intertidal to continental shelf depths, and comprise large numbers of both free-living and commensal species. A spectrum of commensal relationships has been documented involving diverse invertebrate hosts including echinoderms, crustaceans, and annelids (Boss 1965, Chavan 1960, 1969, Dall 1884, 1899, Gage 1966, 1979, Goto et al. 2012, Morton and Scott 1989). This commensal lifestyle is robustly correlated with living in soft sediments, and the evolution of biotic associations with infaunal bioturbating hosts may have been a prerequisite for the diversification of Galeommatoidea in soft-bottom benthos (Li et al. 2012).

An undescribed galeommatid species was discovered in the late 1980's in two regions of the northeastern Pacific Ocean: Vancouver Island, British Columbia and Santa Barbara, California. The new species lives commensally with the heart urchin, *Brisaster latifrons* (Agassiz 1898) and is morphologically distinct from other known irregular echinoid commensals (Coan et al. 2000, Coan and Valentich-Scott 2012, Gage 1966, Morton and Scott 1989, Oliver 2012, Ponder 1967, 1971, Yamamoto and Habe 1974, Zelaya and Ituarte 2002, 2013).

Coan et al. (2000) provisionally identified the specimens as *"Divariscintilla"* sp. A, but this generic placement was thrown in doubt after Zelaya and Ituarte's (2002) redescription of a very similar species, *Waldo parasiticus* (Dall 1876), the type species of genus *Waldo*. Moreover, Zelaya and Ituarte (2002) described a new congener in the Southern Ocean, *Waldo trapezialis*, which is also lives on the spines of irregular echinoids and is morphologically similar to the new northeastern Pacific species. Two additional new South Atlantic *Waldo* species have recently been discovered (Zelaya and Ituarte 2013) and specimens were kindly forwarded to us for genotyping and morphologic examination. This, together with the receipt of fresh specimens from British Columbia, has prompted us to formally describe this species and test its phylogenetic relationships with South Atlantic *Waldo* species.

Materials, methods, abbreviations

Specimens of the heart urchin *Brisaster latifrons* were dredged in Barkley Sound, British Columbia, Canada by invertebrate biology classes held at the Bamfield Marine Sciences Centre on two occasions: in June 1989 from off Sandford Island at 80 m depth (48°51.47'N, 125°08.95'W), and in August 2011 from subtidal depths in the Imperial Eagle Channel (48°55.052'N, 125°13.657'W). On both occasions, live specimens of *Waldo* were observed attached to the ventral surface of the urchins. The bivalves were removed from their urchin hosts for study using dissecting microscopy and scanning electron microscopy and some were preserved in 95% ethyl alcohol for molecular characterization.

In 1986, independent sampling via box corer off Santa Barbara, California yielded additional specimens of the new species. It has subsequently been collected off Monterey Bay, Point San Luis, Los Angeles, and San Diego, California. None of the California *Waldo* specimens were directly collected from a host, but in several instances *Brisaster latifrons* was also found in the same samples. All California specimens were preserved in 4% formalin and then transferred to 70% ethyl alcohol.

For the molecular phylogeny, specimens of *Waldo arthuri*, collected in British Columbia in 2011, were genotyped together with specimens from two recently discovered species of *Waldo* from Puerto Deseado, Argentina: *Waldo digitatus* Zelaya & Ituarte, 2013, and *Waldo paucitentaculatus* Zelaya & Ituarte, 2013. Two *Lasaea* lineages were used as outgroups: *Lasaea australis* (from Esperance, Australia) and an unidentified direct-developing *Lasaea* sp. (from Hong Kong). Ethanol-preserved voucher material of the genotyped *Waldo* and *Lasaea* species have been deposited into the Museum of Zoology, University of Michigan (UMMZ 203919, 203927, 203928).

DNA amplification

A small piece of mantle tissue from each specimen was isolated for genomic DNA extraction using the Omega Biotek E.Z.N.A. Mollusc DNA Kit (Omega. tech). Fragments of two ribosomal genes, the mitochondrial large subunit 16S and the nuclear large subunit 28S, were used to reconstruct the phylogenetic relationships of the North American and Argentine *Waldo* taxa. For all species except *Waldo paucitentaculatus*, the 16S gene fragment was amplified using the *Lasaea* spp. primer set 16SLasF (5'-TAGATTAAGGGTTGGGCCTG-3')/16SLasR (5'-GCCTAAATGGTAAGACTGTTCG-3') (Li et al. unpublished data) following a touchdown PCR protocol. The initial annealing temperature (55°C) was decreased by 2°C per cycle until the final annealing temperature (48°C) was reached, then the reaction was continued for an additional 35 cycles. Because the target gene of *Waldo paucitentaculatus* failed to amplify with this protocol, an internal, *Waldo* specific primer set was developed and a doubly-nested amplification procedure was adopted to improve the PCR process. The first round of PCR was performed as above using the 16SLasF/16SLasR primer set. Products from the first PCR were then used as templates for a second round touchdown PCR using the newly developed internal primers 16SWaldoF (5'-GGCCTGCCCGGTGATAA-3')/16SWaldoR1 (5'-CAACATCGAGGTCGCAAAC-3'). The target 28S fragment for all species was amplified using the primer combination D23FLas (5'-CCGCATAGAGGCAAACGGGT-3') (Li et al. 2013) / D6R (5'-CGAAGTTTCCCTCAGGATAGCTGG-3') (Park and Ó Foighil 2000), following a standard PCR protocol with an annealing temperature at 50°C. All PCR products were sequenced at the University of Michigan Sequencing Core facility and all *Waldo* spp. sequences were deposited in GenBank under the accession numbers JX646678-JX646693.

Phylogenetic analyses

The 16S and 28S sequences were aligned respectively using ClustalW (Thompson et al. 1994) implemented in CodonCode Aligner 3.1.7 (CodonCode Corporation), and corrected by eye. The 16S gene segments amplified using the *Waldo* specific primers (339 nt) were shorter than the ones using 16SLasF/16SLasR primers (394 nt). Thus the homologous 339 nt fragment was used for further analyses. The 28S gene segment has a length of 707 nt.

Bayesian and maximum likelihood (ML) inferences were used to reconstruct the *Waldo* phylogeny for both genes fragments. For each dataset, the appropriate substitution model was selected by JModelTest 2.0.2 (Guindon and Gascuel 2003; Darriba et al. 2012) using the Akaike information criterion. Bayesian analysis was performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The Markov chain Monte Carlo (MCMC) was run for 1 million iterations with trees sampled every 1000 iterations. Two independent runs were performed with two cold and two heated chains in each run. The cumulative split frequencies were observed to be below 0.01 and all parameters were examined in Tracer 1.5 (Rambaut and Drummond 2009) to ensure convergence and proper mixing. The first 250 trees were burned in according to the convergence diagnosis and a 50% majority consensus tree was obtained. Maximum likelihood analyses were conducted with 100 bootstrap replicates using the RAxML (Randomized Axelerated Maximum Likelihood) 7.2.8 (Stamatakis 2006; Stamatakis et al. 2008) online serves hosted at the Vital-IT (http://www.vital-it.ch) Center for high-performance computing of the SIB Swiss Institute of Bioinformatics. The best-scoring trees from the analyses were obtained to represent the phylogeny.

Abbreviations: SBMNH, Santa Barbara Museum of Natural History, Santa Barbara, California, USA; UMMZ, University of Michigan, Museum of Zoology, Ann Arbor, Michigan, USA.

Systematic account

Superfamily Galeommatoidea Gray, 1840
Family Galeommatidae Gray, 1840

***Waldo* Nicol, 1966**
http://species-id.net/wiki/Waldo

Waldo Nicol 1966. Type species (original designation) *Lepton parasiticus* Dall, 1876. Recent, Antarctica.

Description. Shell small (length less than 5 mm), ovate to trapezoidal, extremely thin, fragile, translucent to opaque, gaping ventrally and on anterior and posterior ends; sculpture of commarginal striae, weak radial ribs in some; periostracum thin to thick,

translucent to white; hinge plate narrow, adults edentate; ligament internal; mantle papillate, reflected, covering most of outer shell surface; long, slender mantle tentacles extend well past shell margin; foot elongate, thin, triangular to cylindrical, heel strong to absent; with one demibranch on each side.

Discussion. Zelaya and Ituarte (2002) revived the use and understanding of this genus, with the redescription of the type species, *Waldo parasiticus,* and the description of a new species: *Waldo trapezialis*. They described, for the first time, the gross anatomy of members of the genus and suggested a possible position within the Galeommatoidea. All species are likely to be obligate commensals with echinoid echinoderms. Two additional species were described from the southwestern Atlantic Ocean (Zelaya and Ituarte 2013).

***Waldo arthuri* Valentich-Scott, Ó Foighil & Li, sp. n.**
urn:lsid:zoobank.org:act:1000CA2C-56A1-4846-B8B0-91D3AD0199CE
http://species-id.net/wiki/Waldo_arthuri
Figures 1A–H, 2A–C

"*Divariscintilla*" sp. A Coan et al. 2000: 314

Description. Shell extremely thin, fragile, moderately inflated, translucent; equilateral to slightly longer posteriorly, anterior end slightly flared to gently sloping (Figure 1 A-C); shell margins only weakly gaping if at all. **Prodissoconch** non-umbonate, D-shaped, with a greatly reduced PII comprised of a small number of faint commarginal striae bordering the metamorphic prodissoconch/dissoconch boundary (Figure 1D), prodissoconch length ranged from 338 to 357 μm (n=8) (Figure 1B). **Dissoconch sculpture** of commarginal striae, plus low broad irregular radial ribs; external sculpture variable, radial ribs absent to moderately strong, especially on anterior and posterior ends in some specimens. Beaks low, wide. **Hinge plate** extremely narrow, edentulous (Figures 1E, F). Length to 5 mm.

Mantle large, reflected, covering approximately 80% of outer shell surface when fully extended, not covering umbones (Figure 1G); mantle can be completely retracted into the shell; reflected portion papillate (Figure 1H); fused posteroventrally; facultative exhalant siphon, trumpet-shaped, non-papillate; anterior end thin, non-papillate.

Mantle tentacles long, extend well past shell margins (Figure 1G). Adult with projecting anterior pair, two laterally projecting pairs just posterior to anterior tentacles (one pair on each side); lateral tentacles not present on individuals less than 1 mm in length; ventral pair of tentacles just anterior of exhalant siphon (largest of all tentacles, in adults up to length of shell); single posterior tentacle projects dorsally to the exhalant opening. When animals are actively crawling, it appears that the tentacles might be used as levers to navigate between the urchin spines.

Foot large, exceeds the length of the shell when fully extended, vermiform, without heel (Figure 1G); long ventral byssal groove extending to end of smooth foot tip. This species is an active crawler, and can also attach to the host by byssal threads.

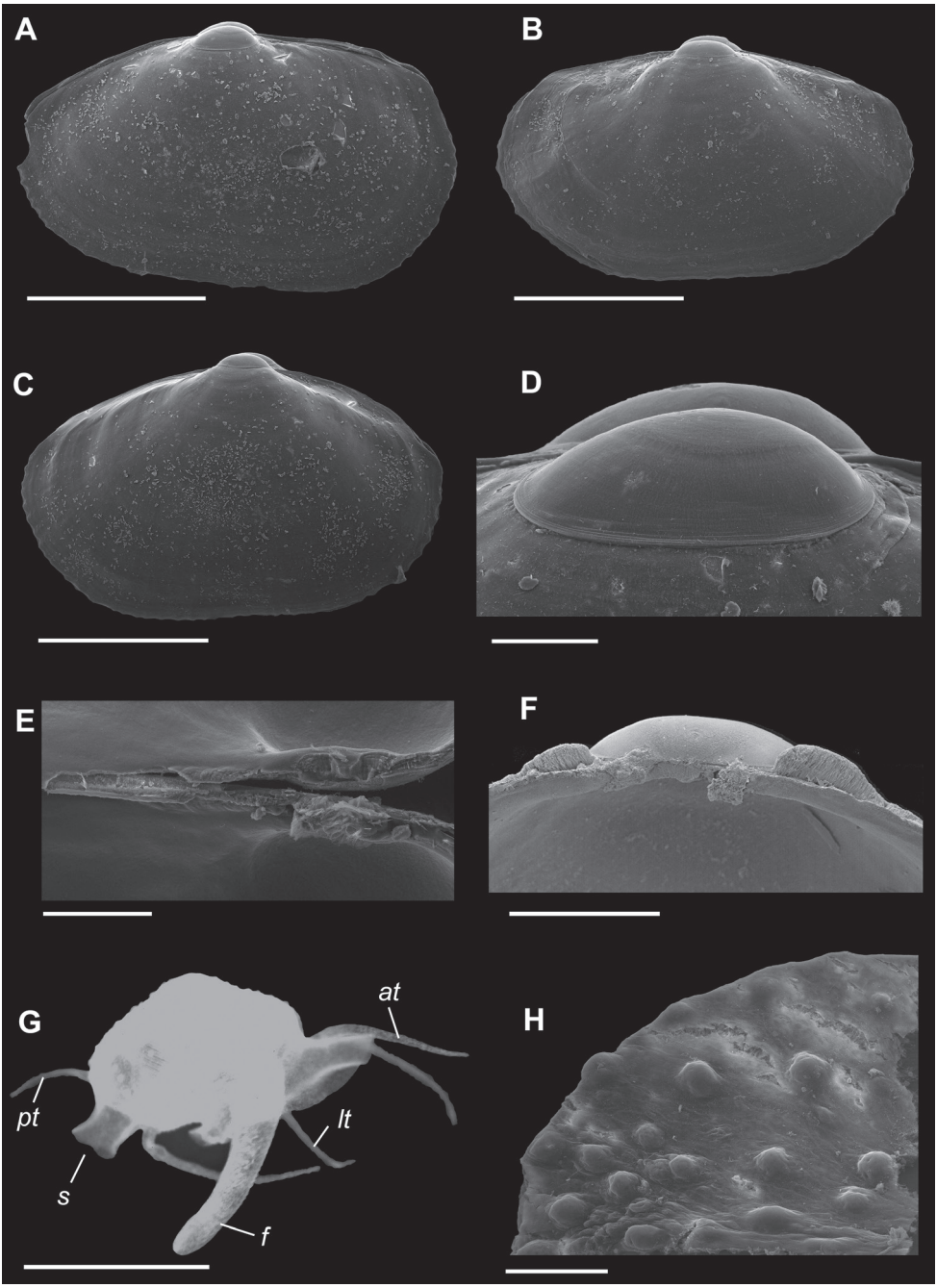


Figure 1. A–H *Waldo arthuri* new species **A–E** paratypes, SBMNH 149934 **A–C** Exterior of left valve **D** Prodissoconch **E** Close up of hinge of both valves **F** Close up of hinge of right valve **G** Live animal with extended mantle and mantle tentacles; posterior mantle tentacle (*pt*); siphon (*s*), foot (*f*), lateral mantle tentacle (*lt*), anterior mantle tentacle (*at*) **H** Detail of mantle papillae. **A–C, G** scale bar = 1 mm; **D–F, H** scale bar = 100 μm.

Ctenidia with one demibranch on each side, comprised of about 12-15 widely spaced filaments in larger specimens.

Development. The reproduction is typical of galeommatoideans, in that the animal is hermaphroditic, and the young are brooded in the ctenidia. Two brooding individuals sampled in 1989 showed early and mid developmental stages respectively. Fecundity was low; the early developmental stage individual (3.8 mm length) had 160 yolky embryos all at the blastula stage (approximately 200 μm in diameter) (Figure 2A). The second specimen was brooding mid-late stage shelled embryos (~ 270 μm length) with a protruding unciliated velum containing partially depleted yolk reserves, a larger dense mass of yolk present in anterior visceral mass, a papillate mantle that extended outside of the valve margins, and a protruding foot. The smallest non-brooded individual observed (370 μm length) byssally attached to its urchin host, had attained a modest (20 μm) increment of dissoconch growth, but notably still had visible yolk reserves dispersed across its visceral mass (Figure 2C). Although we have not observed early ontogeny, these characteristics, together with the non-umbonate prodissonch, point unambiguously toward a non-pelagic developmental mode.

Type locality. USA, California, San Luis Obispo County, off Pt. San Luis; 35°05'18'N, 121°00'54"W; 409 m.

Type material. Holotype, SBMNH 235142, conjoined shell and anatomy, length 2.5 mm, height 1.5 mm. Holotype comprises two conjoined valves, with anatomy, preserved in 70% ethyl alcohol. Given its wet preservation and small size we were unable to capture high quality photographs of the holotype.

7 Paratypes, SBMNH 149934, same locality as holotype (Figures 1A–E), specimens mounted on SEM stub; Figure 1A length 2.45 mm, height 1.45 mm; Figure 1B length 2.55 mm, height 1.45 mm; Figure 1C length 2.61 mm, height 1.63 mm.

3 Paratypes, SBMNH 235142, same locality as holotype (preserved in 100% EtOH)

4 Paratypes, SBMNH 149933, Canada, British Columbia, Sanford Island, Barkley Sound; 48°51'28"N, 125°08'57"W; 80 m, attached to *Brisaster latifrons.*

34 Paratypes, UMMZ 303919, Canada, British Columbia, Imperial Eagle Channel; 48°55.052'N, 125°13.657'W (preserved in 100% EtOH).

Distribution and habitat. Canada, British Columbia, Barkley Sound, Sanford Island, 80 meters, and Imperial Eagle Channel in soft sediments; and United States, California, from Monterey Bay to La Jolla, from 113 to 444 meters [SBMNH].

Ten juvenile specimens from the intertidal zone of Smeaton Bay, Alaska (55.4°N, 130.6°W) [SBMNH 149330] are too small to be identified to species, but might also be *Waldo arthuri.*

Commensal relationship. Crawling on the oral surface of the heart urchin *Brisaster latifrons*, primarily near the peristome. In 1989, most Barkley Sound heart urchins examined had a single bivalve although up to 3 specimens were collected on a single host. In 2011, the commensals were more plentiful: 22/33 urchins bore at least 1 commensal (mean = 2.7 clams/urchin); the maximum number on an individual host was 23 clams.

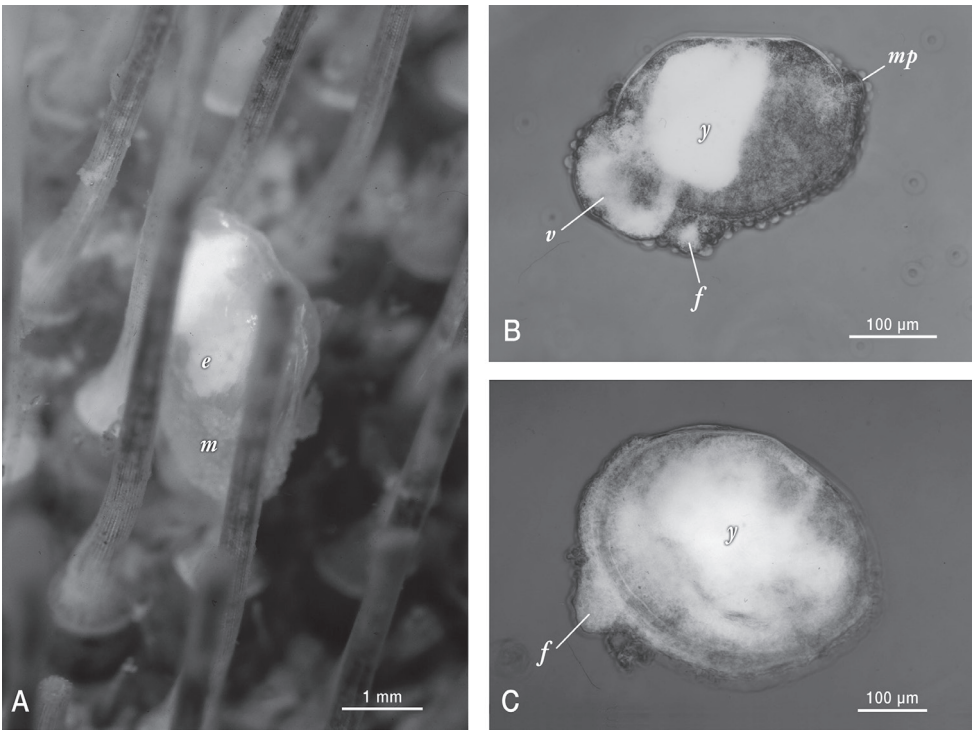


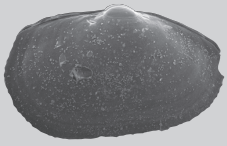
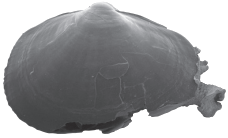
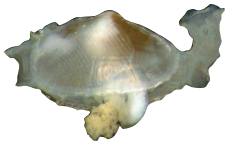
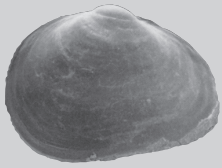
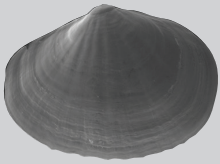
Figure 2. Photographs of live *Waldo arthuri* material sampled in Barkeley Sound in 1989. **A** Brooding adult attached to its host. Note the papillated mantle (*m*) that is partially retracted and the presence of ~ 200 µm diameter white yolky early embryos (*e*) in its ctenidia, visible through the transparent shell **B** Micrograph of mid-late development embryo (equivalent to the pediveliger stage in pelagic developing bivalves) that was dissected from its brooding parent's ctenidia. Labels indicate protruding foot (*f*), modified non-ciliated velum (*v*) with partially consumed yolk reserves (white areas) and mantle papillae (*mp*) in addition to a dense mass of yolk (y) sequestered in the anterior shelled half of the embryo **C** Micrograph of smallest/youngest (20 µm of dissoconch growth) specimen observed attached to an urchin host. Note the protruding foot (*f*) and the apparent presence of persistent yolk reserves (*y*) dispersed throughout much of the juvenile's visceral mass.

Discovery. Independently discovered in the late 1980's by Arthur Fontaine and Diarmaid Ó Foighil in British Columbia and Paul Valentich-Scott and Donald Cadien in southern California.

Etymology. This species is named after Dr. Arthur Fontaine, Professor Emeritus of Biology at the University of Victoria, British Columbia, Canada.

Comparisons. Table 1 provides characteristics to separate *Waldo arthuri* from other members of the genus. The Antarctic *Waldo parasiticus* is subequilateral, has a distinct anterior gape, and lacks the elongate anterior and posterior tentacles. *Waldo trapezialis*, has a strong saddle shaped internal ligament, is subequilateral, and lacks strong radial sculpture. *Waldo digitatus* Zelaya & Ituarte, 2013 lacks the radial sculpture and has a large number of mantle tentacles ventrally. *Waldo arthuri* is closest to

Table 1. Comparison of morphologic characteristics of members of the genus *Waldo*.

Taxa	Shell shape	Living animal	Pedal mantle tentacles	Crenulate ventral margin
Waldo arthuri			1 pair	no
Waldo parasiticus			5 pair	yes
Waldo trapezialis		unfigured	3 pair	no
Waldo paucitentaculatus			1-3 pair	yes
Waldo digitatus			5-15 pair	slightly

Waldo paucitentaculatus Zelaya & Ituarte, 2013, which has wider, stronger radial ribs, a strongly crenulate ventral margin, and a much narrower anterior end.

Scintillona bellerophon Ó Foighil & Gibson, 1984 is the only other galeommatid from the northeast Pacific that has been recorded as an epibiont on echinoderms. This species attaches externally to the sea cucumber, *Leptosynapta clarki* (Heding 1928). *Scintillona bellerophon* has cardinal teeth in both valves. The shell is much thicker, and not transparent, when compared with *Waldo arthuri*.

A species from Japan and Hawaii, *Scintillona stigmatica* (Pilsbry 1921), has been collected on the heart urchin, *Brissus latecarinatus* (Leske 1778). Yamamoto and Habe (1974) illustrate this bivalve on the ventral surface of the urchin, in an arrangement very similar to *Waldo arthuri*. However *S. stigmatica*, as with *S. bellerophon*, has a

cardinal tooth in each valve. In addition, *S. stigmatica* has a red-brown stripe of color running laterally from the umbones to the posteroventral margin.

In the eastern Atlantic Ocean, Gage (1966) documented two species of "*Montacuta*" attached to spantangoid urchins. Both species have a dentate hinge, and are easily separated from the new species.

Other similar North American species include those belonging to *Divariscintilla.* Mikkelsen and Bieler (1989, 1992) describe five species of *Divariscintilla* from Florida. Externally these species all have a papillate, reflected mantle, and long mantle tentacles, similar to the new species. However members of *Divariscintilla* have distinct cardinal teeth.

Molecular analysis

Results from the phylogenetic analyses are shown in Figure 3. For the mitochondrial 16S gene, we successfully amplified sequences from four individuals each of *Waldo arthuri* and *Waldo paucitentaculatus*, and two sequences from *Waldo digitatus*. Both Bayesian and ML analyses gave congruent topologies. All three species of *Waldo* formed their own monophyletic clade with relatively high statistical support. *Waldo arthuri* nested among the two South Atlantic congeners, placing robustly sister to *Waldo paucitentaculatus* (Figure 3) and thereby rendering *Waldo digitatus* basal. The mean genetic distances among the three species for the mt 16S rDNA gene fragment were: 2.2% (*W. arthuri* / *Waldo paucitentaculatus*), 11.6% (*Waldo digitatus* / *Waldo paucitentaculatus*) and 12.4% (*Waldo digitatus*

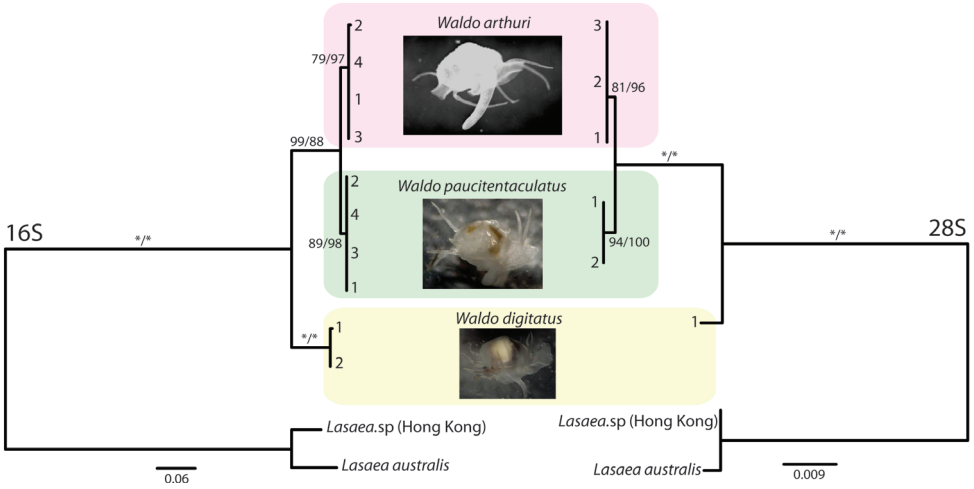


Figure 3. 16S and 28S phylogenies of three *Waldo* species. Numbers at branch tips represent specimen ID numbers. Support values along branches are reported as Bayesian posterior probabilities and ML bootstrap values respectively. An asterisk indicates a support value of 100. The scale bars represent numbers of substitutions per site.

/*W. arthuri*). Mean genetic distance within *Waldo digitatus* is very modest (0.3%). No intraspecific genetic variation was detected for *W. arthuri* and *Waldo paucitentaculatus*.

Specimens of *Waldo arthuri* (N=3), *Waldo paucitentaculatus* (N=2) and *W. digitatus* (N=1) were also genotyped for the more conserved large nuclear ribosomal (28S) gene fragment and their phylogenetic analyses (Figure 3) corroborated the among-*Waldo* relationships inferred from the mt 16S marker: (*Waldo digitatus* (*W. arthuri*, *Waldo paucitentaculatus*)). The mean genetic distances for this gene fragment were: 0.3% (*W. arthuri* /*Waldo paucitentaculatus*), 2.2% (*Waldo digitatus* /*Waldo paucitentaculatus*), and 2.1% (*Waldo digitatus* /*W. arthuri*). No intraspecific variations were detected for all three species.

Discussion

The molecular phylogenetic and morphological data concur that the new species here described is a member of the genus *Waldo* and is sister to the closely related southwestern Atlantic *Waldo paucitentaculatus*. Although it is not uncommon for marine invertebrates to have sister taxa in different ocean basins, it is a little surprising in this case because all other records for this genus are in high latitude southern hemisphere locations and all studied congeners apparently also lack pelagic larval development (Zelaya and Iuarte 2002, 2013). The large majority of commensal galeommatoideans brood their young to a straight-hinged veliger stage and then undergo a prolonged free-swimming obligatory planktotrophic phase prior to metamorphosis. However, absence of pelagic larvae does not seem to constrain the geographic range of species of *Waldo*. *Waldo parasiticus* has a circum-Antarctic distribution (Zelaya and Iuarte 2002) and *W. arthuri* has attained an extensive geographic range (Vancouver Island to San Diego). It is conceivable that *Waldo arthuri* will also be found throughout the range of its host, *Brisaster latifrons*, which has a documented distribution from the Bering Sea, Alaska to the Galapagos Islands, Ecuador (Lissner and Hart 1996).

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RESEARCH ARTICLE

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New species of Brachystomellidae and characterization of *Micronella porcus* (Denis, 1933) from Brazil

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Abstract

Three new species of Brachystomellidae from high altitude fields of southeast Brazil are described and illustrated and additions made to the description of *Micronella porcus* (Denis, 1933). The new species are *Neorganella rotundatae* **sp. n.**, the second for the genus, *Micronella itacaman* **sp. n.** and *M. longisensilla* **sp. n.** Diagnosis of the genera have been extended. An identification key to the genus *Micronella* Arlé, 1959 is provided.

Keywords

Taxonomy, chaetotaxy, biodiversity, *Neorganella*, Neotropic

Introduction

The cosmopolitan family Brachystomellidae is, currently, comprised of 18 genera and 130 species (Bellinger et al. 2013). However, more than half (i.e. 10) the genera are monospecific and with restricted distributions.

The Neotropical fauna of Brachystomellidae is particularly diverse, especially for a group of euedaphic pale species that presents reductions of sense organs and appendages, such as eyes and/or furca. It is the case, for instance, of the Neotropical genera *Folsomiella* Bonet, 1930 (six species), *Maricaella* Mendonça & Fernandes, 1997 (monospecific), *Micronella* Arlé, 1959 (two species), *Neorganella* Rapoport & Rubio, 1963 (monospecific) and *Winterella* Massoud, 1967 (monospecific). The first three genera occurs in different habitats, such as sandy seashores and its surrounding vegetation, tropical forests and high altitude in mountains of the Andes, while *Neorganella* and *Winterella* are only found at high altitude mountains of the Andes (above 2,000 m a.s.l.).

In Brazil, the Brachystomellidae fauna comprises 19 species in seven genera (Abrantes et al. 2012). Of these, seven species belong to the group mentioned above: *Folsomiella albida* (Arlé, 1959), *F. caeca* (Folsom, 1927), *F. intermedia* (Arlé, 1939), *F. pseudocaeca* Mendonça et al. 2005, *F. trisetosa* Mendonça et al., 2005, *Maricaella duna* Mendonça & Fernandes, 1997 and *Micronella porcus* (Denis, 1933).

Recent expeditions, in order to sample the collembolan biodiversity from summits of three of the highest mountain plateaus of southeastern Brazil, always over than 2,000 m a.s.l., have revealed three new pale Brachystomellidae species which are herein described and illustrated: *Micronella itacaman* sp. n., *Micronella longisensilla* sp. n. and *Neorganella rotundatae* sp. n. In addition, a new record of *Micronella porcus* from the State of Minas Gerais, Brazil, and, due to its succinct original description, that lacks body chaetotaxy and other characters, these specimens are characterized and illustrated.

Abbreviations used in text

Abd–abdominal segment; Ant–antennal segment; a.s.l.–above sea level; Cx–coxa; Fe–femur; ICMBio–Instituto Chico Mendes da Biodiversidade; MG–Minas Gerais State; MNHN–Muséum National D'Histoire Naturelle; MNRJ–Museu Nacional do Rio de Janeiro; PAO–postantennal organ; RJ–Rio de Janeiro State; Scx–subcoxa; Th–thoracic segment; Tita–tibiotarsus; Tr–trochanter.

Remarks on *Micronella* and *Neorganella*

The genus *Micronella* was erected by Arlé (1959) in order to separate the species *Salmonella porcus* (Denis, 1933), originally described as a *Brachystomella*, from its congeners. Both *Micronella* Arlé, 1959 and *Setanodosa* Salmon, 1942 (*Salmonella* Stach 1949 was synonymized with *Setanodosa* by Massoud, 1967) are devoid of furca and the main difference between them is the absence of eyes and pigmentation of *Micronella*. Latter, a species from high altitude (2,400–4,200 m a.s.l.) in the Peruvian Andes, *M. checayensis* Winter, 1962 nom.nud., was validated by Massoud (1967), after examination of the type material.

Table of localities

Species	Latitude	Longitude
Micronella itacaman sp. n.	22°22'59"S	44°40'01"W
	22°27'38"S	43°01'45"W
	20°26'07"S	41°47'54"W
Micronella longisensilla sp. n.	22°27'38"S	43°01'45"W
Micronella porcus (Denis, 1933)	20°26'07"S	41°47'54"W
Neorganella rotundatae sp. n.	22°22'59"S	44°40'01"W

Both *Micronella* species were briefly described, without any mention to dorsal body and also the furcal area chaetotaxy, which contains a set of chaetae that can be of taxonomic importance. Nevertheless, the analysis of Brachystomellidae made by Najt et al. (2005), which includes information on *M. porcus*, the following characterization of Brazilian specimens of *M. porcus* and of other two new species allow the expansion of the diagnosis of the genus.

Concerning *Neorganella* Rapoport & Rubio, 1963, the only species of the genus *N. nothofagutalis* Rapoport & Rubio, 1963 was described based on a single specimen from a mountain called "El Roble", of about 2,000 m a.s.l. and 50km from the Pacific Ocean. In 1967, Massoud synonymized *Neorganella* with *Folsomiella* and this remained until recently, when Najt et al. (2005), in an analysis of Brachystomellidae, revalidated the genus *Neorganella*.

As for the first two species of *Micronella*, there is no reference to head and most of the dorsal body chaetotaxy, regardless of the drawing of Abd III–VI in the original description, which is not elucidative. Nevertheless, the genus is well established among the Brachystomellidae, due to the presence of a reduced furca without mucro. The analysis of Najt et al. (2005) and the description of *N. rotundatae* sp. n. supports the genus and allows the expansion of its diagnosis.

Micronella Arlé, 1959

http://species-id.net/wiki/Micronella

Syn. *Brachystomella* Agren, 1903 ad. part.
Salmonella Stach 1949 ad. part.

Type species. *Brachystomella porcus* Denis, 1933.

Diagnosis. Pigmentation absent. Antennae shorter than head diagonal. Ant IV with dorsolateral microsensillum and round subapical organite; apical vesicle simple. Eyes absent. PAO with 6–15 vesicles. Maxilla typical of *Brachystomella*, with 5–7 teeth. Unguis tooth present or absent; tenent hair acuminated. Ventral tube with 3+3 chaetae. Furcal area delimited by a circular region of primary granulation of the tegument and a set of six chaetae within it. Table 1 summarizes the main characters of the species of the genus.

Table 1. Main characters of species of *Micronella* Arlé, 1959.

Species	***checayensis*** **Massoud, 1967**	***porcus*** **(Denis, 1933)**	***itacaman*** **sp. n.**	***longisensilla*** **sp. n.**
Ant IV sensilla	?	6	5	4
Shape of sensilla of Ant III organ	curved, opposite sense	"club"	one bilobed, one "club"	"cloverleaf"
PAO vesicles	15	6–8	7–8	12–13
Ratio ordinary chaetae: sensilla	?	1:1.3	1:1	1:2
Serrated chaetae on body	?	–	+	–
Unguis tooth	+	–	+†	–
Type Locality	Peru	Costa Rica	Brazil (southeast)	Brazil (RJ)

†Seen only on unguis of Tita I and II.

Key to the species of *Micronella* Arlé, 1959

1 PAO with up to 13 vesicles; unguis without or with minute inner tooth **2**

– PAO with 15 vesicles, unguis with inner tooth ..... ***Micronella checayensis*** **Massoud, 1967**

2 PAO with up to 8 vesicles; ratio chaetae: sensilla approximatelly 1:1 ..... **3**

– PAO with 12–13 vesicles; ratio chaetae: sensilla = 1:2..... ***Micronella longisensilla*** **sp. n.**

3 Ant IV with six sensilla; smooth chaetae on body; unguis without inner tooth ..... ***Micronella porcus*** **(Denis, 1933)**

– Ant IV with five sensilla; serrated chaetae on body; unguis of Tita I and II with minute inner tooth ..... ***Micronella itacaman*** **sp. n.**

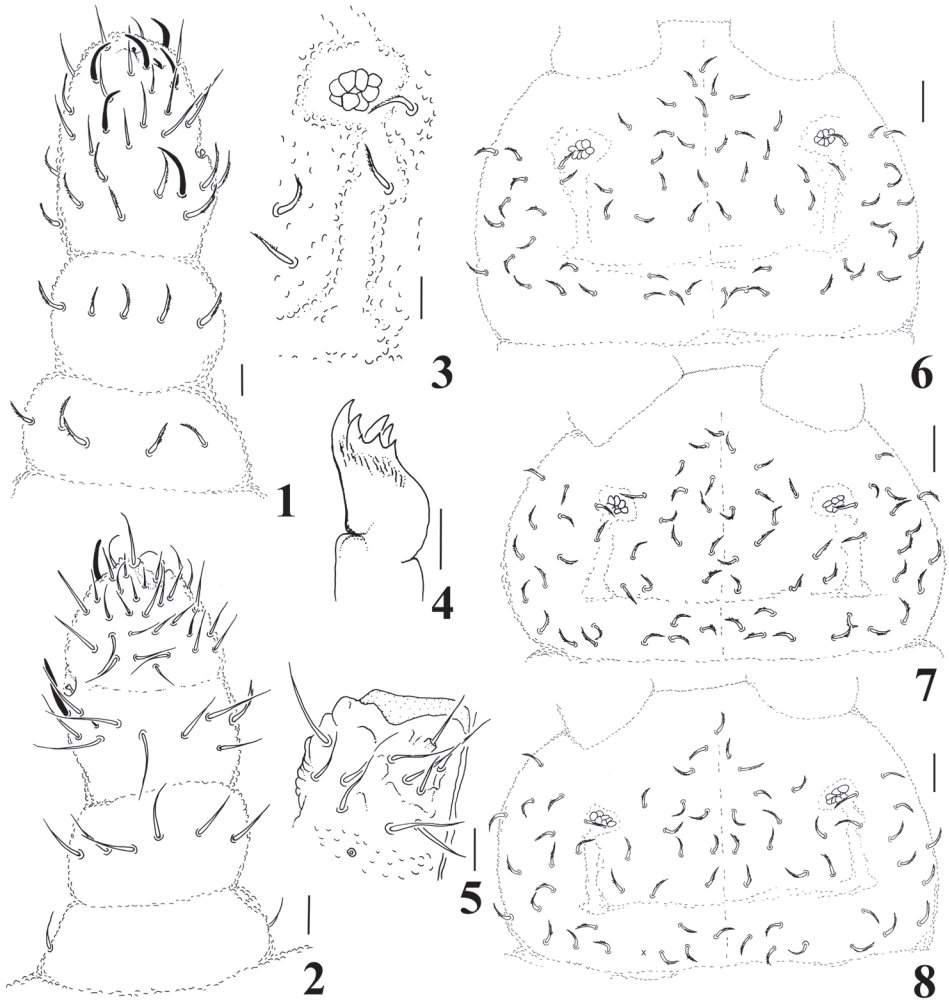
Micronella itacaman sp. n.

urn:lsid:zoobank.org:act:E02CF5EA-2148-496C-BC05-253533ABFACB
http://species-id.net/wiki/Micronella_itacaman
Figs 1–14

Type material. Holotype: female, on slide. Label: Nº 2332 CM/MNRJ, Itatiaia, RJ, Brasil, Queiroz, G.C. leg, 27.iii.2012, 22°22'59"S, 44°40'1"W. Paratype: 1 female on slide, Label: Nº 2138 CM/MNRJ (D), Itatiaia, RJ, Brasil; Queiroz, G.C. leg, 14.vii.2011, 22°22'59"S, 44°40'1"W. Deposited at MNRJ, Rio de Janeiro, Brazil.

Type locality. Brasil, Rio de Janeiro: Itatiaia municipality, Parque Nacional de Itatiaia (ICMBio), 22°22'59"S, 44°40'1"W, leaf litter and soil of "campos de altitude", 2,400 m a.s.l.

Other material. One female on slide, Label: Nº 2153 CM/MNRJ (A), Alto Caparaó, MG, Brasil, Queiroz, G.C. leg, 27.vii.2011, 20°26'7"S, 41°47'54"W. Deposited at MNRJ, Rio de Janeiro, Brazil. One specimen deposited at MNHN, Paris, Fran-

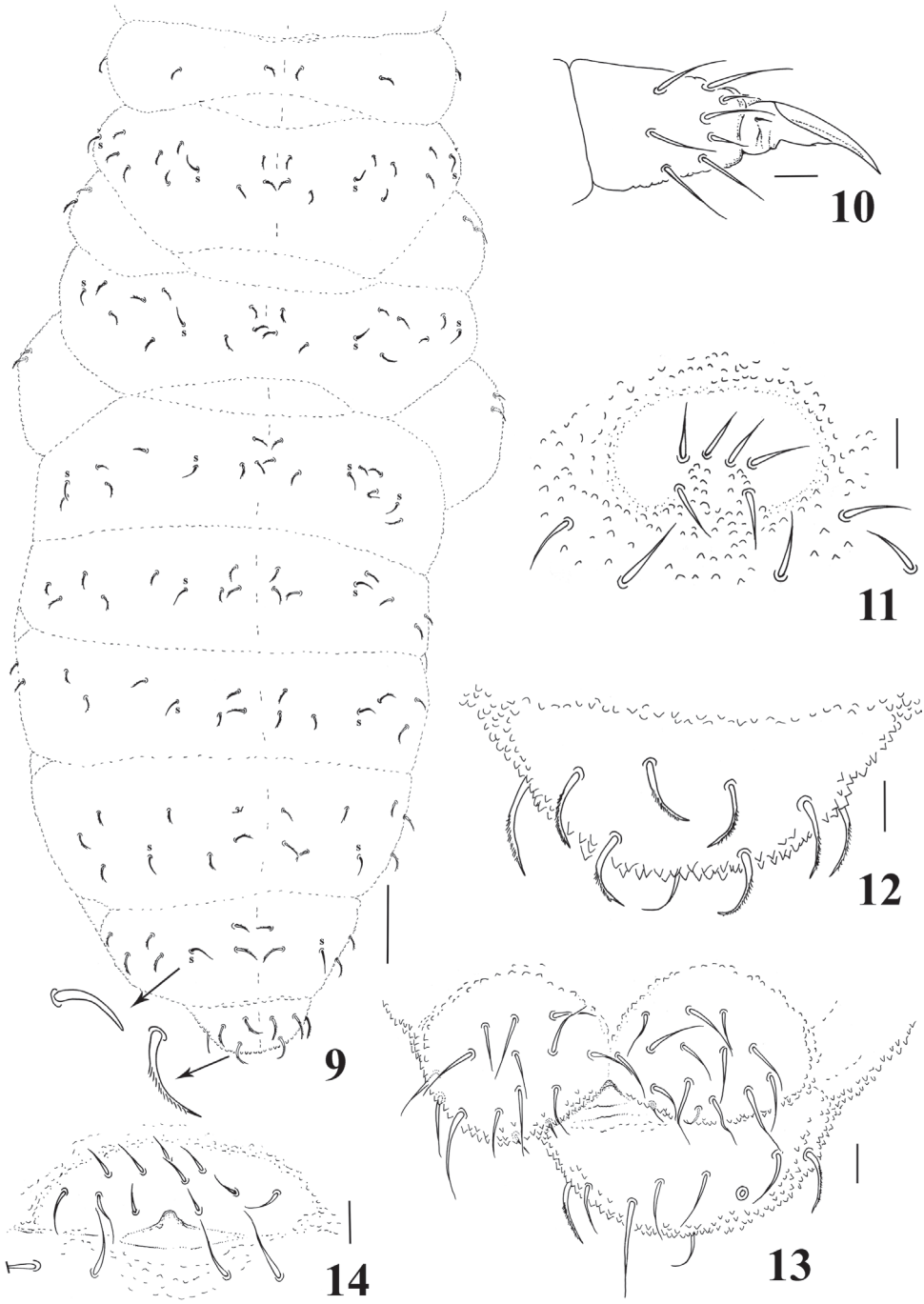


Figures 1–8. *Micronella itacaman* sp. n. **1** Dorsal view of Ant I–IV 2. Ventral view of Ant I–IV **3** PAO and its surrounding chaetae **4** Maxilla **5** Labium **6** Head chaetotaxy of specimen from Itatiaia **7** Head chaetotaxy of specimen from Teresópolis **8** Head chaetotaxy of specimen from Alto Caparaó. Scale bars: 10μm (**1–5**); 20 μm (**6–8**).

ce: female, on slide, MNHN-EA011504, Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 30.iii.2011, 22°27'38"S, 43°1'45"W.

Description. Habitus typical of the genus. Body length of holotype: 0.88 mm; body length range of paratypes: 0.63–0.90 mm. Color in ethanol: white, no pigmentation.

Ratio head diagonal: antenna = 1:0.63. Ant I with 7 chaetae. Ant II with 12 chaetae. Ant III and IV fused dorsally, ventral separation marked. Sensory organ of Ant III with two small club shaped sensilla, the mid-ventral one with a bilobed apex; two longer and subcylindrical guard sensilla; ventral microsensillum present (Figs 1–2). All dorsal chaetae of Ant I–III are serrated, ventral chaetae smooth and longer than those from dorsal



Figures 9–14. *Micronella itacaman* sp. n. 9. Dorsal body chaetotaxy with details of sensilla and chaetae **10** Tita of leg I **11** Furcal area **12** Dorsal view of Abd VI **13** Anal valves and ventral view of Abd VI **14** Female genital plate. Scale bars: 10μm (10–14); 50μm (9).

side (10–13 µm dorsal; 13–15 µm ventral). Ant IV with simple apical bulb and five sensilla, three weakly differentiated from ordinary chaetae; dorsolateral microsensillum present; subapical organite round; with about 30 ventral chaetae (Fig. 2).

Without eyes. PAO bearing 7–8 vesicles disposed as a rosette (Fig. 3). Maxilla quadrangular with 6–7 teeth (Fig. 4). Labral formula: 2/2334. Labium typical of *Brachystomella*, with one papillated chaeta (L) and four proximal chaetae (Fig. 5).

Head chaetotaxy as in Figs 6–8; asymmetries in the number of axial chaetae. Chaetae a0 present; Oc chaetae 3+3. Dorsal chaetotaxy composed of ordinary serrated chaetae and sensilla subequal in size, becoming longer towards the distal segments of the body (15 µm in Th I and 25 µm in Abd VI) (Fig. 9). Ratio of body ordinary chaetae: sensilla = 1:1. Th I with 2+2 chaetae; sensillar formula by half tergum: 022/211110.

Chaetotaxy of legs I–III as follows: Scx I– 1, 2, 2; Scx II– 0, 2, 2; Cx– 3, 6, 7; Tr– 5, 5, 5; Fe– 12, 10, 10; Tita– 19, 19, 18. All chaetae of Scx I of legs I–III are serrated. Tenent hair on tibiotarsi acuminate; unguis of legs I and II with one extremely minute median inner tooth; tooth not seen on unguis of leg III (Fig. 10). Ventral tube with 3+3 chaetae. Without tenaculum. Furca completely absent, but with a well-defined furcal area with six chaetae arranged in two rows: anterior row with four chaetae and posterior row with two chaetae (Fig. 11). Abd VI with 4+4 serrated chaetae and one unpaired smooth chaetae on dorsal side (Fig. 12). Each anal valve with 12–13 chaetae and 2 hr chaetae; Abd VI with 3+3 smooth chaetae on ventral side (Fig. 13). Female genital plate as in Figure 14.

Etymology. "Itakamã" (pronounced itakaman) means "high stone" or "rocky mountain" in the indigenous language Tupi, spoken by the Brazilian natives, reference to the three highest mountain plateaus of southeast Brazil, where the species was found.

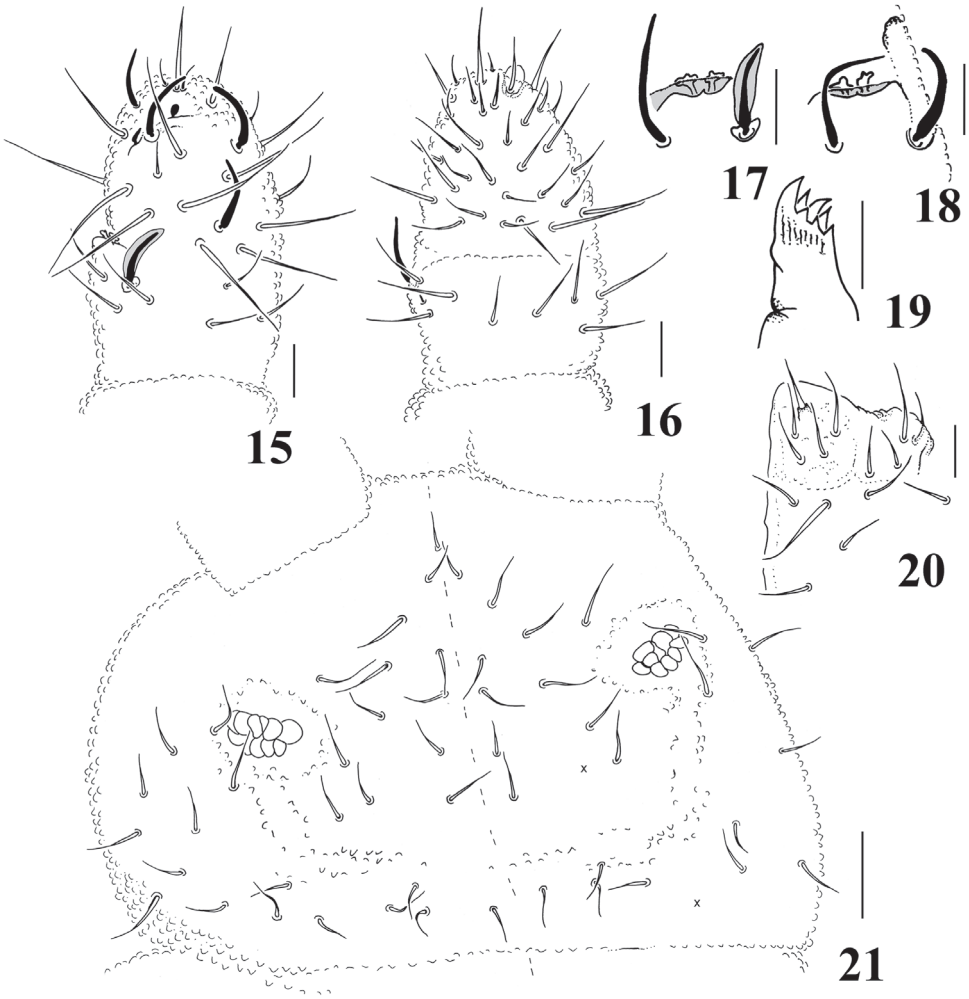
Discussion. The new species, *Micronella itacaman* sp. n., is well characterized in the genus, as all the species share euedaphic characters such as absence of eyes and furca, but with PAO. It can be distinguished from its congeners by characters such as serrated chaetae on body and five sensilla on Ant IV. In relation to number of vesicles on PAO and ratio of ordinary chaetae: sensilla, the new species is most similar to *M. porcus*, as they have 6–8 vesicles and a ratio of ordinary chaetae: sensilla of approximately 1:1.

Micronella longisensilla sp. n.

urn:lsid:zoobank.org:act:B4515695-AD7E-4436-A3BF-FF131F3C9F76
http://species-id.net/wiki/Micronella_longisensilla
Figs 15–28

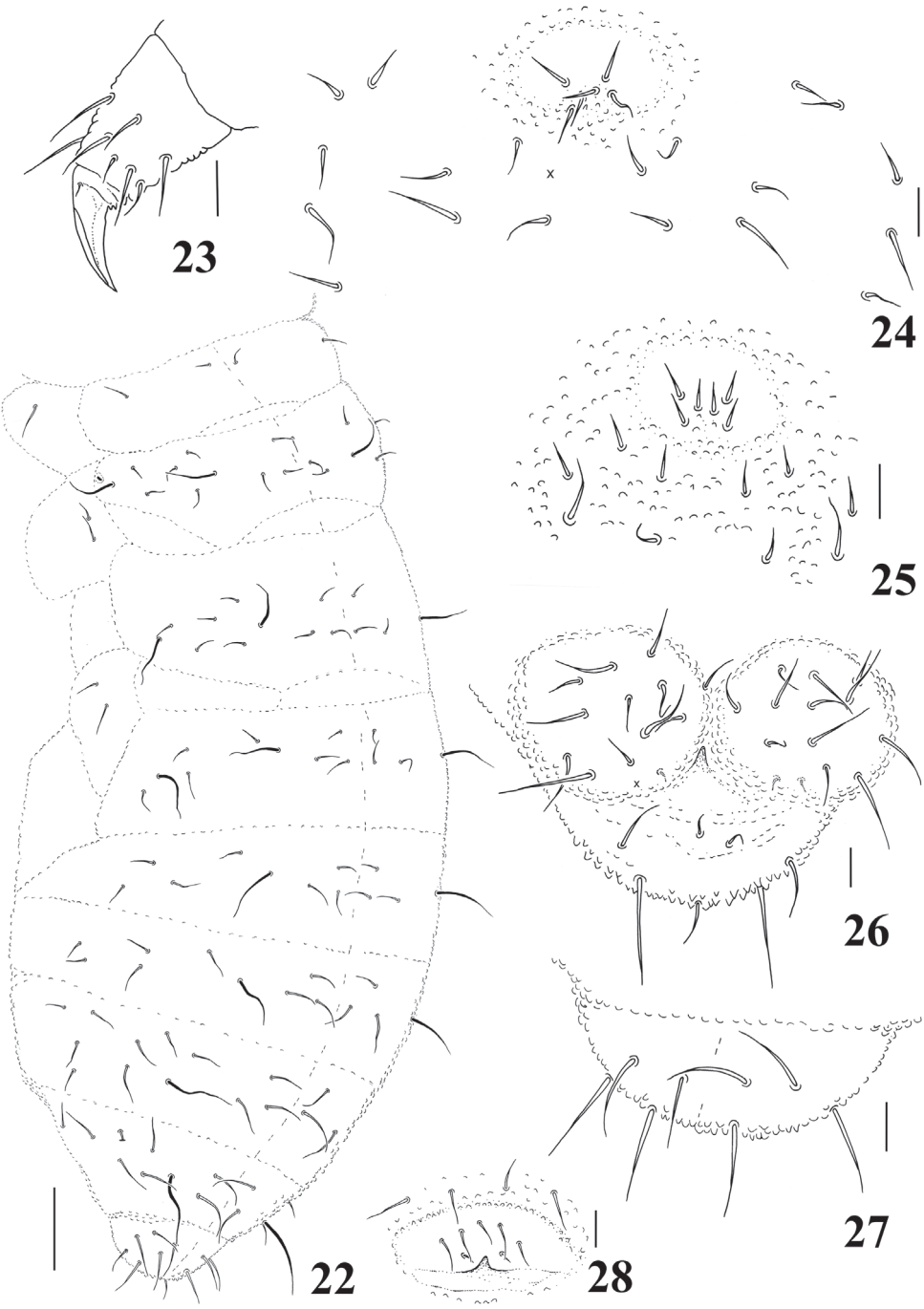
Type material. Holotype: female, on slide. Label: Nº 2207 CM/MNRJ (B), Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 09.xi.2011, 22°27'38"S, 43° 1'45"W. Paratypes: 4 females on slides and 1 specimen in ethanol, 2207 CM/MNRJ (A and C), same data as holotype. Deposited at MNRJ, Rio de Janeiro, Brazil.

Other material. One female on slide. Label: Nº 2020 CM/MNRJ, Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 30.iii.2011, 22°27'38"S, 43°1'45"W; 1 specimen in



Figures 15–21. *Micronella longisensilla* sp. n. **15** Dorsal view of Ant III–IV **16** Ventral view of Ant III–IV **17** Detail of Ant III organ **18** Detail of Ant III organ (same specimen of Fig. 17, right antennae) **19** Maxilla **20** Labium **21** Head. Scale bars: 10μm (**15–20**); 20μm (**21**). x represents missing chaeta.

ethanol, 2023 CM/MNRJ, Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 30.iii.2011, 22°27'38"S, 43°1'45"W; 2 females on slide, Nº 2092 CM/MNRJ (C), Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 29.vi.2011, 22°27'38"S, 43°1'45"W; 1 female on slide and 3 specimens in ethanol, Nº 2211 CM/MNRJ (A), Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 10.xi.2011, 22°27'38"S, 43°1'45"W; 1 female on slide, Label: Nº 2212 CM/MNRJ (D), Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 10.xi.2012, 22°27'38"S, 43°1'45"W; 1 female on slide, Label: Nº 2302 CM/MNRJ (A), Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 14.iii.2012, 22°27'38"S, 43°1'45"W; 1 specimen in ethanol, Nº 2307 CM/MNRJ, Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 14.iii.2012, 22°27'38"S, 43°1'45"W; 1 specimen in ethanol, Nº 2314 CM/MNRJ, Teresópolis, RJ. Brasil,



Figures 22–28. *Micronella longisensilla* sp. n. **22** Dorsolateral body chaetotaxy **23** Tita of leg I **24** Furcal area and its surrounding chaetae (adult) **25** Furcal area and its surrounding chaetae (juvenile) **26** Anal valves and ventral view of Abd VI **27** Dorsal view of Abd VI **28** Female genital plate. Scale bars: 10μm (**23–28**); 50μm (**22**). x represents missing chaeta.

Queiroz, G.C. leg; 15.iii.2012, 22°27'38"S, 43°1'45"W; 1 specimen in ethanol, Nº 2317 CM/MNRJ, Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 15.iii.2012, 22°27'38"S, 43°1'45"W. Deposited at MNRJ, Rio de Janeiro, Brazil. Two specimens deposited at MNHN, Paris, France: 1 female on slide, MNHN-EA011506, Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 10.xi.2011, 22°27'38"S, 43°1'45"W; 1 female on slide, MNHN-EA011505, Teresópolis, RJ. Brasil, Queiroz, G.C. leg; 14.iii.2012, 22°27'38"S, 43°1'45"W.

Type locality. Brasil, Rio de Janeiro, Teresópolis municipality, Parque Nacional da Serra dos Órgãos (ICMBio), 22°27'38"S, 43°1'45"W, leaf litter and soil of "campos de altitude", 2,100 m a.s.l.

Description. Habitus typical of the genus. Body length of holotype: 0.62 mm; body length range of paratypes: 0.40–0.75 mm. Color in ethanol: white, no pigmentation.

Ratio head diagonal: antenna = 1:0,.66. Ant I with 7 chaetae. Ant II with 11 chaetae. Ant III and IV fused dorsally, ventral separation marked. Sensory organ of Ant III with two cloverleaf-shaped sensilla partially covered by a fold of the integument; two longer and subcylindrical guard sensilla, the dorsal one is shorter but greatly enlarged in its width, in relation to the ventral one; ventral microsensillum present (Figs 15–18). Ant IV with simple apical bulb and four sensilla; dorsolateral microsensillum present; subapical organite round; with about 30 ventral chaetae (Fig. 16).

Without eyes. PAO bearing 12–13 vesicles disposed as a rosette. Maxillae quadrangular with 6–7 teeth (Fig. 19). Labral formula: 2/2334. Labium typical of *Brachystomella*, with one papillated chaeta (L) and four proximal chaetae (Fig. 20).

Head chaetotaxy as in Fig. 21. Chaetae a0 present; Oc chaetae 3+3, sometimes asymmetric of 2+3. Dorsal chaetotaxy composed of smooth ordinary chaetae (10–25µm) and long sensilla (25–50µm), that becomes longer towards distal segments of the body. Ratio body ordinary chaetae: sensilla = 1:2. Th I with 2+2 chaetae; sensillar formula by half tergum: 022/211110 (Fig. 22).

Chaetotaxy of legs I–III as follows: Scx I– 1, 2, 2; Scx II– 0, 2, 2; Cx– 3, 6, 7; Tr– 5, 5, 4; Fe– 12, 11, 10; Tita– 19, 19 , 18. Tenent hair on tibiotarsi acuminate; unguis without tooth (Fig. 23). Ventral tube with 3+3 chaetae. Without tenaculum. Furca completely absent, but with a well-defined furcal area with six chaetae (Figs 24–25). Each anal valve with 11–12 chaetae and 2 hr chaetae; Abd VI with 3+3 chaetae on ventral side, 4+4 chaetae on dorsal side and one unpaired chaetae (Figs 26–27). Female genital plate as in Fig. 28.

Etymology. In a reference to the size of the sensilla in relation to ordinary chaetae on body of the new species.

Discussion. The new species, *Micronella longisensilla* sp. n., is well characterized in the genus (see Table 1). It differs from its congeners in relation to the ratio of ordinary chaetae: sensilla, that is 1:2, only four sensilla on Ant IV, a PAO with 12–13 vesicles, the Ant III organ with two cloverleaf-shaped sensilla under a fold of the tegument and the dorsal guard sensilla which is greatly enlarged in its width, in relation to the ventral one.

***Brachystomella porcus* Denis, 1933**
http://species-id.net/wiki/Brachystomella_porcus
Figs 29–41

Examined material. One female on slide, Label: Nº 2037 CM/MNRJ (C), Alto Caparaó, MG, Brasil, Queiroz, G.C. leg, 12.iv.2011, 20°26'7"S, 41°47'54"W; 1 female on slide and 2 specimens in ethanol, Nº 2041 CM/MNRJ (D), Alto Caparaó, MG, Brasil, Queiroz, G.C. leg, 13.iv.2011, 20°26'7"S, 41°47'54"W; 1 female and 1 juvenile on slides, Label: Nº 2353 CM/MNRJ (C and E), Alto Caparaó, MG, Brasil, Queiroz, G.C. leg, 11.iv.2012, 20°26'7"S, 41°47'54"W; 1 young female and 1 juvenile on slides, Label: Nº 2354 CM/MNRJ (A and B), Alto Caparaó, MG, Brasil, Queiroz, G.C. leg, 11.iv.2012, 20°26'7"S, 41°47'54"W. Deposited at MNRJ, Rio de Janeiro, Brazil. Two specimens deposited at MNHN, Paris, France: 1 female on slide MNHN-EA011501; 1 female on slide, Label: MNHN-EA011500, Alto Caparaó, MG, Brasil, Queiroz, G.C. leg, 13.iv.2011, 20°26'7"S, 41°47'54"W.

Locality. Brasil, Minas Gerais: Alto Caparaó municipality, Parque Nacional do Caparaó (ICMBio), 20°26'7"S, 41°47'54"W, leaf litter and soil of "campos de altitude", 2,700 m a.s.l.

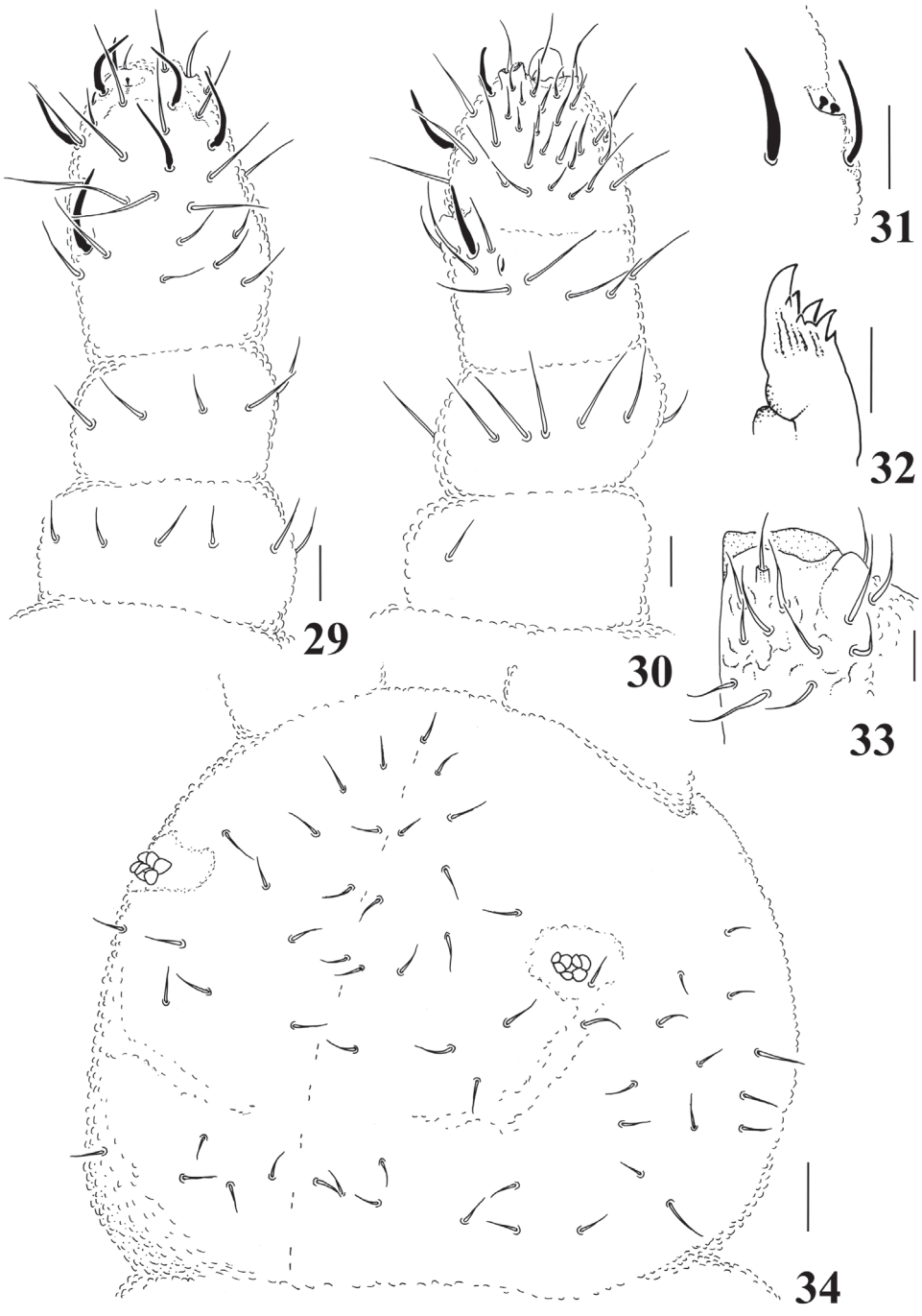
Characterization of Brazilian specimens. Habitus typical of the genus. Body length range of specimens: 0.45–0.95 mm. Color in ethanol: white, no pigmentation.

Ratio head diagonal: antenna = 1:0.57. Ant I with 7 chaetae. Ant II with 12 chaetae. Ant III and IV fused dorsally, ventral separation marked. Sensory organ of Ant III with two club-shaped sensilla; two longer and subcylindrical guard sensilla, the dorsal is stouter than ventral guard sensilla; ventral microsensillum present (Figs 29–31). Ant IV with simple apical bulb and six slender sensilla; dorsolateral microsensillum present; subapical organite round; with about 30 ventral chaetae (Fig. 30).

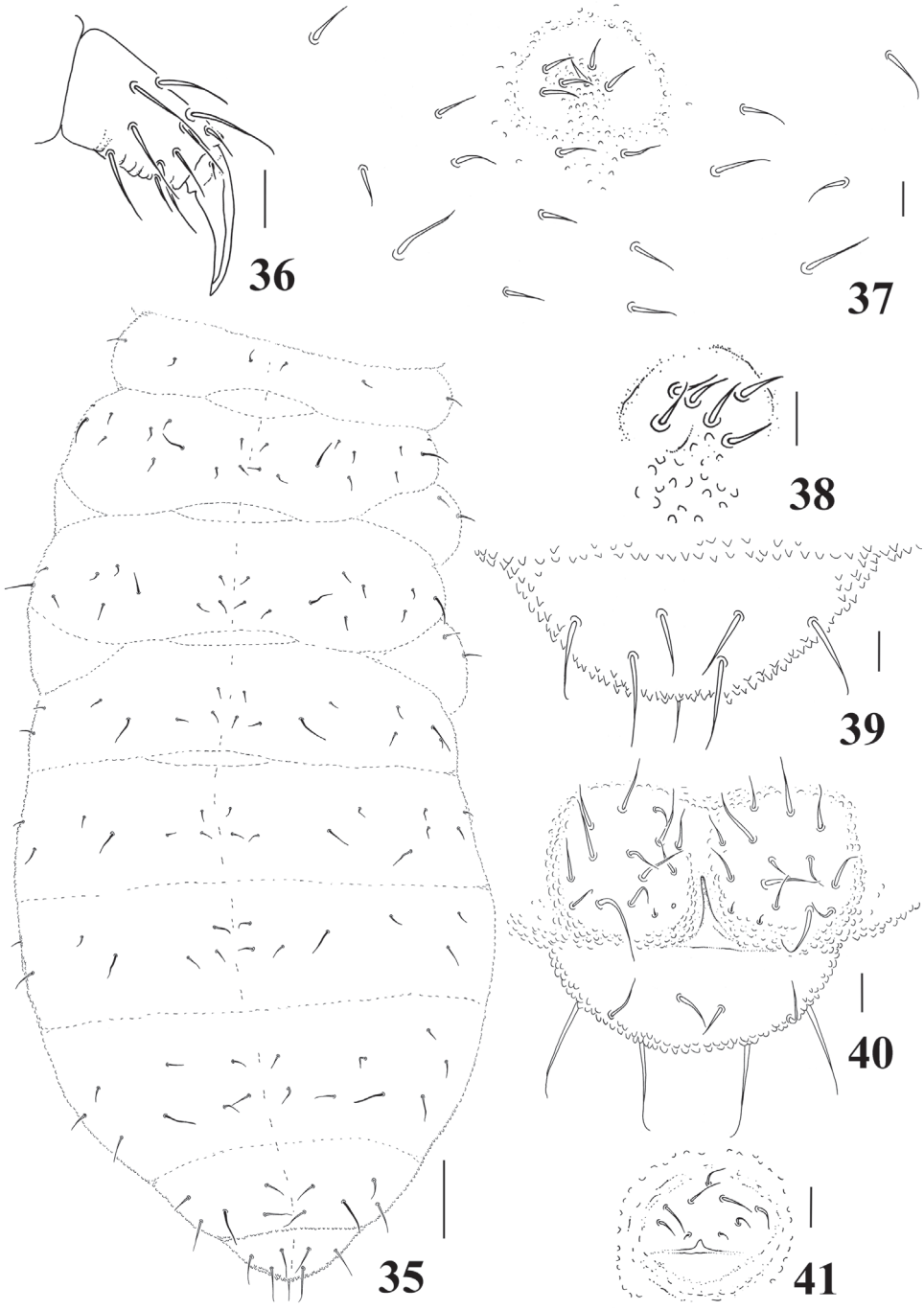
Without eyes. PAO bearing 7–8 vesicles disposed as a rosette. Maxilla quadrangular with 6–7 teeth (Fig. 32). Labral formula: 2/2334. Labium typical of *Brachystomella*, with one papillated chaeta (L) and four proximal chaetae (Fig. 33).

Head chaetotaxy as in Fig. 34. Chaetae a0 present, but some specimens with asymmetries; Oc chaetae 3+3. Dorsal chaetotaxy composed of smooth ordinary chaetae (15–20 μm) and longer sensilla (20–25 μm) that becomes longer towards distal segments of the body. Ratio ordinary chaetae: sensilla = 1:1.3. Th I with 2+2 chaetae; sensillar formula by half tergum: 022/211110 (Fig. 35).

Chaetotaxy of legs I–III as follows: Scx I– 1, 2, 2; Scx II– 0, 2, 2; Cx– 3, 6, 7; Tr– 5, 5, 4; Fe– 12, 11, 10; Tita– 19, 19 , 18. Tenent hair on tibiotarsi acuminated; unguis without tooth (Fig. 36). Ventral tube with 3+3 chaetae. Without tenaculum. Furca completely absent, but with a well-defined furcal area with six chaetae (Figs 37–38). Abd VI with 4+4 chaetae on dorsal side and one unpaired chaetae; with 3+3 chaetae on the ventral side (Fig. 39). Each anal valve with 12–13 chaetae and 2 hr chaetae; (Fig. 40). Female genital plate as in Fig. 41.



Figures 29–34. *Micronella porcus* (Denis, 1933). 29. Dorsal view of Ant I–IV **30** Ventral view of Ant I–IV **31** Detail of Ant III organ **32** Maxilla **33** Labium **34** Head chaetotaxy. Scale bars: 10μm (**29–33**); 20μm (**34**).



Figures 35–41. *Micronella porcus* (Denis, 1933). **35** Dorsal body chaetotaxy **36** Tita of leg I **37** Furcal area and its surrounding chaetae **38** Detail of furcal area **39** Dorsal view of Abd VI **40** Anal valves and ventral view of Abd VI **41** Female genital plate. Scale bars: 10μm (**36–41**); 50μm (**35**).

Remarks. The examined specimens from Minas Gerais State, Brazil, fit the description of the Neotropical species *Micronella porcus*. The six sensilla on Ant IV, the club-shaped sensilla on Ant III organ, the 6–8 vesicles on PAO and the toothless unguis are the main characters that define the species. The description above adds important characters such as head and dorsal body chaetotaxy and also the number of chaetae on furcal area to the original description.

Neorganella Rapoport & Rubio, 1963

http://species-id.net/wiki/Neorganella

Diagnosis. Pigmentation absent, pale aspect. Antennae shorter than head diagonal. Ant IV with dorsolateral microsensillum and round subapical organite; apical vesicle simple. Eyes absent. PAO with 4–12 vesicles. Maxilla typical of *Brachystomella*, with 5–7 teeth. Unguis tooth present or absent; tenent hair acuminate. Ventral tube with 3+3 chaetae. Tenaculum present. Reduced furca: without mucro, but with two small rounded or globular dens, each with 3–4 chaetae.

Neorganella rotundatae sp. n.

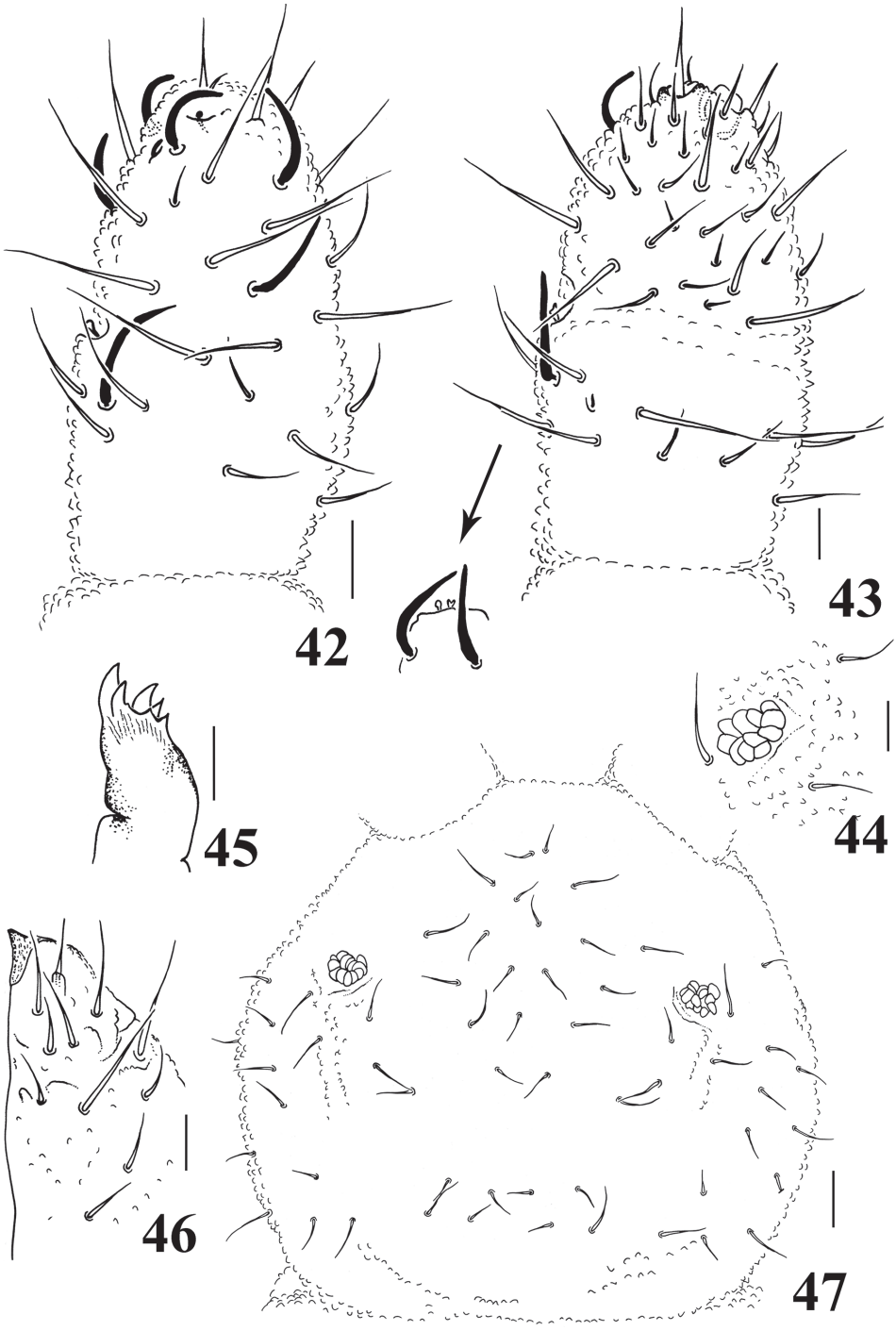
urn:lsid:zoobank.org:act:0AA68A6D-A503-4695-89E2-64893DEE33B3
http://species-id.net/wiki/Neorganella_rotundatae
Figs 42–55

Type material. Holotype: male, on slide, Label: Nº 1984 CM/MNRJ, Itatiaia, RJ, Brasil, Queiroz, G.C. leg, 14.iii.2011, 22°22'59"S, 44°40'1"W. Paratypes: 1 female and 4 juveniles on slides, Label: Nº 2133 CM/MNRJ (C and D), Itatiaia, RJ, Brasil, Queiroz, G.C. leg, 13.vii.2011, 22°22'59"S, 44°40'1"W. Deposited at MNRJ, Rio de Janeiro, Brazil. Two specimens deposited at MNHN, Paris, France: 1 female on slide, MNHN-EA011502, Itatiaia, RJ, Brasil, Queiroz, G.C. leg, 13.vii.2011, 22°22'59"S 44°40'1"W1 juvenile on slide, MNHN-EA011503, Itatiaia, RJ, Brasil, Queiroz, G.C. leg, 25.x.2011, 22°22'59"S, 44°40'1"W.

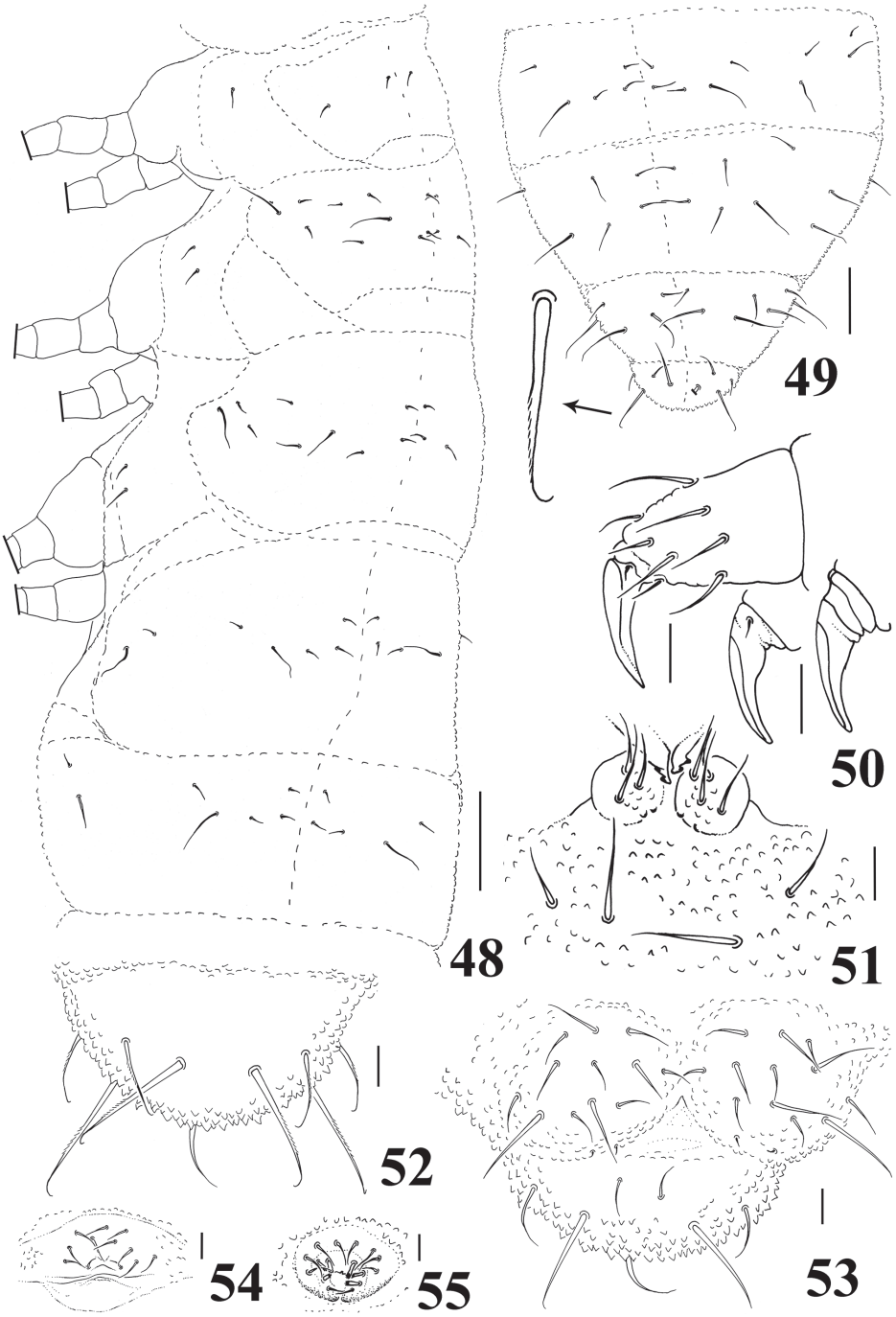
Type locality. Brasil, Rio de Janeiro: Itatiaia municipality, Parque Nacional de Itatiaia (ICMBio), 22°22'59"S, 44°40'1"W, leaf litter and soil of "campos de altitude", 2,400 m a.s.l.

Description. Habitus typical of the family. Body length of holotype: 0.88 mm; body length range of paratypes: 0.47–1.20 mm. Color in ethanol: white, no pigmentation.

Ratio head diagonal: antenna = 1:0.63. Ant I with 7–8 chaetae. Ant II with 12 chaetae. Ant III and IV fused dorsally, ventral separation marked. Sensory organ of Ant III with two small club-shaped sensilla, the mid-ventral one with a bilobed apex; two longer and subcylindrical guard sensilla; ventral microsensillum present (Figs 42–43). Ant IV with simple apical bulb and five sensilla; dorsolateral microsensillum present; subapical organite round; about 30 ventral chaetae (Figs 42–43).



Figures 42–47. *Neorganella rotundatae* sp. n. **42** Dorsal view of Ant II–IV **43** Ventral view of Ant III–IV with detail of Ant III organ **44** Detail of PAO **45** Maxilla **46** Labium **47** Head chaetotaxy. Scale bars: 10μm (**42–46**); 20μm (**47**).



Figures 48–55. *Neorganella rotundatae* sp. n. **48** Dorsolateral chaetotaxy of Th I–Abd II **49** Dorsolateral chaetotaxy of Abd III–VI with detail of chaetae **50** Tita of leg II with detail of two unguis (left: unguis III; right: unguis II) **51** Tenaculum and reduced furca **52** Dorsal view of Abd VI **53** Anal valves and ventral view of Abd VI **54** Female genital plate **55** Male genital plate. Scale bars: 10μm (**50–55**); 50μm (**48–49**).

Without eyes. PAO bearing 10–12 vesicles disposed as an elongated rosette (Fig. 44). Maxilla quadrangular with 6–7 teeth (Fig. 45). Labral formula: 2/2334. Labium typical of *Brachystomella*, with one papillated chaetae (L) and four proximal chaetae (Fig. 46).

Head chaetotaxy as in Fig. 47. Chaetae a0 absent; Oc chaetae 3+3. Dorsal chaetotaxy composed of slightly serrated chaetae and longer sensilla (Fig. 48); Abd V with some longer chaetae, subequal to sensilla, and Abd VI with 4+4 serrated chaetae with a tendency to have bent tips (Fig. 49). Th I with 2+2 chaetae; sensillar formula by half tergum: 022/211110. All dorsal and lateral chaetae are slightly serrated.

Chaetotaxy of legs I–III as follows: Scx I – 1, 2, 2; Scx II – 0, 2, 2; Cx – 3, 6, 7; Tr – 5, 5, 5; Fe – 12, 12?, 10; Tita – 18, 18 , 17. Tenent hair on tibiotarsi acuminated; unguis of legs I and II with one extremely minute median inner tooth; tooth not seen on unguis of leg III (Fig. 50). Ventral tube with 3+3 chaetae. Tenaculum small with 2 teeth on each ramus. Furca reduced to two small globular dens with 3–4 chaetae on each side and without mucro (Fig. 51). Abd VI with 4+4 serrated chaetae with bent tips, of which 2+2 are longer than others (25μm to 20μm), and one unpaired smooth chaetae on dorsal side (Fig. 52). Each anal valve with 12 chaetae and 2 hr chaetae; Abd VI with 3+3 smooth chaetae on ventral side (Fig. 53). Female and male genital plate as in Figs 54 and 55, respectively.

Etymology. The Latin word *rotundatae* means roundish, spherical, referring to dens shape of the new species.

Discussion. The new species *N. rotundatae* sp. n. is well characterized in the genus, mainly due to the facts that it shares a reduced furca without mucro, dens with 3+3 chaetae, and the presence of tenaculum with the other species *N. nothofagutalis* Rapoport & Rubio, 1963 (according to original description and after Najt et al. 2005). The new species differs from its congener by the presence of 10–12 vesicles on PAO, while *N. nothofagutalis* has only 4 vesicles. It is also noteworthy that *Neorganella rotundatae* sp. n. presents a reduction in the number of chaetae on Tita of legs I–III, being 18, 18, 17, respectively, while *N. nothofagutalis* has 19, 19, 18 (see Najt et al. 2005).

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