

**Molecular systematics of Thaumaleidae (Insecta: Diptera):
The first phylogeny depicting intergeneric relationships
and other taxonomic discoveries**

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Robert John Pivar
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ABSTRACT

Thaumaleidae, commonly referred to as madicolous midges, are a small family of aquatic Diptera (true flies). The larvae are restricted in their habitat to thin films of vertically flowing water, such as the margins of waterfalls, rock face seeps and cascading mountain streams. The restricted larval habitat and inconspicuous adults have led to thaumaleids being uncommonly encountered, under-collected and thus, understudied. The monophyly of the family is well supported, but generic concepts and relationships have never been rigorously tested, no phylogenies have been published and the validity of several genera is questionable. The overarching goal of this research is to produce the first comprehensive phylogeny for Thaumaleidae to ascertain generic relationships and test current generic concepts. Additional goals include alpha taxonomy and diversity studies. A new genus from Brazil, *Neothaumalea* Pivar, Moulton and Sinclair, is described. This is the first thaumaleid known from Brazil and east of the Andes. The Chilean thaumaleid fauna is also revised, bringing the known South American fauna from six species to seventeen. In addition to new species descriptions, all available life stages for previously known Chilean species are redescribed and illustrated, and distribution maps and keys to adults are provided. New larval morphotypes for *Niphtha* spp. Theischinger are described and phylogenetic relationships hypothesized. Finally, the first phylogeny depicting thaumaleid generic relationships is constructed. Nucleotide sequences from three nuclear genes (big zinc finger, molybdenum cofactor sulfuryase and elongation complex protein 1) were acquired from at least one representative of each genus and several outgroup taxa and analyzed with Bayesian methods. Results support the recognition of six genera within two clades: a Northern Hemisphere clade and a

Southern Hemisphere clade. The Northern Hemisphere clade includes *Trichothaumalea* Edwards as sister to *Thaumalea* Ruthe (expanded to include *Androprosopa* Mik and *Protothaumalea* Vaillant). The Southern Hemisphere clade includes *Afrothaumalea* Stuckenberg as sister to *Austrothaumalea* Tonnoir (expanded to include *Oterere* McLellan) + (*Neothaumalea* + *Niphta*). This first comprehensive phylogeny for Thaumaleidae establishes a predictive framework with which to erect a stable classification and test myriad evolutionary hypotheses.

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CHAPTER 1
GENERAL INTRODUCTION

OPENING REMARKS ABOUT DIPTERA

The Diptera (“true flies”) are one of the most diverse and abundant insect orders. They occupy every niche imaginable and as a result, have developed an astonishing variety of behavioral, ecological and morphological adaptations. The general population tends to have a negative view when they hear the word ‘fly’, and for good reason: they are responsible for the transmission of numerous disease organisms affecting humans and animals, damaging crops and being a general nuisance. Many flies have a bad reputation simply because of their diet, which can include some vile and objectionable food sources. There is another side to flies, though.

Many species have enormous ecological roles that tend to be overlooked. They are important pollinators, predators, and decomposers. Many species act as biocontrol agents, parasitizing pests, and unbeknownst to the fly, they can even help solve criminal cases via their forensic applications. One of their most underrated qualities is something few people attribute to flies: beauty. They come in all shapes and sizes; some are metallic and shiny with striking coloration. Others are densely hairy, while some have long eyestalks. The combinations are endless. Generations of researchers are responsible for countless publications exploring the world of Diptera, and yet the opportunity for discovery is ever-present. Whether they are explorations into biology, ecology or diversity, flies will continue to reveal incredible life history stories. Some families of Diptera are not as pretty and charismatic, and have no known impact on humans. This often leads to these groups being understudied, leaving much to be uncovered about their diversity and biology. One such group is the family Thaumaleidae.

INTRODUCTION TO THAUMALEIDAE

Thaumaleidae, commonly referred to seepage or madicolous midges, are well supported as sister to Simuliidae (black flies) (Pawłowski *et al.* 1996; Moulton 2000; Bertone *et al.* 2008; Wiegmann *et al.* 2011; Borkent 2012; Kutty *et al.* 2018). They are similar in appearance to black flies: 2–5 mm long, slightly humpbacked, and mainly black or brown in coloration, although there are some light yellow or orange species (Pivar *et al.* 2018a). Unlike black flies, however, adult thaumaleids do not feed on blood. Madicolous midges, are so-called due to their highly specialized larval habitat: thin films of vertical flowing or seeping water over either rocky (Vaillant 1956; Sinclair and Marshall 1987; Pivar *et al.* 2018a) or vegetative (Pivar, Chapter 3) substrates. Once potential habitat is found, sweeping above the madicolous substrate and in adjacent riparian vegetation is the best method to collect adult thaumaleids. Sweeping for adults should be attempted first if possible, as searching for larvae may cause adults to fly deeper into vegetation or into more difficult-to-sweep areas. After sweeping, immatures can be collected by using forceps to pull them off the substrate or by pouring water over the substrate and flushing immatures into a white pan (Sinclair and Saigusa 2002). Immatures usually exhibit a distinctive side-winding motion, likely to avoid predation; this response can be triggered through direct contact or by gently blowing on suspected habitat (Sinclair and Stuckenberg 1995; Sinclair 2000). Upon collection, all life stages should be placed directly into 95% ethanol to preserve specimens and DNA until further processing.

Their restricted habitat and inconspicuous adults has led to thaumaleids being under-collected and thus, an understudied group. There are approximately 200 described

species among 8–9 recognized genera: *Afrothaumalea* Stuckenberg, *Androprosopa* Mik, *Austrothaumalea* Tonnoir, *Neothaumalea* Pivar, Moulton & Sinclair gen. n., *Niphta* Theischinger, *Oterere* McLellan, *Thaumalea* Ruthe and *Trichothaumalea* Edwards. Some researchers recognize *Protothaumalea* Vaillant as a ninth genus (Wagner 2002), while others consider it to be a synonym of *Androprosopa* (Sinclair 1996). For a complete review of thaumaleid classification history, refer to Pivar (2015).

Thaumaleids are found on all continents except Antarctica (Vaillant 1977; Sinclair and Saigusa 2002). Their diversity is fairly well known in the Australasian (Theischinger 1986; McLellan 1988; Sinclair 2008a, 2008b; Pivar *et al.* 2016), Nearctic (Arnaud and Boussy 1994; Sinclair 1996; Pivar *et al.* 2018a) and Palearctic regions (Edwards 1929; Wagner 2002). In contrast, knowledge of the Afrotropical and Neotropical fauna is substantially weaker. Only three species have been described from the Afrotropics, all from South Africa (Stuckenberg 1960; Sinclair and Stuckenberg 1995; Sinclair 2015, 2017). The Neotropics are also understudied, with only six described species (Edward 1930; Schmid 1970). The lack of knowledge is likely explained by the absence of focused collecting in those regions, and forms the basis for the first two objectives of this dissertation.

The vast majority of thaumaleid research has been descriptive in nature. Species-level relationships have been proposed for various genera (McLellan 1988; Wagner 2002; Sinclair 2008b; Pivar *et al.* 2018a), some of which have been tested molecularly (Haubrock *et al.* 2017; Pivar in prep.). While no phylogenies depicting thaumaleid generic relationships have been published, hypotheses have been proposed.

Sinclair (pers. comm.) hypothesized that *Thaumalea* + *Androprosopa* is the sister group to all remaining genera. Both of these Holarctic genera are distinguished from all others by the presence of a complete subcostal (Sc) vein. Most described species of *Androprosopa* were initially placed in the genus *Thaumalea* until Sinclair (1996) redefined *Androprosopa* to include all species with obliquely to dorsoventrally directed gonostyli. Edwards (1929), Vaillant (1953) and Sinclair (1996) all noted that both genera are nearly identical and can only be accurately separated on the basis of male genitalia. The validity of *Protothaumalea* is also disputed, as it was synonymized with *Orphnephilina* (= *Androprosopa*) by Vaillant and Vinçon (1988) on the basis of undisclosed intermediate characters possessed by *An. demandana* (Vaillant and Vinçon). Wagner (2002) rejected this synonymy, while Sinclair (1996) accepted it. Haubrock *et al.* (2017) used molecular data to test relationships between European species and found *Protothaumalea* nested within *Thaumalea*. This controversy, along with the generic limits of *Androprosopa* and *Thaumalea* must be rectified in order to provide a stable classification system.

Sinclair (pers. comm.) also hypothesized the abbreviated Sc vein as a synapomorphy supporting a lineage comprised of *Trichothaumalea* + *Afrothaumalea* + *Niphta* + *Neothaumalea* + *Oterere* + *Austrothaumalea*. Sinclair noted that the abbreviated Sc vein terminates at the sc-r crossvein in all Southern Hemisphere genera forming a well-supported monophyletic lineage (Sinclair 2008a). In this hypothesis, *Trichothaumalea* would either be placed with the Northern Hemisphere genera, or on its own, as the Sc vein terminates opposite the branching of Rs (Sinclair and Saigusa 2002). Previously, Sinclair and Stuckenberg (1995) suggested close affinities between

Afrothaumalea, *Austrothaumalea* and *Niphta*, citing several larval characters. Sinclair (2000) suggested that the absence of apical, spine-like gonostylar setae in *Afrothaumalea*, *Niphta*, *Oterere* and *Austrothaumalea* further supported this hypothesis, but the recent discoveries of *Af. stuckenbergi* Sinclair and *Ne. atlantica* Pivar and Pinho put this hypothesis into question as they both possess gonostylar spines. *Niphta* and *Afrothaumalea* were also considered to be sister genera based on reduced macrotrichia on wing vein R₁ (Sinclair and Stuckenberg 1995; Sinclair 2000), but Sinclair (2015) described the setae running the entire length of this vein in *Af. stuckenbergi*, calling into question the value of this character. The validity of the genus *Oterere* is also suspect, as several characters used to diagnose the genus are also found in species of *Austrothaumalea*, perhaps rendering it paraphyletic (Sinclair 2008a,b). The relationship between *Trichothaumalea* and other genera is also in question, as it shares derived characters with *Niphta* (presence of a prominent antealar ridge), *Afrothaumalea* and *Neothaumalea* (dorsoventrally flattened pupa), but these are thought to be due to homoplasy (Sinclair and Saigusa 2002; Sinclair 2015; Pivar *et al.* 2018b). Pivar *et al.* (2018b) proposed that *Neothaumalea* was closely allied to *Niphta* based on morphological characters.

OBJECTIVES

The absence of a stable phylogeny for Thaumaleidae is a glaring void in the literature. Likewise, the lack of knowledge about the diversity and biology of the Neotropical fauna must be addressed. The objectives of this dissertation are divided into two main sections: (1) diversity and morphology, and (2) molecular systematics. The

diversity and morphology objectives fall under Chapters 2 and 3, where the Neotropical thaumaleid fauna, in particular Brazil and Chile, are investigated. The goals of both chapters are to examine and revise the diversity, and discover new information about the life history of species in the region. Also, fresh specimens from the Neotropics were needed for use in the molecular portion of this research. Chapter 4 is the molecular systematics portion of this dissertation, where molecular data is used to test all generic concepts and relationships within Thaumaleidae, in particular, deciphering relationships among the difficult-to-assess Gondwanan genera. This phylogeny will give insight into relationships, evolutionary trends and biogeography within the family. A rigorously tested and comprehensive phylogeny is imperative for setting the groundwork for future thaumaleid studies.

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CHAPTER 2

A NEW GENUS AND SPECIES, AND FIRST RECORD OF THE FAMILY

THAUMALEIDAE (DIPTERA) FROM BRAZIL

This chapter was originally published by Robert J. Pivar, Luiz C. Pinho, William E. Klingeman, John K. Moulton and Bradley J. Sinclair:

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ABSTRACT

Neothaumalea atlantica new genus, new species (Diptera: Thaumaleidae), is described from the state of Santa Catarina in southern Brazil. This represents the first thaumaleid collected east of the Andes mountain range. The egg, larva, pupa, and both adults are described and illustrated, distribution map presented, and phylogenetic affinities discussed. A key to the genera of South America is also provided.

INTRODUCTION

Thaumaleidae is a family of Diptera with roughly 190 described species and, as their common name seepage or madicolous midges suggests, thaumaleids are restricted to thin films of vertically flowing water (Vaillant 1956; Pivar *et al.* 2018). This specialised environment and poor dispersal ability of adults have led them to be poorly collected and consequently understudied. Found along cascading streams, rock face seeps, and margins of waterfalls, thaumaleid adults are best collected by sweeping above the madicolous habitat and adjacent riparian vegetation, while immatures can be plucked from the substrate using forceps or flushed into a white pan (Sinclair and Saigusa 2002).

The Southern Hemisphere fauna consists of four genera: *Afrothaumalea* Stuckenberg, *Austrothaumalea* Tonnoir, *Niphta* Theischinger, and *Oterere* McLellan. Prior to this study, six described species were recorded from South America, all from Chile and Argentina. Edwards (1930) described five species now assigned to *Austrothaumalea* (two species), *Niphta* (two species), and *Oterere* (one species), while Schmid (1970) described an additional species of *Austrothaumalea*. An undescribed thaumaleid species from Ecuador was reported by Röder (1886), but this record remains unverified as the specimen has not been located despite multiple attempts by the authors. A revision of Chilean and Argentinean fauna by R.J.P. was well underway when material of *Neothaumalea atlantica* new genus, new species, described herein, was discovered and made available. This species represents the first record of Thaumaleidae in Brazil and first east of the Andes Mountains. Both sexes and preimaginal stages are described.

MATERIALS AND METHODS

Terms used for adult structures follow Cumming and Wood (2017), except wing venation where the interpretation of Sinclair (2015) is accepted. Homology of the male terminalia follows Sinclair (1992). Terms used for larval and pupal structures follow those of Courtney *et al.* (2000) and Borkent (2012), respectively. The numbering system for larval head capsule setae and sensory pits follows Sinclair and Stuckenberg (1995). The distribution map was created using SimpleMappr (Shorthouse 2010).

Thaumaleidae were collected directly into 95% ethanol and later dried using hexamethyldisilazane, as outlined by Brown (1993). Adult genitalia were cleared using hot, 85% lactic acid. Representative males and larvae were also cleared with the

GeneJET Genomic DNA Purification Kit #K0722 (ThermoScientific, Waltham, Massachusetts, United States of America) in order to study structures cleared less aggressively while simultaneously extracting DNA for future molecular study. The lysate preparation protocol was followed and cleared voucher specimens were stored in 70% ethanol. Specimens were viewed under a Meiji Techno RZ stereomicroscope mounted with a Progres Gryphax® Naos camera (Jenoptik, Jena, Germany) to take light micrographs of pinned adults, and immatures in alcohol. Images were taken using iSolution Lite x64 (Focus Precision Industries, Victoria, Minnesota, United States of America) and stacked using Helicon Focus 6.7.1 (HeliconSoft, Roseau Valley, Dominica). Cleared terminalia and larval head capsules in glycerine were viewed with an Olympus BH-2 compound microscope and images were taken following the protocol described above. Line drawings were first traced from stacked micrograph images taken on the Olympus microscope (micrographs on their own failed to clearly illustrate critical characters), then inked and scanned for publication. Samples for scanning electron microscopy were dried using hexamethyldisilazane, mounted on gold-plated metal supports, sputter-coated, and scanned using a JEOL JSM-6390LV scanning electron microscope (Akishima, Tokyo, Japan) at the Central Laboratory of Electronic Microscopy. Cleared terminalia were preserved either in slide mounts made following the procedures outlined by Sæther (1969) and mounted in Euparal, or placed in glycerine filled microvials pinned beneath the specimen.

Label data for primary types are presented exactly as they appear. Data are listed from the top downward on the staging pin, with data from each label enclosed in quotation marks; lines are delimited by a forward slash mark. The repository is given in

parentheses. Specimens are deposited in the following repositories: Canadian National Collection of Insects, Ottawa, Ontario, Canada (CNC), Coleção Entomológica da Universidade Federal de Santa Catarina, Brazil (UFSC), Museu Nacional do Rio de Janeiro, Brazil (MNRJ), Museu de Zoologia da Universidade de São Paulo, Brazil (MZSP), the National Museum of Natural History, Smithsonian Institution, Washington, District of Columbia, United States of America (USNM), and the University of Tennessee, Knoxville, Tennessee, United States of America (UTK).

TAXONOMY

***Neothaumalea* Pivar, Moulton, and Sinclair, new genus**

Type species. *Neothaumalea atlantica* **new species.**

Etymology. From the Greek ‘neo-’ (new) in reference to the discovery of a new genus from the New World.

Diagnosis. Eye bridge narrow, tapered to 2–3 facets; head clothed in setae of uniform length. Mesoscutum with prominent antealar ridge; scutum clothed in short, undifferentiated setulae; proepisternal setae absent. Wing with R_{2+3} crossvein absent. Male abdomen with sternites 4–6 modified with medial heavily setose, sclerotised flap-like projections; female abdomen unmodified. Male terminalia with hypandrium narrow, paired parameres and membranous gonocoxal plate.

***Neothaumalea atlantica* Pivar and Pinho, new species**

Type material. Holotype: slide-mounted male with larval and pupal exuviae labelled: “BRAZIL, Santa Catarina State, Grão Pará / Parque Nacional de São Joaquim, Serra do Corvo Branco / 28°03'21"S 49°22'01"W, 1245.m a.s.l. / 10.ix.2016, seepage, L.C. Pinho

leg.” (MZSP). **Allotype:** female, same data as holotype except: “L.C. Pinho & A.P. Amaral leg.” (MZSP). **Paratypes:** Brazil: Santa Catarina State: Alfredo Wagner, BR282 km 111, 27°44'00"S 49°22'02"W, 915 m, 29.ix.2016, L.C. Pinho (two pupal exuviae (slide mount), UFSC); Brazil: Santa Catarina State: Grão Pará, Parque Nacional de São Joaquim, Serra do Corvo Branco, 28°03'21"S 49°22'01"W, 1245 m, 10.ix.2016, sweeping, L.C. Pinho and A.P. Amaral (two males, CNC; two males, MNRJ; two males, UFSC; two males, USNM; one male, UTK); Brazil: Santa Catarina State: Grão Pará, Parque Nacional de São Joaquim, Serra do Corvo Branco, 28°03'21"S 49°22'01"W, 1245 m, 7.x.2014, seepage, L.C. Pinho and A. Pitaluga (three larvae, MNRJ; two females (with larval and pupal exuviae), four larvae (slide mount), UFSC); Brazil: Santa Catarina State: Grão Pará, Parque Nacional de São Joaquim, Serra do Corvo Branco, 28°03'21"S 49°22'01"W, 1245 m, 28.ix.2016, seepage, L.C. Pinho and A. Pitaluga (10 larvae, one pupa, CNC; seven larvae (slide mount), MZSP); Brazil: Santa Catarina State: Lauro Müller, Serra do Rio do Rastro, 28°23'57"S 49°32'54"W, 1326 m, 29.ix.2016, seepage, L.C. Pinho (25 larvae, six pupae, CNC; one pupal exuviae, UFSC); Brazil: Santa Catarina State: Urubici, Parque Nacional de São Joaquim, Rio Italianinho, Cachoeira, 28°08'49"S 49°37'06"W, 1530 m, 23.viii–5.ix.2014, malaise, L.C. Pinho *et al.* (one female, UFSC); Brazil: Santa Catarina State: Urubici, SC370, São Pedro, 28°03'14"S 49°25'22"W, 980 m, 10.ix.2016, seepage, L.C. Pinho and A.P. Amaral (two pupae (slide mount), MNRJ).

Recognition. *Neothaumalea atlantica* males are distinguished from other genera by sternites 4–6 bearing densely setose flap-like extensions, hypandrium narrow, and gonostyli bearing five long, apical, spine-like setae. Both sexes also have an antealar ridge, a narrow eye bridge comprising only 2–3 facets, as well as uniform lengthened

setae on both the head and scutum.

Description of male. $n = 10$ (Fig. 2.1). *Length.* 2.00–2.05 mm. *Colouration.* Head dull, brown; pronotum light brown; postpronotum brown; postpronotal lobe light brown; mesonotum and pleura brown and somewhat shiny; scutellum and mediotergite brown, shiny; katapisternum light brown; halter pale and legs pale brown, legs becoming slightly darker on apical tarsomeres; abdomen brown; terminalia pale brown. *Head.* Eyes above antennae closely approximated, tapered to 2–3 facet width, exposing triangular frons above antennae (Figs. 2.2, 2.5). Clothed in setae of uniform length, lacking long orbital setae. *Thorax.* Mesoscutum with prominent antealar ridge, bearing strong medial seta flanked by one weak seta above and two below (Fig. 2.2). Scutum clothed in short, undifferentiated setulae; scutellum with row of marginal setae. Proepisternal setae absent. All legs with fourth tarsomere bilobed. *Wing.* (Fig. 2.2): Wing length: 2.03–2.32 mm. Slightly infusate throughout, apex somewhat narrowed; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with uniseriate row of microtrichia along entire length, remaining veins bare; R flexed into cell br; R_{2+3} crossvein absent; bend in R_{4+5} well defined; R_{4+5} and M_1 running parallel toward margin; M_1 straight, terminating at wing apex; CuA with short basal appendage. *Abdomen.* (Figs. 2.3, 2.5): Abdominal sternite 1 narrow, spectacle shaped; sternite 2 reduced to slender median sclerite, few setae restricted to laterad on posterior third; sternite 3 rectangular, weakly sclerotised, bearing setae on laterad and middle areas of median third; sternites 4–6 rectangular, weakly sclerotised with lateral setae, posterior margins modified with medial heavily setose, sclerotised flap-like projection, projection on sternite 4 overlapping sternite 5, projection on sternite 6 smallest; sternite 7 rectangular, weakly sclerotised, with row of anterior marginal setae and few setulae

medially; sternite 8 trapezoidal, with setae and more sclerotised than preceding sternites.

Male terminalia. (Figs. 2.4, 2.5): Epandrium with posterior margin rounded, short, not extending past gonostyli; without lobes or projections. Hypandrium narrow, same width as gonostylar spine-like setae. Gonocoxites bulbous, not much longer than wide; closely approximated medially. Gonostylus 1.5 times longer than wide, conical; bearing five long apical spine-like setae. Parameres paired, blade like; short, not extending past gonocoxal plate, diverging toward apex in ventral view. Gonocoxal plate membranous with cleft, setose posterior margin; basally not fused to hypandrium. Aedeagus membranous, sac-like. Cercus narrow, transverse band.

Description of female. $n = 4$ (Fig. 2.1). Similar to male except as follows: *Length:* 2.2 mm. Abdominal sternites 4–6 unmodified. *Female terminalia* (Figs. 2.4): Hypogynial valve short, not projecting beyond tergite 9; divergent in ventral view; densely setose. Tergite 9 subquadrate in lateral view, longer than tergite 8, lacking lateral projections. Sternite 9 (genital fork) slender, anchor shaped, swollen medially; lateral arms forming sclerotised semicircle. Cercus triangular, slightly scalloped on ventral margin toward apex, longer than wide, projecting posteroventrally; bearing numerous setae, single strong seta at apex. Spermathecal ducts not observed.

Description of pupa. $n = 14$ (Figs. 2.6, 2.7). *Length.* 2.85–3.80 mm. *Colouration.* Light brown; black spot above eyes in developing adult; caudal hook with black medial spot.

Head. Maxillary sheath short, posteromedially directed, apex truncate; three short, slender setae on small tubercle above black spot over eye; single slender seta below inner margin of eye in ventral aspect. *Thorax.* Prothoracic and mesothoracic leg sheaths projecting straight and shorter than wing sheaths; metathoracic leg projecting beyond

wing sheath and touching medially. Wing sheaths extending nearly to posterior margin of abdominal sternite 2. Respiratory organ three times as long as maxillary sheath; cylindrical with medial arch; spiracular openings encircling apex. Three slender setae on small tubercle anterior to respiratory organ. Mesothorax with three pairs of short, slender dorsocentral setae and one pair of long setae; dorsolateral tubercle bare. *Abdomen.*

Dorsoventrally flattened; broadest at segment 2. Spiracles very weakly developed, most distinct on segments 5–7, forming slight swelling, not projecting. Segments 1–7 with two pairs of dorsal setae on raised keel projecting posteriorly; lateral margins crenulate, bearing numerous small blade-like setae and four interspersed long, hook-tipped setae. Segment 8 with dorsolateral keel, posterior margin ending in small tubercle bearing pair of slender setae; posterior lateral margins bearing three slender setae; lateral margins crenulate, not as expanded as preceding segments, bearing numerous blade-like setae and two interspersed long setae. Caudal segment truncate, terminating in pair of large, stout anteroventrally curved hooks; caudal hooks with pair of sub-basal fine setae on outer margin. Sternites 2–7 with row of setulae complete around margin; sternite 8 with row of setulae incomplete along posterior margin, faint along anterior margin; caudal sternite without setulae.

Description of larva. *n* = 44 (Figs. 2.8–2.11). *Length of final instar.* 4.66–5.33 mm.

Colouration. Head capsule varies, may be pale brown to dark brown. Body brownish red dorsally, with creamy protuberances; cream coloured ventrally. *Head capsule.* Two large, circular eye spots; antenna on tubercle, with three finger-like processes; with 10 tubercles, length of which may vary between individuals from short (Fig. 2.8) to long (Fig. 2.10); 15 pairs of unbranched setae; five sensory pits (13, 14, 18, 19, 20), sensory

pit 13 above antennal tubercle. *Thorax*. Prothorax with single pair of protuberances bearing single spiracle; spiracular protuberance bearing one pair of dorsal setae anterior to spiracle and single dorsolateral seta; single long mid-lateral seta; group of four anterolateral setae situated near head capsule, three long and one short; three closely approximated setae near base of prothoracic leg (Keilin's organ). Mesothorax and metathorax with one pair of lateral and dorsolateral protuberances and an unpaired dorsomedial protuberance; dorsomedial protuberance naked; dorsolateral protuberance bearing two setae, anterior seta thickened; lateral protuberance bearing five setae, three short, two long; single antero-ventrolateral seta and one pair of mid-ventrolateral setae; mesothorax with additional seta between dorsolateral and lateral protuberance. *Abdomen*. Ventral abdominal sternites flat, smooth. Segments 1–7 with many protuberances and setae; unpaired small dorsomedial protuberance on anterior and posterior margins, not bearing setae; anterodorsolateral pair large, with single short seta beneath; dorsolateral pair small, bearing single short seta; five ventrolateral setae. Segment 8 with unpaired small dorsomedial protuberance, not bearing setae; pair of large dorsolateral protuberances flanking posterior spiracular plate, bearing single small medial seta and single long seta at base; three short posterolateral setae and two long ventrolateral setae; ventral sternite bearing two pairs of setae. Posterior spiracular plate with sclerite encircling procerci; procercus shorter than length of spiracular plate, less than twice as long as wide, bearing pair of single small plumose seta and four long setae; without cone-like protuberance on either side of cerci. Terminal segment with single small dorsomedial protuberance, not bearing seta; four short lateral setae, one long; single long ventrolateral seta; two pairs of long setae on posterior margin, above pair of anal papillae; ventral

sternite bearing single pair of setae.

Description of egg. (Figs. 2.12). Broadly ovate; ventral surface thin, half width of dorsal surface, smooth and flattened; dorsal surface thickened, reticulate. Embryo seemingly exposed.

Distribution. (Figs. 2.13, 2.14) This species is currently only known from five localities in Brazil's southern state of Santa Catarina, at elevations ranging from 915–1530 m in Aparados da Serra Geral, a mountain range with steep slopes running parallel to the Atlantic coastal plain. Aparados da Serra Geral, the coldest region in Brazil, is carved from volcanic rocks that date from the Mesozoic era (Wildner *et al.* 2004). A possible record of the genus was recently discovered in Serra do Caraça (Minas Gerais), however this is based only on a single pupal exuviae and additional material is required to confirm this determination.

Etymology. The specific epithet *atlantica* is in reference to the Atlantic Forest where this species is found. The name is to be regarded as a noun in apposition.

Bionomics. The larvae of this species are somewhat flattened ventrally compared to typical thaumaleid larvae. This is perhaps an adaptation to the madicolous zone of streams subject to rapid changes in water level and velocity; *i.e.*, regions exposed to more variable seasonal rainfall. The recurved caudal pupal hooks could also aid in anchoring the pupa during changes in flow conditions.

DISCUSSION AND CONCLUSIONS

Neothaumalea is most similar to the Gondwanan genus *Niphta* based on morphological characters. Both genera share a developed antealar ridge, which is absent

in all other Southern Hemisphere genera (Theischinger 1986). This ridge is present, however, in the Holarctic genus *Trichothaumalea* Edwards. Both *Neothaumalea* and *Niphta* also lack proepisternal setae adjacent to the anterior spiracle present in all other genera. *Neothaumalea atlantica* differs from *Niphta* by the presence of paired parameres (fused in *Niphta*) and hypandrium (absent in *Niphta*).

Among the few species with associated immature stages, the long hook-tipped pupal abdominal setae found in *Neothaumalea atlantica* are also present in *Austrothaumalea* and *Niphta* (Sinclair 2000). Recurved pupal caudal hooks are present in *Neothaumalea atlantica* and *Niphta collessi* Theischinger (Sinclair 2000), but are absent among known Chilean species of *Niphta* (R.J.P., unpublished data). The pupa is dorsoventrally flattened, as in *Trichothaumalea* and *Afrothaumalea stuckenbergi* Sinclair (Sinclair 2015). Unfortunately the immature stages of most thaumaleid species remain unknown and confident phylogenetic trends remain elusive.

Neothaumalea atlantica has several unique characteristics. Perhaps the most obvious of these are the heavily setose, sclerotised, flap-like projections on male abdominal sternites 4–6. These projections represent the first abdominal modifications described in Thaumaleidae and the second sexually dimorphic character in Thaumaleidae to be described; the other being the enlarged first palpal segment of male *Androprosopa larvata* Mik (Schmid 1958, figs. 34–36). While their function remains unknown, the presence of these projections only on males indicates that they may be used during mating. *Neothaumalea atlantica* also has a very narrow eye bridge that reveals a small triangular frons, unlike any other species in the family. In all remaining genera of Thaumaleidae, the eyes meet broadly, though a small frons may still be visible in some

species. The holoptic condition of the eyes in both sexes has been viewed as a family level synapomorphy, but it remains uncertain whether the reduced holoptic condition (*i.e.*, narrowed eye bridge) is a secondary reduction or plesiomorphic compared to the fully holoptic condition in the remaining genera. *Neothaumalea* is also the only genus in the Southern Hemisphere to have setae of uniform length both on the head and the scutum; although *Trichothaumalea* also exhibits this trait, as well as on the scutellum. The large, stout setae on the gonostyli are unique among South American thaumaleids, but this feature appears to be somewhat homoplastic given the presence or absence of stout setae among species of other genera (*e.g.*, *Afrothaumalea*; see Sinclair 2015).

The above characters support the erection of a genus for this new thaumaleid from Brazil. Future studies should focus on the collection of material from the southern region of Brazil and adjacent localities. Though this represents the first record of thaumaleids east of the Andes Mountains, this is unlikely to be the only species present given the extensive area that likely holds suitable habitat. Future collection attempts throughout South America are strongly encouraged and will undoubtedly yield many new discoveries.

Key to adults of South American genera of Thaumaleidae

- 1 Antealar ridge present (Fig. 2.2); proepisternal setae absent ... 2
- Antealar ridge absent; proepisternal setae present ... 3
- 2 $R_1(+R_{2+3})$ with several widely spaced weakenings or depigmented gaps; eye bridge broad, more than five facets; male terminalia without hypandrium and parameres fused ... *Niphta*

- $R_1(+R_{2+3})$ without widely spaced weakenings or depigmented gaps; eye bridge narrow, comprising 2–3 facets (Figs. 2.2, 2.5); male terminalia with hypandrium narrow and parameres separate, not fused (Figs. 2.4) ... *Neothaumalea*
- 3 Macrotrichia absent on wing vein R_{4+5} ... *Austrothaumalea*
- Macrotrichia present on wing vein R_{4+5} ... *Oterere*

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APPENDIX

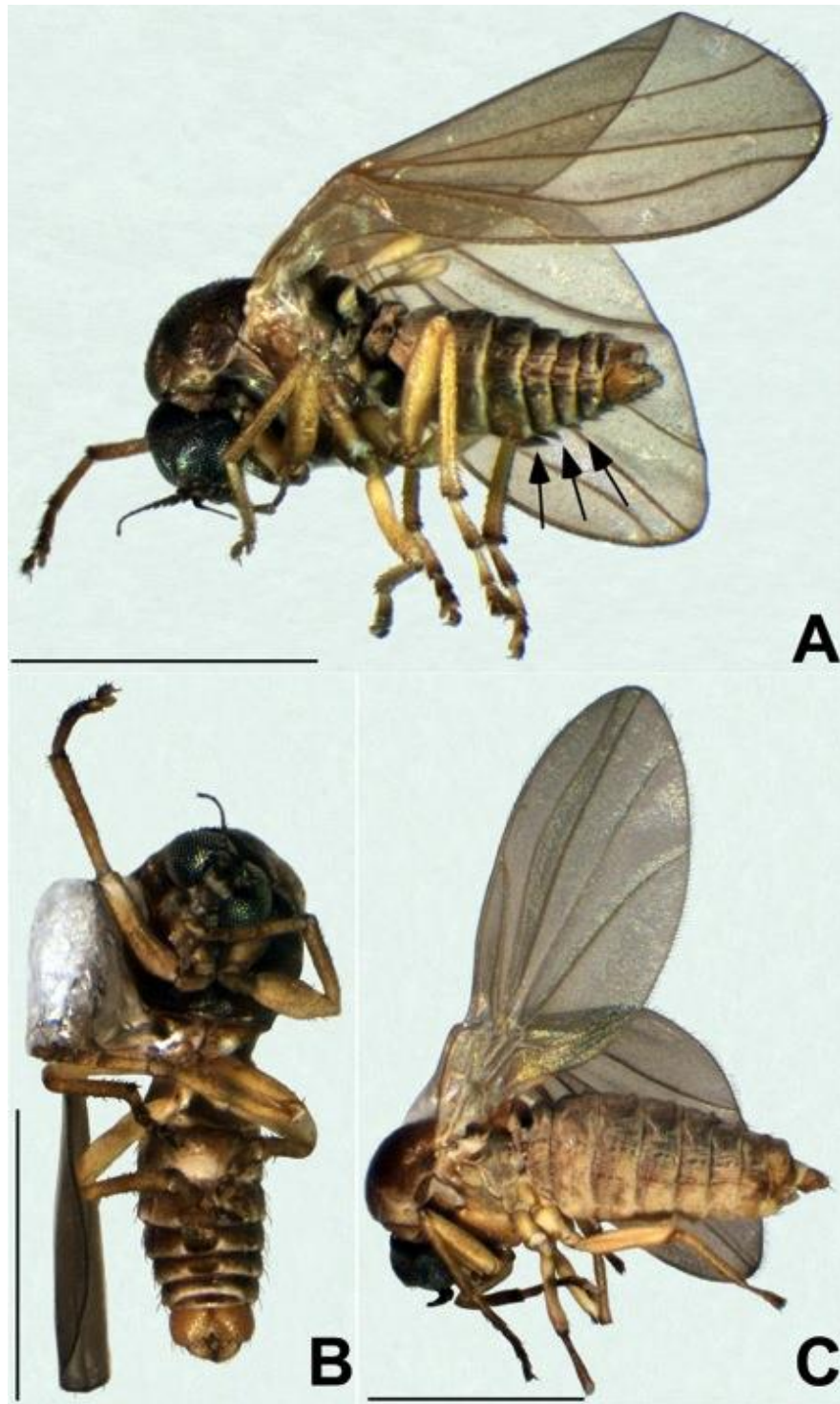


Figure 2.1. Light micrographs of adult *Neothaumalea atlantica* habitus: A, male lateral view with arrows indicating abdominal modifications; B, male ventral view; C, female lateral view. Scale bar = 1.0 mm.

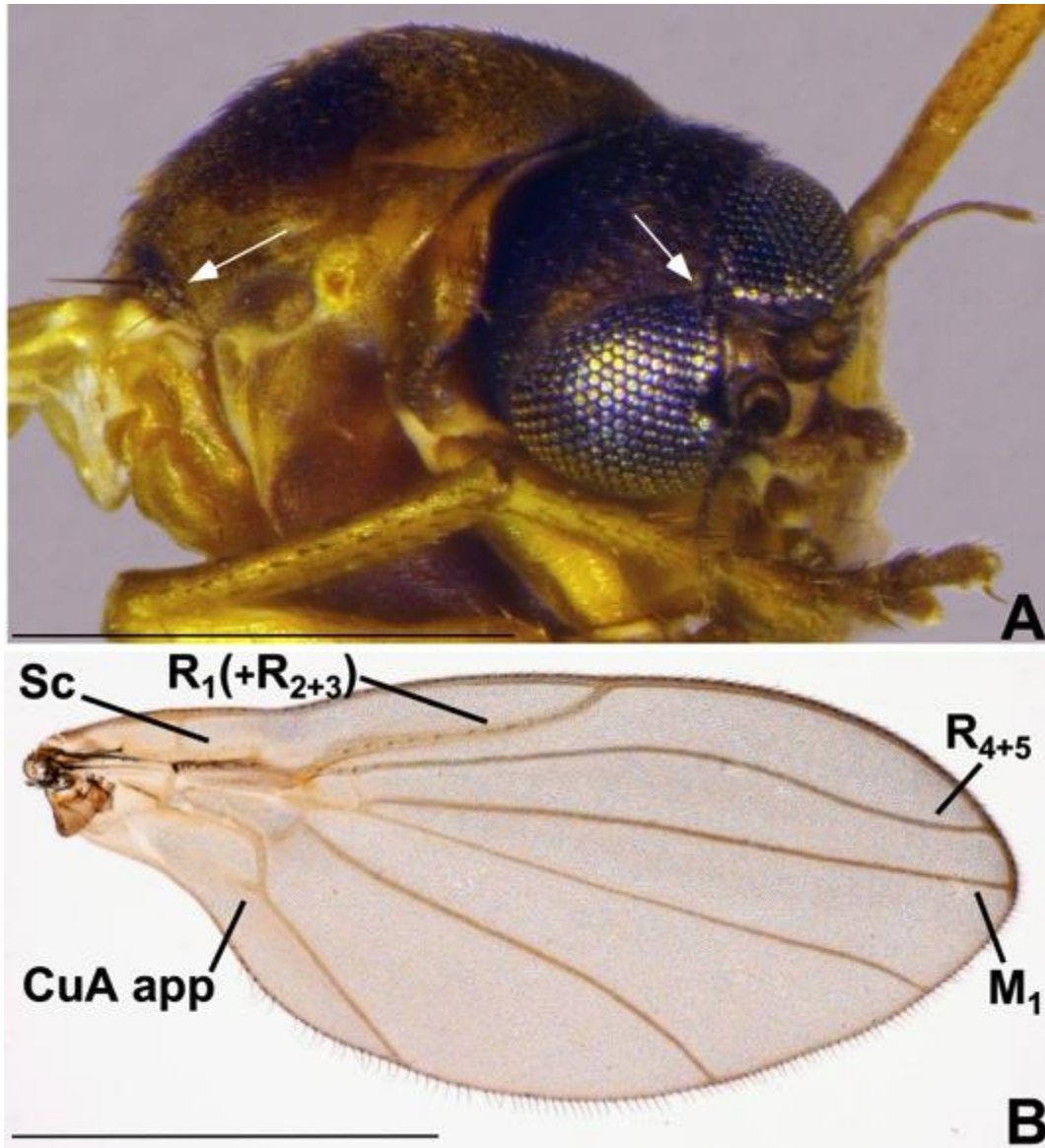


Figure 2.2. Light micrographs of adult *Neothaumalea atlantica*: A, arrows pointing to antealar ridge and narrow eye bridge (scale bar = 0.5 mm); B, right wing (scale bar = 1.0 mm). Abbreviations: CuA app, cubital vein with appendage; M, medial vein; R, radial vein; Sc, subcosta.



Figure 2.3. Light micrographs of cleared abdomen of *Neothaumalea atlantica* to illustrate modified sternites 4–6: A, lateral view; B, ventral view. Abbreviations: S, sternite. Scale bar = 0.1 mm.

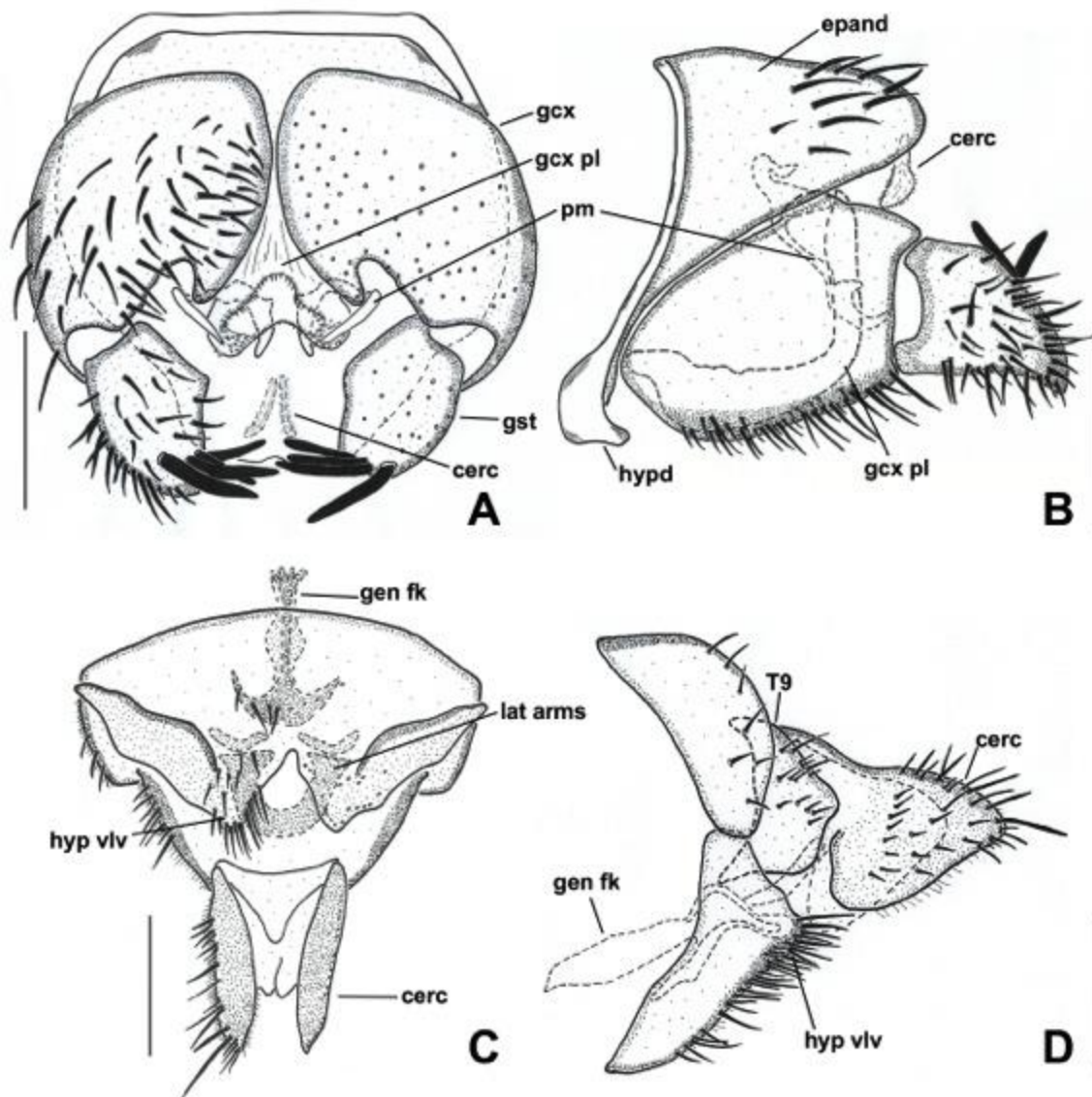


Figure 2.4. Illustrations of *Neothaumalea atlantica* genitalia: A, male ventral view; B, male lateral view; C, female ventral view; D, female lateral view. Abbreviations: cerc, cercus; epand, epandrium; gen fk, genital fork; gcx, gonocoxite; gcx pl, gonocoxal plate; gst, gonostylus; hypd, hypandrium; hyp vlv, hypogynial valve; lat arms, lateral arms; pm, paramere; T, tergite. Scale bars = 0.1 mm.

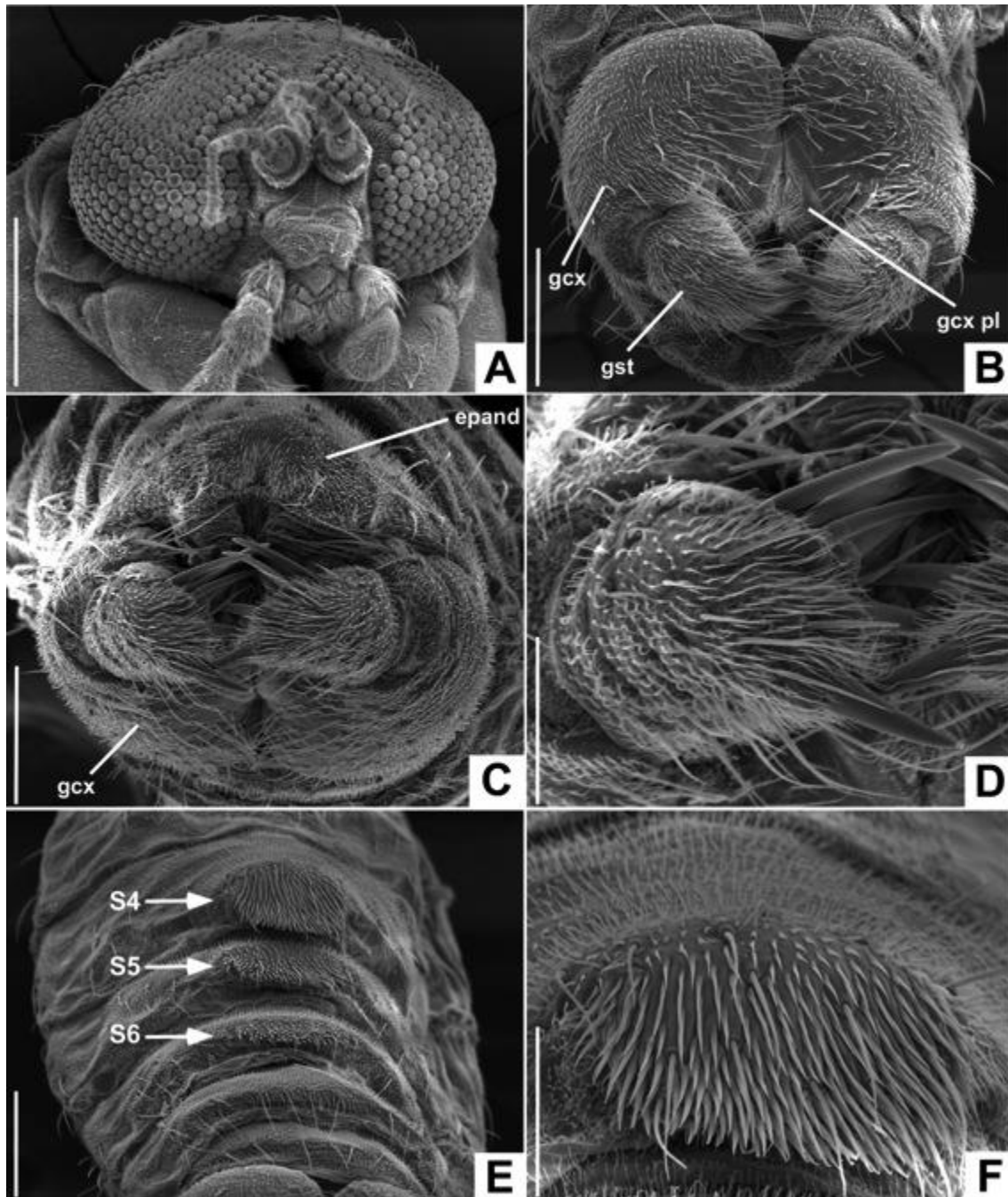


Figure 2.5. Scanning electron micrographs of adult male *Neothaumalea atlantica*: A, anterior view of head; B, ventral view of genitalia; C, caudal view of genitalia; D, caudal view of left gonostylus; E, ventral view of abdomen illustrating modified sternites 4–6; F, close-up of modified sternite 4. Abbreviations: epand, epandrium; gcx, gonocoxite; gcx pl, gonocoxal plate; gst, gonostylus; S, sternite. Scale bars: Figs. A–C, E = 0.1 mm; Figs. D, F = 0.05 mm.

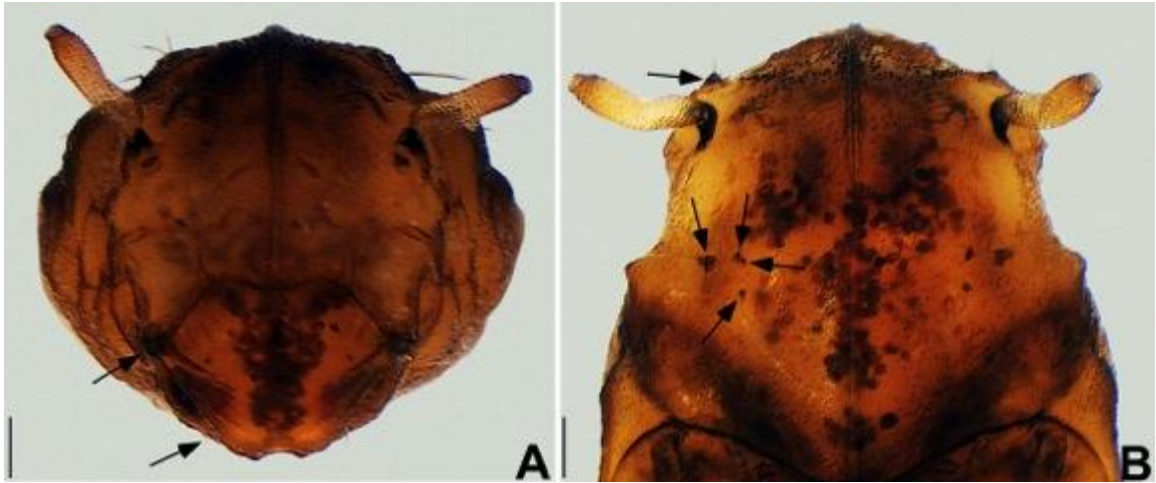


Figure 2.6. Light micrographs of anterior region of *Neothaumalea atlantica* pupa. Arrows depicting location of setae: A, anterior view of head; B, dorsal view of thorax and respiratory organ. Scale bar = 0.1 mm.



Figure 2.7. Light micrographs of *Neothaumalea atlantica* pupa: A, dorsal view; B, lateral view; C, ventral view; D, close-up of caudal segments and hooks. Scale bar = 1.0 mm.

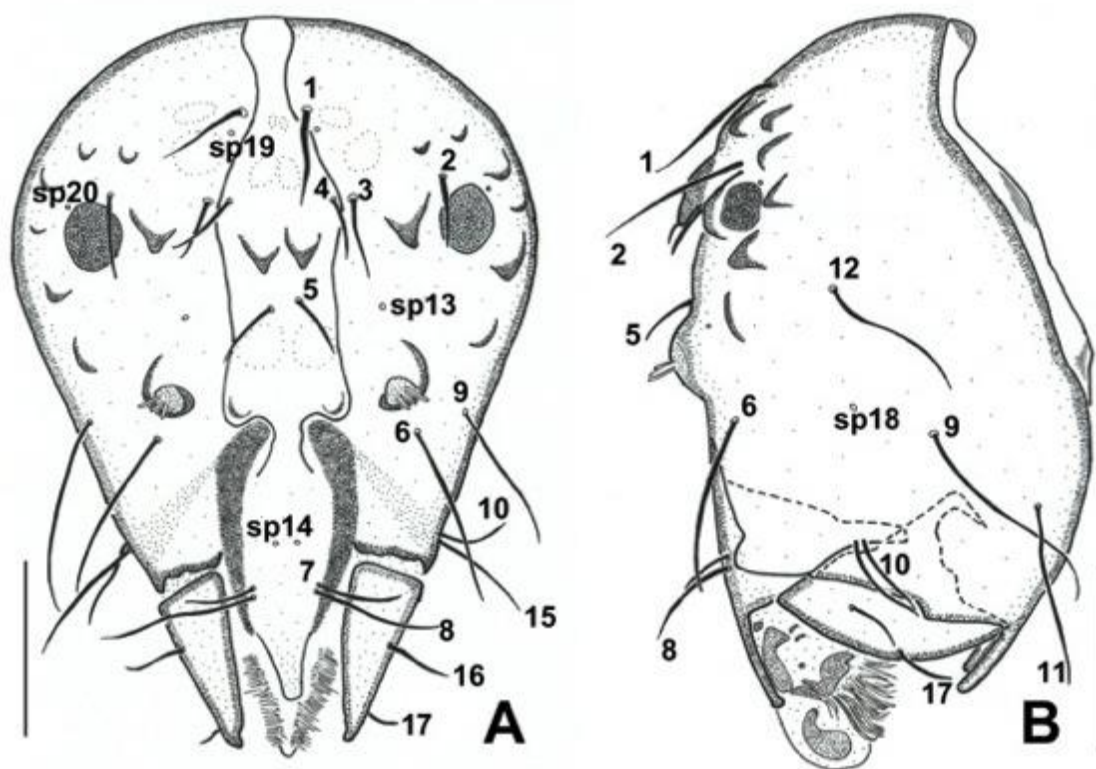


Figure 2.8. Illustrations of *Neothaumalea atlantica* larval head capsule: A, anterior view; B, lateral view. Abbreviations: sp, sensory pit. Scale bar = 0.1 mm.



Figure 2.9. Light micrographs of *Neothaumalea atlantica* larva: A, dorsal view; B, lateral view; C, ventral view. Scale bar = 1.0 mm.



Figure 2.10. Light micrographs of *Neothaumalea atlantica* larva, anterior half: A, dorsal view; B, lateral view; C, ventral view. Abbreviations: sp pro, spiracular protuberance. Scale bar = 1.0 mm.



Figure 2.11. Light micrographs of *Neothaumalea atlantica* larva, posterior half: A, dorsal view; B, lateral view; C, ventral view. Scale bar = 1.0 mm.

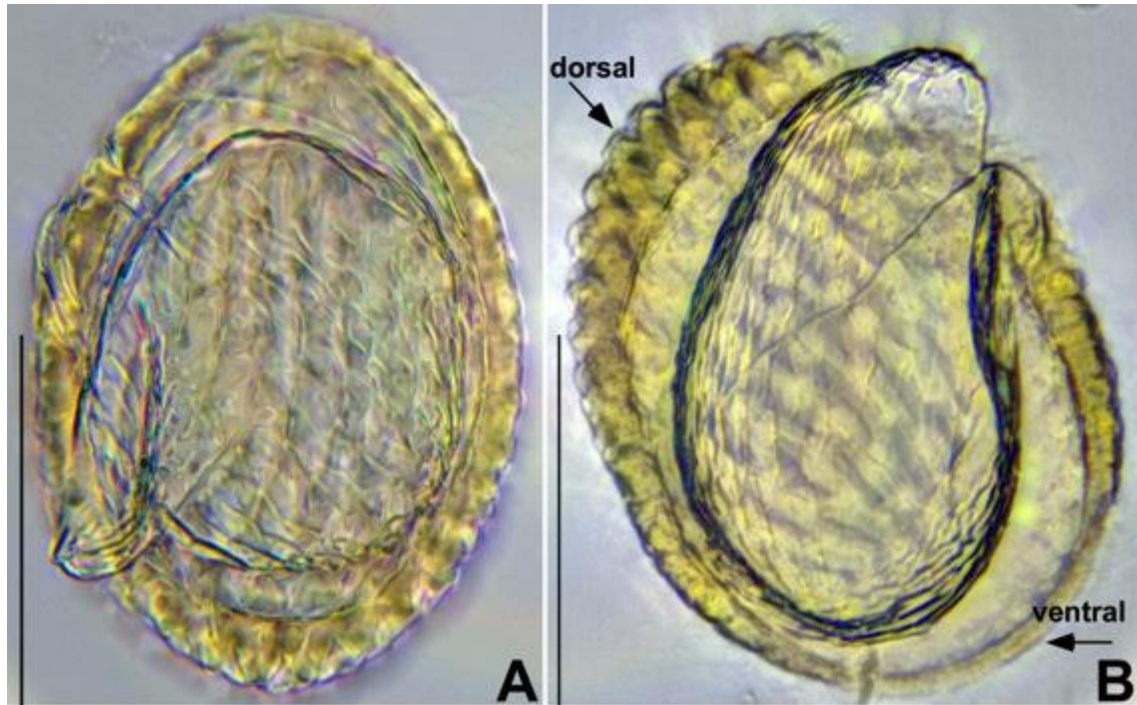


Figure 2.12. Light micrographs of *Neothaumalea atlantica* egg: A, dorsal view; B, cross section. Scale bar = 0.1 mm.



Figure 2.13. Distribution map of *Neothaumalea atlantica*.



Figure 2.14. Habitat of *Neothaumalea atlantica*: A, Serra do Corvo Branco, Santa Catarina, Brazil (28°03'21"S, 49°22'01"W); B, rock face seepage, Serra do Corvo Branco site; C, Serra do Rio do Rastro site (28°23'57"S, 49°32'54"W).

CHAPTER 3

A REVISION OF THE THAUMALEIDAE (DIPTERA) OF SOUTHERN CHILE

ABSTRACT

The thaumaleid fauna of southern Chile is examined and revised to include 16 species, 10 of which are described as new to science (*Austrothaumalea fredericki* **sp.n.**, *Niphta acus* **sp.n.**, *Ni. brunnea* **sp.n.**, *Ni. courtneyi* **sp.n.**, *Ni. daniellae* **sp.n.**, *Ni. downesi* **sp.n.**, *Ni. eurydactylus* **sp.n.**, *Ni. mapuche* **sp.n.**, *Ni. moultoni* **sp.n.** and *Ni. sinclairi* **sp.n.**). Additionally, *Oterere* is synonymized with *Austrothaumalea*, the genus *Niphta* is redefined, the male of *A. apicalis* Edwards is described for the first time, and all previously described Chilean species are redescribed. Females are also described and redescribed where possible. The first descriptions of immature South American *Niphta* Theischinger are provided, which represent a new larval morphotype in Thaumaleidae; larvae and pupae possess ventral adhesive structures. Illustrations and micrographs are provided for all species, and SEM images are provided for select immatures. Distribution maps, keys to genera and species, and discussions regarding larval cuticular sculpture, phylogenetic affinities and habitat are also included.

INTRODUCTION

An uncommonly encountered family of lower Diptera, Thaumaleidae consists of slightly fewer than 200 species classified in eight genera. Considered to be the sister family to Simuliidae (black flies) (Moulton 2000; Bertone *et al.* 2008; Wiegmann *et al.* 2011; Borkent 2012; Kutty *et al.* 2018), thaumaleids are significantly less studied due to their restricted larval habitat, but also because they are of no known medical or economic importance to humans. They are commonly referred to as seepage or solitary midges; however, madicolous midge is a more apt name as it encompasses all of their potential

habitats, including rock-face seepages, margins of waterfalls and splash zones of cascading streams (Sinclair 2000; Pivar *et al.* 2018a; Pivar *et al.* 2018b). Madicolous habitats are characterized to have thin films of water, frequently flowing over rocky surfaces (Mackie 2004; Shimabukuro and Trivinho-Strixino 2018). Thaumaleid larvae are restricted to these habitats, and until this study, were thought to be limited to rocky substrates. Once candidate habitat is found, sweeping above the madicolous substrate and adjacent riparian vegetation with an aerial net is the best method to collect adult thaumaleids. Sweeping for adults should be attempted first if possible, as searching for larvae may cause adults to fly deeper into vegetation or into more difficult-to-sweep areas. Special attention should be given to dead branches and leaf apices, where adults have frequently been observed resting (Pivar, personal observation). After sweeping, immatures can be collected by using forceps to pull them off the substrate, or by pouring water over the substrate and flushing immatures into a white pan (Sinclair and Saigusa 2002). Once collected, all life stages should be placed directly into 75% non-denatured ethanol for morphological studies or 95% non-denatured ethanol for molecular studies.

The South American thaumaleid fauna, along with that of Africa, is presently the least known. Between both continents, only 10 species have been described within five genera. Africa has the fewest described species, with only three species of *Afrothaumalea* Stuckenberg (Sinclair 2017). Until now, South America had seven described species in four genera, known from only three countries: *Austrothaumalea* Tonnoir (three species, Chile and Argentina), *Neothaumalea* Pivar, Moulton and Sinclair (one species, Brazil), *Niphta* Theischinger (two species, Chile) and *Oterere* McLellan (one species, Chile). Edwards (1930) was the first to describe thaumaleids from South America, describing

two species each of *Austrothaumalea* and *Niphta*, and a single species of *Oterere*. Schind (1970) later described an additional species of *Austrothaumalea*. While these publications were important starting points for South American thaumaleid studies, the species descriptions were very brief and the illustrations lack important details. Furthermore, little was written in the way of phylogenetic discussion, no keys were provided, and immature stages were unknown. Pivar *et al.* (2018b) most recently described *Neothaumalea atlantica* Pivar & Pinho from Brazil. *Oterere* was also transferred back into *Austrothaumalea* based on both morphological and molecular evidence (Pivar, Chapter 4). In addition to the described species above, Röder (1886) noted an undescribed species from the Ecuadorian Andes, though this record remains unverified by the authors despite attempts to locate the specimen. Stone (1966) published a catalogue of thaumaleids south of the United States, but this needs updating to reflect recent discoveries and generic assignment. The vastness of South America and its many mountain ranges, including the enormous Andes Mountains, beckon a study of thaumaleid diversity.

Colleagues of the authors collected in southern Chile in 2008 and 2013, and returned with few, but important, thaumaleid specimens. Of the four species collected, three were undescribed, and immatures that looked very different from any other known thaumaleid were also collected. Furthermore, the temperate rainforest habitat abutting the high Andes of southern Chile, with its abundance of cascading creeks and waterfalls, is similar to that of the Pacific Northwest of North America, which is home to the highest diversity of thaumaleids in the Nearctic Region (Pivar *et al.* 2018a). These two factors, plus the lack of published and museum material, led the authors to hypothesize that

thaumaleid diversity is likely greater than is presently known. In December 2016, Pivar and Moulton travelled to central Chile south to the Island of Chiloé in order to better assess the Chilean fauna (Fig. 3.1). This paper is the result of that collecting expedition.

Herein, 10 new species are described (one *Austrothaumalea* and nine *Niphta*), the male for *A. apicalis* Edwards is described, all of Edwards and Schmid species are redescribed, females are described or redescribed for all available species and *Niphta* is redefined. Additionally, immature stages of South American *Niphta* are described for the first time. Distribution maps, keys to genera and species, and discussions regarding phylogenetic affinities are also provided.

MATERIALS AND METHODS

Although original type labels were vague, collection attempts in suitable habitats from as near as possible to the perceived type localities were made in hopes of recollecting fresh typotypical material. This allowed for greater certainty when comparing fresh material to the type specimens.

Adult genitalia were cleared with hot, 85% lactic acid. Representative adults and immatures were also cleared with the GeneJET Genomic DNA Purification Kit #K0722 (ThermoScientific, Waltham, MA) in order to study structures cleared less aggressively and to extract DNA for subsequent molecular study. The GeneJET lysate preparation protocol was followed and cleared voucher specimens were stored in 70% non-denatured ethanol. Positive identifications of females and immatures were made by comparing their DNA sequences to those of identified males (see Chapter 4 for methods). Specimens identified through molecular means are denoted by an asterisk (“*”) in the Type Material

and Additional Material Examined sections. Pinned specimens were dried using Brown's (1993) hexamethyldisilazane (HMDS) method. Cleared terminalia were preserved in glycerine-filled microvials pinned beneath the specimen.

Specimens were viewed under a Meiji Techno RZ stereomicroscope mounted with a Progres Gryphax® Naos camera (Jenoptik, Jena, Germany) to take light micrographs of pinned adults and immatures in alcohol. Images were taken using iSolution Lite x64 (Focus Precision Industries, Victoria, MN, USA) and stacked using Helicon Focus 6.7.1 (HeliconSoft, Roseau Valley, Dominica). Cleared terminalia and larval head capsules in glycerine were viewed with an Olympus BH-2 compound microscope and images were taken following the same steps as above. Line drawings were first traced from stacked micrograph images taken on the Olympus microscope (micrographs on their own failed to clearly illustrate critical characters), then inked and scanned for publication. The left gonocoxite and gonostylus were intentionally omitted for *Niphta nudipennis* group species to allow for clearer visualization of remaining genitalic characters.

Samples were prepared for scanning electron microscopy by placing specimens from 95% non-denatured ethanol into a 12 mm × 30 µm microporous specimen capsule (Electron Microscopy Services, Hatfield, PA). Capsules were then subjected to the following HMDS dehydration series, each step lasting 20 minutes: 100% ethanol, 1:1 ethanol to HMDS, 1:75 ethanol to HMDS, then two steps of 100% HMDS. Dried specimens were mounted on carbon tape affixed to a 45°/90° aluminum stubs and sputter-coated with gold for 10 sec at 20 µA in a SPI-Module Sputter Coater (West Chester, PA). Specimens were viewed with a Hitachi TM3030 electron microscope (Tokyo, Japan) at a

voltage of 15 kV.

Terms used for adult structures follow Cumming and Wood (2017), except wing venation where the interpretation of Saigusa (2006) and Sinclair (2015) are accepted. Homology of the male terminalia follows Sinclair (1992). Terms used for larval and pupal structures follow those of Courtney *et al.* (2000) and Borkent (2012), respectively. The numbering system for larval head capsule setae and sensory pits follows Sinclair and Stuckenberg (1995). Distribution maps were created with SimpleMapper (Shorthouse 2010).

Label data for primary types are presented exactly as they appear. Data are listed from the top downward on the staging pin, with data from each label enclosed in quotation marks; lines are delimited by a forward slash mark. The repository is given in parentheses. Unless otherwise stated, non-primary types collected by the authors will be distributed amongst the CNC, USNM, UTK and Pivar's personal collection.

Specimens will be deposited in the following repositories: Canadian National Collection of Insects, Ottawa, Canada (CNC); Instituto de Entomología, Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile (UMCE); Museo Nacional de Historia Natural, Santiago, Chile (MNHC); National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM); University of Tennessee, Knoxville (UTK).

Key to adults of South American genera of Thaumaleidae

Modified from Pivar *et al.* (2018b).

1. Antealar ridge present (Fig. 3.10H) ... 2

- Antealar ridge absent ... *Austrothaumalea*

2. $R_1(+R_{2+3})$ with several widely spaced weakenings or depigmented gaps (Fig. 3.2B); eye bridge broad, more than five facets; male terminalia without hypandrium and parameres fused ... *Niphta*

- $R_1(+R_{2+3})$ without widely spaced weakenings or depigmented gaps; eye bridge narrow, comprising 2–3 facets; male terminalia with hypandrium narrow and parameres separate, not fused ... *Neothaumalea*

TAXONOMY

Genus *Austrothaumalea* Tonnoir

Austrothaumalea Tonnoir, 1927: 109. Type-species: *Austrothaumalea neozealandica*

Tonnoir (original designation).

Oterere McLellan, 1988: 573. Type-species: *Oterere oliveri* McLellan (original designation) **syn. nov.**

Diagnosis. The genus *Austrothaumalea* is characterized as follows: absence of a distinct antealar ridge; proepisternal setae present; microtrichia of first vein ($R_1(+R_{2+3})$) extending length of vein; R_{2+3} crossvein usually situated closer to apex of $R_1(+R_{2+3})$ than to origin of R_{4+5} ; R_{4+5} often with arch strongly produced; basal appendage of CuA often present; gonocoxites slender, longer than wide; hypandrium broad; parameres fused medially.

Distribution. Argentina, Australia (including Tasmania), Chile, New Caledonia and New Zealand.

Notes on synonymy. *Oterere* was synonymized on the basis of shared characters with

numerous species of *Austrothaumalea* (a well-developed appendage on CuA, macrotrichia on R₄₊₅ and patterned wings), as well as through molecular data presented in Chapter 4.

Key to adult males of South American *Austrothaumalea*

See Figure 3.2 for micrograph of wing and Figures 3.4A (ventral) & 3.5A (lateral) for genitalic illustrations with pertinent structures labelled.

1. Macrotrichia present on wing vein M₁ (Fig. 3.3) ... *A. setipennis* Edwards
 - Macrotrichia absent on wing vein M₁ (Fig. 3.2A) ... 2
2. Epandrium with posterolateral projections (Figs. 3.4A, 3.5A) ... *A. apicalis* Edwards
 - Epandrium without posterolateral projections (Figs. 3.4B–E & 3.5B–E) ... 3
3. Paramere spatulate-shaped, without lateral projections (Figs. 3.4E , 3.5E) ... *A. spatulata* Schmid
 - Paramere cruciate-shaped, with lateral projections (Figs. 3.4B,C, 3.5B,C) ... 4
4. Lateral projections on paramere located toward apex, not concealed behind gonocoxal plate (Figs. 3.4B, 3.5B) ... *A. chilensis* Edwards
 - Lateral projections on paramere located medially, concealed behind gonocoxal plate (Figs. 3.4C, 3.5C)... *A. fredericki* Pivar **sp.n.**

Species diagnoses and descriptions

Austrothaumalea apicalis Edwards

Austrothaumalea apicalis Edwards, 1930: 113.

Stone, 1966: 1 (catalogue); Arnaud, 1977: 284 (distribution); Theischinger, 1986: 316 (phylogenetic discussion); Sinclair, 2008b: 12 (species groups).

Type material examined. Holotype: ♀, minuten pinned with right wing mounted on plastic card mounted beneath specimen and abdomen mounted in resin, labelled: “Bariloche./ 1.xii.1926.”; “Austrothaumalea/ apicalis Edw./ F.W. Edwards/ det. 1930.”; “Argentina:/ Terr. Rio Negro./ F. & M. Edwards./ B.M. 1927 – 63.”; “HOLO-/ TYPE”; “NHMUK010210687” (BMNH). **Paratype:** same label data as holotype (1♀, BMNH).

Recognition. This species is the only described species of South American *Austrothaumalea* to possess posterior epandrial projections (which are present in some Australian species). This feature, along with the more rounded apex of the paramere and concave posterior margin of the gonocoxal plate distinguish it from *A. spatulata*.

Description of male. *n* = 1.

Length 2.1 mm.

Colouration (Fig. 3.3A). Head dull, dark brown; pronotum and postpronotum brown; postpronotal lobe brown; prescutum with lateral margins brown; mesoscutum uniformly yellowish-brown, shiny; scutellum and mediotergite pale brown, shiny; pteropleuron brown with ill-defined darker markings; halter yellowish-brown; legs pale brown, apices

of femora and tarsi darkened; abdomen dark brown; terminalia brown.

Head. Eyes above antennae broadly joined, barely exposing frons above antennae.

Flagellomeres 1–3 expanded, subquadrate; 1 as long as 2 and 3 combined; flagellomeres 4–10 thin, cylindrical, becoming progressively more elongate. Vertex clothed in golden setae of uniform length, with sparse, long, black orbital setae.

Thorax. Pronotal setae long. Mesoscutum without antealar ridge. Scutum clothed dorsally in short setulae; notopleural, supra-alar and postscutellar setae long, black. Scutellum with row of long, black marginal setae. Proepisternal setae present. Pteropleuron bare. All legs with tarsomere 4 bilobed.

Wing. Wing length: 2.7 mm. Lightly infusate throughout except for distinct dark, apical spot encircling apex of R_{4+5} and M_1 (Fig. 3.3); apex broadly rounded; C and posterior wing margin with fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ without weakenings or depigmented gaps, with uniseriate row of microtrichia along entire length, remaining veins bare; R flexed into cell br; R_{2+3} crossvein very weak or absent, at most a faint infuscation, situated slightly beyond midpoint of $R_1(+R_{2+3})$; bend in R_{4+5} gentle; R_{4+5} and M_1 running parallel toward margin; M_1 and M_2 straight; M_4 slightly sinuous; CuA with indistinct, short basal appendage.

Abdomen. Abdominal sternites 1–4 not observable due to dissection; sternites 5–6 rectangular, weakly sclerotized, anterior margin well sclerotized, setae restricted to posterior third; sternite 7 rectangular, a few setae on posterior third, weakly sclerotized, anterior margin well sclerotized, arched slightly into preceding sternite; sternite 8 crescent-shaped, without setae, weakly sclerotized except for anterior margin slightly more sclerotized and arched slightly into preceding sternite.

Male terminalia (Figs. 3.4A, 3.5A). Epandrium broadly triangular from ventral aspect; apical third slightly tapered; posterior margin narrow, slightly indented, with single small tooth-like projecting from posterolateral margin. Hypandrium broad, wider than width of base of gonostylus; anterior margin not concave. Gonocoxite broadest basally, inner margins nearly parallel, smooth. Gonostylus shorter than gonocoxite, basal quarter straight then gently curved; broadest at basal quarter, apical three-quarters equal in width; apex truncate. Parameres distally fused, three-quarters length of epandrium; expanding irregularly toward apex. Gonocoxal plate weakly sclerotized, apical third tapered, posterior margin slightly concave with posterolateral projection on each corner; nearly half-length of gonocoxite; basally not fused to hypandrium. Cercus weakly sclerotized, situated medially.

Redescription of female. *n* = 1.

Similar to male except as follows: *Length*: 2.3 mm. *Abdomen*: Tergites 8–9 noticeably more sclerotized than preceding segments; only anterior margin of sternite 7 well sclerotized. *Female terminalia* (Figs. 3.6A, 3.7A): Hypogynial valve not projecting beyond tergite 9; posterior margin rounded with shallow cleft in ventral view; densely setose. Tergite 9 rectangular in lateral view, wider than tergite 8, lacking lateral projections. Sternite 9 (genital fork) thin, rod-shaped, posteriorly divided into two triangular-shaped sclerites; distal margin of lateral arms extended beyond hypogynial valve, arms nearly meeting along midline, golf club-head shaped. Hypoproct arched, thin medially. Cercus rounded, projecting slightly posterodorsally; bearing numerous setae. Spermathecal ducts not observed.

Immature Stages. Unknown.

Additional Material Examined. ARGENTINA. RIO NEGRO: Bariloche, xi.1926, R&E Shannon, USNMENT01115807 (1♂, USNM); CHILE. REGION X (Los Lagos): Casa Pangué, Llanquihue, xii.1926, R&E Shannon, USNMENT01115806 (1♀, USNM).

Distribution. Known from adjacent localities on the Chile-Argentina border in the southern Andes (Fig. 3.8).

Notes. The male specimen was not described by Edwards (1930), presumably because he did not have access to Shannon's material. Stone identified the specimen as *A. apicalis* (year not specified on determination label), but he never formally described it. Since it was collected in the same region as the holotype female, and it is unique from all other described males, the authors have described it as the putative male.

***Austrothaumalea chilensis* Edwards**

Austrothaumalea chilensis Edwards, 1930: 111.

Stuardo, 1946: 42 (catalogue); Stone, 1966: 1 (catalogue); Arnaud, 1977: 284 (distribution); Theischinger, 1986: 316 (phylogenetic discussion); Sinclair, 2008b: 11 (species groups).

Type material examined. Holotype: ♂, minuten pinned with abdomen mounted in resin, labelled: "Ancud./ 17–19.xii.1926."; "Austrothaumalea/ chilensis Edw./ F.W. Edwards/ det. 1930."; "S. Chile:/ Chiloe I./ F. & M. Edwards./ B.M. 1927 – 63."; "HOLO-/ TYPE"; "NHMUK010210688" (BMNH). **Allotype:** ♀, same label data as holotype (BMNH). **Paratypes:** CHILE. REGION X (Los Lagos): Casa Pangué,

Llanquihue prov., 4–10.xii.1926, F. & M. Edwards (1♂, BMNH); Chiloé, Ancud, 17–19.xii.1926, F. & M. Edwards (3♂, 1♀, BMNH).

Recognition. This species is recognized by its gonocoxal plate bearing lateroapical teeth and cruciate paramere. The lateral, cross-like projections on the paramere are located apically, not concealed by the gonocoxal plate and are much shorter than those of *A. fredericki*. This is also the only known South American species to have the gonocoxal plate secondarily fused to the ventral surface of the hypandrium.

Redescription of male. *n* = 4.

Length 2–2.3 mm.

Colouration (Fig. 3.3C). Head dull, dark brown; pronotum and postpronotum brown; postpronotal lobe brown; mesoscutum and pleura brown, somewhat shiny; scutellum and mediotergite brown, shiny; katapisternum dark brown; halter brown, dark at apex; legs pale brown, apices of femora and tarsi darkened; abdomen dark brown; terminalia brown.

Head. Eyes above antennae broadly joined, frons above antennae barely exposed.

Flagellomeres 1–3 expanded, subquadrate; 1 as long as 2 and 3 combined; flagellomeres 4–10 thin, cylindrical, becoming progressively more elongate. Vertex clothed in golden setae of uniform length, with sparse, long, black orbital setae.

Thorax. Pronotal setae long. Mesoscutum without antealar ridge. Scutum clothed dorsally in short, golden setulae; notopleural, supra-alar and postscutum setae long, black.

Scutellum with row of long, black, marginal setae. Proepisternal setae present.

Pteropleuron bare. Fore- and mid legs with tarsi simple, hind leg with tarsomere 4 bilobed.

Wing. Wing length: 2.2–3.0 mm. Lightly infuscate throughout, apex broadly rounded; C

and posterior wing margin with fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ without weakenings or depigmented gaps, with uniseriate row of microtrichia along entire length, remaining veins bare; R flexed into cell br; R_{2+3} crossvein strong, situated toward apex of $R_1(+R_{2+3})$; bend in R_{4+5} well defined; R_{4+5} and M_1 running parallel toward margin; M_1 and M_2 straight; M_4 slightly sinuous; CuA with distinct short basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, few setae on posterior margin; sternites 3–6 rectangular, weakly sclerotized, setae largely restricted to posterior half; sternite 7 a semi-circle, few setae on posterior margin, weakly sclerotized except for anterior margin, arched slightly into preceding sternite; sternite 8 crescent-shaped, without setae, well sclerotized, strongly arched into preceding sternite.

Male terminalia (Figs. 3.4B, 3.5B). Epandrium broadly triangular from ventral aspect; apical third slightly tapered; posterior margin broad and rounded; lacking pointed posterolateral processes. Hypandrium broad, wider than width of base of gonostylus; anterior margin not concave. Gonocoxite broadest basally, inner margins parallel, smooth. Gonostylus shorter than gonocoxite, gently curved throughout, tapering to a point. Parameres distally fused, three-quarters length of epandrium, apex cruciform. Gonocoxal plate with well sclerotized base dividing into two lateral, sclerotized arms terminating in 2–4 apical teeth, arranged irregularly; nearly three-quarters length of gonocoxite; inner basal third lightly sclerotized, apical two-thirds membranous; secondarily fused to ventral surface of hypandrium. Cercus weakly sclerotized, situated medially.

Redescription of female. *n* = 2.

Similar to male except as follows: *Length*: 1.8–2.1 mm. *Abdomen*: Tergites 8–9 noticeably more sclerotized than preceding segments; only anterior margin of sternite 7 well sclerotized. *Female terminalia* (Figs. 3.6B, 3.7B): Hypogynial valve slightly shorter than tergite 9; posterior margin with rounded with shallow cleft in ventral view; densely setose. Tergite 9 rectangular in lateral view, wider than tergite 8, posterodorsal corner projecting posteriorly. Sternite 9 (genital fork) connected anteriorly, then dividing into two thin arms, projecting laterally before cleft of hypogynial valve and expanding into rounded sclerite; lateral arms extending slightly beyond hypogynial valve, arms nearly meeting along midline; spectacle-shaped in lateral view, with anterior portion circular and posterior portion trapezoidal. Cercus rounded, projecting posteriorly; bearing numerous setae. Spermathecal ducts not observed; spermathecal pump located in middle of lateral arm, short, thickened.

Immature Stages. Unknown.

Additional Material Examined. CHILE. REGION RM (Santiago): El Canelo,

10.i.1967, 33°37'S 71°35'W, 950m, E.I. Schlinger (1♂, CAS).

REGION IX (Araucanía): Rte. 71, 15.xii.2016, 38°13'20.3"S 71°44'41.1"W, elev. 1047

m, seep, J.K. Moulton & R.J. Pivar (1♂); REGION X (Los Lagos): Isla Chiloé, Ancud,

xii.1926, R&E Shannon (1♂ (USNMENT01115810), 2♀ (USNMENT01115808-09),

USNM).

Note. The authors visited the GPS coordinates listed on the Schlinger specimen and arrived at a site with no visible appropriate habitat near the costal town of San Antonio.

The authors also visited a site in a small town named 'El Canelo' in the Andes, which has

a number of mountain creeks with suitable habitat, although the creek, Estero el Canelo (33°34'31"S 70°26'47"W), was fenced off and uncollectable. This may be the actual El Canelo referred to on the label and perhaps the GPS coordinates were incorrectly recorded.

Distribution. Known from the Andes of central Chile, south to Isla Chiloé along the Chilean Coastal Range (Fig. 3.8).

***Austrothaumalea fredericki* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: "CHILE: Region X (Los/ Lagos) East side Lago/ Llanquihue/ small falls on road (nr PN/ VPR) 41°08.47'S/ 72°35.28'W ~100m/ 2.xii.2008 GW Courtney/ (CH08-30)"; "HOLOTYPE/ *Austrothaumalea/ fredericki/ Pivar*" (CNC).

Recognition. This species is recognized by its cruciate parameres. The lateral, cross-like projections on the paramere are located medially, concealed behind the gonocoxal plate and are much longer than those of *A. chilensis*.

Description of male. *n* = 1.

Length 2.8 mm.

Colouration (Fig. 3.3D). Head dull, dark brown; pronotum, postpronotum, postpronotal lobe and lateral margins of prescutum yellowish-brown; prescutum and mesoscutum shiny, primarily yellow, with pleura yellowish-brown; two lateral brown spots above scutoscuteellar suture; scutellum and mediotergite shiny, yellow; paratergite yellowish-brown; katepisternum mainly yellow, remaining pteropleuron yellowish-brown; halter

brown; legs pale brown, tarsi darker; abdomen brown; terminalia light brown.

Head. Eyes above antennae broadly joined, barely exposing frons above antennae.

Flagellomeres 1–3 expanded, subquadrate; 1 as long as 2 3 combined; flagellomeres 4–10 thin, cylindrical, becoming progressively more elongate. Vertex clothed in golden setae of uniform length, with sparse long, black orbital setae.

Thorax. Pronotal setae long. Mesoscutum without antealar ridge. Scutum clothed dorsally in short setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae. Proepisternal setae present. Pteropleuron bare. Fore- and mid legs with tarsomere 4 bilobed; hind tarsi broken, not observed.

Wing. Wing length: 2.9 mm. Lightly infusate throughout, slightly darker where R_{4+5} meets apex; apex rounded; C and posterior wing margin with fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ without three weakenings or depigmented gaps; $R_1(+R_{2+3})$ with uniseriate row of microtrichia along entire length, remaining veins bare; R flexed into cell br; R_{2+3} crossvein very weak, situated slightly before midpoint of $R_1(+R_{2+3})$; bend in R_{4+5} gentle; R_{4+5} and M_1 running parallel toward margin; M_1 and M_2 straight; M_4 slightly sinuous; CuA with distinct short basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 with slender median sclerite and weakly sclerotized posterior trapezoid, few setae on posterior margin; sternites 3–7 rectangular, weakly sclerotized, setae restricted to posterior third; anterior margin of sternites 5–7 thin, well sclerotized, sternite 7 arched slightly into preceding segment; sternite 8 triangular, lacking setae, anterior margin well sclerotized, arched strongly into preceding sternite.

Male terminalia (Figs. 3.4C, 3.5C). Epandrium broadly triangular from ventral aspect;

apical third slightly tapered; posterior margin broad, irregular; lacking pointed posterolateral processes. Hypandrium broad, equal to width of base of gonostylus; anterior margin not concave. Gonocoxite broadest at basal third, tapered toward apex, inner margin smooth. Gonostylus about two-thirds length of gonocoxite, distal half strongly curved; broadest basally, gradually tapered to slightly pointed apex. Parameres distally fused, slightly more than half-length of epandrium, cruciform medially, projecting ventrally. Gonocoxal plate U-shaped, short, about one-third length of gonocoxite, well sclerotized; lateral arms diverging posteriorly into projections, distal inner margins weakly toothed; membrane extended to middle of paramere, with medial invagination; basally not fused to hypandrium. Cercus weakly sclerotized, situated medially.

Female. Unknown.

Immature Stages. Unknown.

Additional Material Examined. Known only from the holotype.

Distribution. Known only from the type locality in the Southern Andes (Fig. 3.8).

Etymology. *Austrothaumalea fredericki* is named in honour of British dipterist Frederick W. Edwards, who was a primary author of the Natural History Museum's (London) series of books entitled "Diptera of Patagonia and South Chile". Specifically, Edwards was heavily involved in writing the lower Diptera chapters and laid the groundwork for future studies of many families, including Thaumaleidae.

***Austrothaumalea setipennis* Edwards**

Austrothaumalea setipennis Edwards, 1930: 113.

Stone, 1966: 1 (catalogue); Arnaud, 1977: 284 (distribution); Theischinger, 1986: 316 (phylogenetic discussion).

Oterere setipennis (Edwards): McLellan, 1988: 575.

Sinclair, 2008b: 11, 12 (species groups).

Austrothaumalea setipennis Edwards: Pivar, 2018: Chapter 4 status revised.

Type material examined. Holotype: ♂, minuten pinned with abdomen mounted in resin, labelled: “Bariloche./ 29.xi.1926.”; “Austrothaumalea/ setipennis Edw./ F.W. Edwards/ det. 1930.”; “Argentina:/ Terr. Rio Negro./ F. & M. Edwards./ B.M. 1927 – 63.”; “HOLO-/ TYPE”; “NHMUK010210691” (BMNH).

Recognition. This species is recognized by the presence of macrotrichia on wing vein M₁ and the rod-shaped parameres.

Redescription of male. *n* = 4.

Length 2.5–2.7 mm.

Colouration (Fig. 3.3E). Head dull, brown; pronotum, postpronotum, postpronotal lobe and lateral margins of prescutum brown; prescutum and mesoscutum shiny, primarily yellow, with three yellowish-brown, longitudinal vittae ending in postscutal spot; pleura yellowish-brown; two lateral brown spots above scutoscutellar suture; scutellum yellowish-brown, sometimes with dark speckles; mediotergite shiny, mainly yellow, slightly darker anteriorly; katapisternum yellow; paratergite dark brown; remaining

pteropleuron yellowish-brown to brown; posterior basalare yellow; halter yellowish; legs yellow, tarsi darker; abdomen brown, hind margins of tergites whitish; terminalia pale brown.

Head. Eyes above antennae broadly joined, slightly exposing frons above antennae.

Flagellomeres 1–3 expanded, subquadrate; 1 as long as 2 and 3 combined; flagellomeres 4–10 thin, cylindrical, becoming progressively more elongate. Vertex clothed in golden setae of uniform length, with sparse long, black orbital setae.

Thorax. Pronotal setae long. Mesoscutum without antealar ridge. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black.

Scutellum with row of long, black marginal setae. Proepisternal setae present.

Pteropleuron bare. Fore- and mid legs with tarsi simple, hind leg with tarsomere 4 bilobed.

Wing. Wing length: 2.8–3.3 mm. Lightly infusate throughout, slightly darker where R_{4+5} meets apex; apex rounded; C and posterior wing margin with fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ without weakenings or depigmented gaps; $R_1(+R_{2+3})$ and M_1 with uniseriate row of microtrichia along entire length, remaining veins bare; R flexed into cell br; R_{2+3} crossvein strong, situated toward apex of $R_1(+R_{2+3})$; bend in R_{4+5} well defined; R_{4+5} and M_1 running parallel toward margin; M_1 and M_2 straight; M_4 slightly sinuous; CuA with distinct short basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 with slender median sclerite and lightly sclerotized posterior trapezoid, few setae on posterior margin; sternites 3–7 rectangular, weakly sclerotized, setae restricted to posterior half; sternite 8 crescent-shaped, with a few setae, weakly sclerotized, arched slightly into preceding

sternite.

Male terminalia (Figs. 3.4D, 3.5D). Epandrium broadly triangular from ventral aspect; apical third slightly tapered; posterior margin broad and rounded; lacking pointed posterolateral processes. Hypandrium broad, nearly subequal to width of base of gonostylus; narrowest at midpoint, anterior margin not concave. Gonocoxite broadest basally, tapered at midpoint toward apex, inner margin smooth. Gonostylus nearly same length as gonocoxite, anterior three-quarters curved inward; nearly same width throughout, gently tapered to distinct tooth at apex. Parameres distally fused, three-quarters length of epandrium, rod-shaped, broadest medially; slowly tapering toward apex, projecting ventrally. Gonocoxal plate formed of two lateral, sclerotized arms terminating in pectinate, saw-like structure; short, about half-length of gonocoxite; plate broad basally; basally not fused to hypandrium. Cercus weakly sclerotized, situated medially.

Description of female. $n = 2$.

Similar to male except as follows: *Length*: 2.5–2.8 mm. *Abdomen*: Tergites 8–9 noticeably more sclerotized than preceding segments; only anterior margin of sternite 7 well sclerotized. *Female terminalia* (Figs. 3.6C, 3.7C): Hypogynial valve not projecting beyond tergite 9; posterior margin rounded with cleft in ventral view; densely setose. Tergite 9 rectangular in lateral view, wider than tergite 8, lacking lateral projections. Sternite 9 (genital fork) connected anteriorly, then divided into two thick arms ending before cleft of hypogynial valve; lateral arms extended beyond hypogynial valve, nearly meeting along midline, triangular in lateral view. Cercus rounded, projecting slightly posteroventrally; bearing numerous setae. Three spermathecal ducts arising near cleft of

hypogynial valve, only two terminating in lateral, irregularly rounded, weakly sclerotized spermathecal receptacle.

Immature Stages. Unknown.

Additional Material Examined. CHILE. REGION IX (Araucanía): Rte. 71, 15.xii.2016, 38°13'20.3"S 71°44'41.1"W, elev. 1047 m, seep, J.K. Moulton & R.J. Pivar (1♀); REGION XI (Aysén): Coihaique-Aysén Rd., 6.ii.1985, J.A. Downes (3♂, 1♀, CNC).

Distribution. Known from the Andes of southern Chile, this species holds the southernmost record of thaumaleids in South America (Fig. 3.8).

***Austrothaumalea spatulata* Edwards**

Austrothaumalea spatulata Schmid, 1970: 501.

Arnaud, 1977: 284 (distribution); Cooper, 1991: 79 (CNC type catalogue);

Sinclair, 1992: 492 (male terminalia studied); Theischinger, 1986: 316

(phylogenetic discussion).

Austrothaumalea spatula Schmid: Sinclair 2008b: 12 (*lapis calami*, species groups).

Type material examined. Holotype: ♂, minuten pinned, with abdomen mounted in resin below specimen, labelled: “Estero la Jaula/ Curico, CHILE/ I.1964, L. Pena/ Nothofagus”; “HOLOTYPE/ CNCNo. 11,305/ Thaumalea spatulata Schm. [red label]”; “CNC/ 803775” (CNC). **Allotype:** ♀, same label data as holotype, except, CNC 803775 (CNC). **Paratypes:** CHILE: REGION VI (O’Higgins): Bosque de los Conservadores Graneros, 1–4.iii.62, 1100m, Pena, CNC #11.035 (1♂, 1♀, CNC).

Recognition. This species is recognized by the quadrate apex of its parameres and the quadrate posterior margin of the gonocoxal plate. It lacks the posterior epandrial projections found in *A. apicalis*.

Redescription of male. *n* = 8.

Length 1.8–2.2 mm.

Colouration (Fig. 3.3B). Head dull, dark brown; pronotum, postpronotum, postpronotal lobe and lateral margins of prescutum brown; prescutum and mesoscutum shiny, primarily yellow, with pleura and posterior margin of mesoscutum brown with a posterior brown rectangle, anterior corners of rectangle extended into two longitudinal brown vittae connecting, or nearly so, to posterior vittae extending from hind margins or prescutal dark patches; scutellum shiny, dorsal aspect brown with lateral yellowish brown patches, ventral aspect yellow; mediotergite shiny, brown; katepisternum yellow near coxa then turning brown, remaining pteropleuron yellowish-brown to brown; posterior basalare yellowish-brown; halter brown; legs pale brown, tarsi darker; abdomen dark brown, hind margins of tergites whitish; terminalia dark brown, gonostyli light brown.

Head. Eyes above antennae broadly joined, barely exposing frons above antennae.

Flagellomeres 1–3 expanded, subquadrate; 1 as long as 2 and 3 combined; flagellomeres 4–10 thin, cylindrical, becoming progressively more elongate. Vertex clothed in golden setae of uniform length, with sparse long, black orbital setae.

Thorax. Pronotal setae long. Mesoscutum without antealar ridge. Scutum clothed dorsally in short, golden setulae; notopleural, supra-alar and postscutum setae long, black.

Scutellum with row of long, black, marginal setae. Proepisternal setae present.

Pteropleuron bare. Fore- and mid legs with tarsi simple, hind leg with tarsomere 4

bilobed.

Wing (Fig. 3.2A). Wing length: 2.8–3.3 mm. Lightly infusate throughout, slightly darker where R_{4+5} meets apex; apex rounded; C and posterior wing margin with fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ without weakenings or depigmented gaps; $R_1(+R_{2+3})$ with uniseriate row of microtrichia along entire length, remaining veins bare; R flexed into cell br; R_{2+3} crossvein very weak or absent, at most a faint infuscation, situated slightly beyond midpoint of $R_1(+R_{2+3})$; bend in R_{4+5} well defined; R_{4+5} and M_1 running parallel toward margin; M_1 and M_2 straight; M_4 slightly sinuous; CuA with short basal appendage, may be distinct or indistinct.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 with slender median sclerite and lightly sclerotized posterior rectangle, few setae on posterior margin; sternites 3–7 rectangular, weakly sclerotized, setae restricted to posterior half and laterad; anterior margin of sternites 5–7 thin, well sclerotized; sternite 8 crescent-shaped, lacking setae, anterior margin well sclerotized, arched slightly into preceding sternite.

Male terminalia (Figs. 3.4E, 3.5E). Epandrium broadly triangular from ventral aspect; apical third slightly tapered; posterior margin broad and rounded; lacking pointed posterolateral processes. Hypandrium broad, nearly subequal to width of base of gonostylus; anterior margin slightly concave. Gonocoxite broadest medially, tapered toward apex. Gonostylus about two-thirds length of gonocoxite, curved gently throughout; broadest basally, gradually tapered with small tooth at apex. Parameres distally fused, slightly more than half-length of epandrium, spatula-like, broadest apically. Gonocoxal plate rectangular, short, about half-length of gonocoxite; broad basally; apical margin truncate; basally not fused to hypandrium. Cercus weakly

sclerotized, situated medially.

Description of female. *n* = 2.

Similar to male except as follows: *Length*: 2.5–2.7 mm. *Abdomen*: Tergites 7–9 noticeably more sclerotized than preceding tergites; only anterior margin of sternite 7 well sclerotized. *Female terminalia* (Figs. 3.6D, 3.7D): Hypogynial valve projecting slightly beyond tergite 9; posterior margin with rounded cleft in ventral view, forming two truncate lobes; densely setose. Tergite 9 subquadrate in lateral view, wider than tergite 8, lacking lateral projections. Sternite 9 (genital fork) slender, weakly sclerotized, with two rod-shaped sclerites anteriorly and two triangular sclerites next to hypogynial valve; lateral arms extending past hypogynial valve, arms closely approximated along midline, thin anteriorly, distal half expanded. Hypoproct thin, divided into pair of sclerites. Cercus rounded, projected posteriorly; bearing numerous setae. Spermathecal ducts not observed.

Immature Stages. Unknown.

Additional Material Examined. CHILE. REGION VI (O'Higgins): Rte. I-45, 16.xii.2016, 34°46'29.2"S 70°40'03.9"W, elev. 827 m, stream, J.K. Moulton & R.J. Pivar (1♂); REGION VII (Maule): Los Queñes, Rte. J-25, 6.xii.2016, 34°59'48.8"S 70°48'37.0"W, elev. 684 m, seep, J.K. Moulton & R.J. Pivar (4♂, 1♀*); REGION VIII (Bío Bío): Rte. Q-689, 8.xii.2016, 37°54'55.6"S 71°35'43.2"W, elev. 552 m, cascading creek, J.K. Moulton & R.J. Pivar (1♂, 1♀*).

Distribution. Known from the Andes of central Chile (Fig. 3.8).

Note. The O'Higgins specimen collected by JKM and RJP differs somewhat in appearance from the other specimens in that the posterior margin is slightly concave.

Molecular fingerprinting matches that of other *A. spatulata* specimens. This is the northern-most locality for this species and likely indicates population variation.

Genus *Niphta* Theischinger

Niphta Theischinger, 1986: 314. Type-species: *Niphta bickeli* Theischinger (original designation).

The genus *Niphta* is characterized as follows: presence of a distinct antealar ridge; proepisternal setae absent; microtrichia of first vein ($R_1(+R_{2+3})$) confined to base near humeral crossvein; R_{2+3} crossvein situated closer to apex of $R_1(+R_{2+3})$ than to origin of R_{4+5} ; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps; R_{4+5} often with arch not strongly produced; basal appendage of CuA absent; gonocoxites broad, not much longer than wide; hypandrium absent; gonocoxal plate extending posterodorsally forming a medial process; parameres fused medially, derived from gonocoxal plate complex.

Description

Adult. Eye bridge broad, comprising more than 5 facets. Scutum clothed in both short and long setae; scutellum with row of marginal setae. Supralar region produced into distinct antealar ridge; ridge with bristles. Proepisternal setae absent. Wing tip narrowly rounded; membrane lacking macrotrichia; C with sparse macrotrichia, more so on remaining wing margin; Sc incomplete; microtrichia of first vein ($R_1(+R_{2+3})$) confined to base near humeral crossvein; R_{2+3} crossvein situated closer to origin of R_{4+5} than to apex of $R_1(+R_{2+3})$; R_1 ; $R_1(+R_{2+3})$ with three weakenings or depigmented gaps; R_{4+5} often with

arch not strongly produced; R₄₊₅ and M₁ running parallel toward margin; M₁ straight; M₂ with gentle bend in apical third; M₄ with slight bend; CuA angulate near base; basal appendage of CuA absent. *Male Terminalia*: Hyandrium absent. Gonocoxites broad, not much longer than wide; gonocoxal plate extended posterodorsally, forming medial process, acting as aedeagal guide; parameres fused medially, emerging from gonocoxal plate complex.

Distribution. Australia and Chile.

Key to adult males of South American *Niphta*

See Figure 3.2 for micrograph of wing, and Figures 3.12C/ 3.15B (ventral) and 3.13A/ 3.16B (lateral) for genitalic illustrations with pertinent structures labelled.

1. Gonocoxite subquadrate or conical, with posteromedial projection broad, rounded, projecting posteriorly (Fig. 3.12); parameres fused medially to apex (Fig. 3.12); typically dark in colouration (Fig. 3.9) ... 2 (*N. halteralis* group)

- Gonocoxite oblong, with posteromedial projection narrow, pointed, projecting medially (Figs. 3.15, 3.17); parameres fused medially then separating into two raptorial-like apical arms (Figs. 3.15, 3.17); typically light in colouration (Figs. 3.10, 3.11) ... 5 (*N. nudipennis* group)

2. Paramere with hooked apex; gonostylus straight (Figs. 3.12C, 3.13C) ... *N. halteralis* (Edwards)

- Paramere without hooked apex; gonostylus arched outwards ... 3

3. Gonostylus bifurcate apically; paramere with sudden, strongly tapered, off-centred needle-like apex (Figs. 3.12A, 3.13A) ... *N. acus* Pivar **sp.n.**

- Gonostylus tapered to single apex; paramere evenly tapered throughout ... 4
- 4. Paramere, in lateral view, two-filamented; dorsal filament, at most, barely reaching posterior margin of epandrium; ventral filament not extending beyond apex of gonostylus, not easily visible (Figs. 3.12D, 3.13D) ... *N. mapuche* Pivar **sp.n.**
- Paramere, in lateral view, three-filamented; dorsal filament extending beyond posterior margin of epandrium; paired ventral filaments extending beyond apex of gonostylus; both ventral filaments easily visible (Figs. 3.12B, 3.13B) ... *N. downesi* Pivar **sp.n.**
- 5. Gonostylus cheliform or bearing a finger-like projection (Figs. 3.15B,C, 3.17) ... 6
- Gonostylus not cheliform, without projections; broad at base, tapered to pointed apex (Figs. 3.15A, 3.16A) ... *N. daniellae* Pivar **sp.n.**
- 6. Gonostylus with finger-like projection (Fig. 3.15B,C) ... 7
- Gonostylus cheliform, resembling that of a crab (Fig. 3.17) ... 9
- 7. Gonostylus with projection broad, tapered slightly at apex, without bend (Figs. 3.15B, 3.16B) ... *N. eurydactylus* Pivar **sp.n.**
- Gonostylus with projection narrow throughout, bent at midpoint (Figs. 3.15C, 3.16C) ... *N. nudipennis* (Edwards)
- 8. Gonostylus with posterior apex bifurcate (Figs. 3.17A,B) ... 9
- Gonostylus with posterior apex bearing single apex (Figs. 3.17C,D) ... 10
- 9. Gonocoxite with two projections, anterior projection bifurcate (Figs. 3.17A, 3.18A); body brown to dark brown in colour (Figs. 3.10, 3.11) ... *N. moultoni* Pivar **sp.n.**
- Gonocoxite with three separate projections (Figs. 3.17B, 3.18B); body yellow in colour (Figs. 3.10, 3.11) ... *N. courtneyi* **sp.n.**
- 10. Gonocoxite with two projections, anterior projection long and bifurcate, posterior

projection small, tooth-like (Figs. 3.17C, 3.18C); body yellowish-brown in colour (Figs. 3.10 & 3.11) ... *N. sinclairi* Pivar **sp.n.**

- Gonocoxite with three projections, two anterior (one small and inconspicuous at base of large one); posterior projection slender, about as long as anterior large projection (Figs. 3.17D, 3.18D); body dark brown in colour (Figs. 3.10, 3.11) ... *N. brunnea* Pivar **sp.n.**

Key to adult female species groups of South American *Niphta*

1. Sternite 8 with distinct projection between hypogynial valves (Figs. 3.19, 3.20)... *N. nudipennis* group

- Sternite 8 without distinct projection between hypogynial valves *N. halteralis* group

Species diagnoses and descriptions

The *Ni. halteralis* group

Niphta acus* Pivar **sp.n.*

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: “CHILE: Region VIII (Bío Bío)/ Rte. Q-61, Estero Agua/ Blanca, 8.xii.2016/ 37°46'30.8"S 71°42'03.9"W/ elev. 552 m, vegetation near/ splash zones, J.K. Moulton &/ R.J. Pivar”; “HOLOTYPE/ *Niphta/ acus/* Pivar” (CNC). **Allotype:** ♀, same label data as holotype (CNC). **Paratypes:** CHILE. REGION RM (Santiago): Quebrada el

Cinco Mil, 17.xii.2016, 33°31'30.4"S 70°13'52.6"W, elev. 1308 m, creek, J.K. Moulton & R.J. Pivar (3♀*); REGION V (Valparaíso): Rte. 60, 18.xii.2016, 32°54'31.3"S 70°18'21.5"W, elev. 1423 m, creek, J.K. Moulton & R.J. Pivar (2♂); REGION VIII (Bío Bío): Rte. N-55, 16.xii.2016, 36°55'02.7"S 71°25'49.6"W, elev. 1449 m, roadside seep, J.K. Moulton & R.J. Pivar (25♂); same label data as previous except, collected from rockface (8 larvae*, 5 pupae*, 8 pupal exuviae); Rte. Q-61, Estero Agua Blanca, 8.xii.2016, 37°46'30.8"S 71°42'03.9"W, elev. 552 m, vegetation near splash zones, J.K. Moulton & R.J. Pivar (41♂, 10♀*, 1 larva*); Rte. Q-61, 8.xii.2016, 37°48'34.7"S 71°40'30.0"W, elev. 390 m, roadside seep, J.K. Moulton & R.J. Pivar (2♂, 1♀*).

Recognition. This species is recognized by the bifurcated apex of the gonostylus and the strongly apically tapered parameres , giving the appearance of a needle-like tip.

Description of male. *n* = 71.

Length 1.6–2.5 mm.

Colouration (Fig. 3.9A). Head dull, blackish-brown; pronotum and postpronotum dark brown; postpronotal lobe brown with dark brown markings, light brown around anterior spiracle; prescutum, mesoscutum and pleura shiny, blackish-brown; scutellum and mediotergite shiny, blackish-brown; katapisternum dark brown with blackish-brown markings, remaining pteropleuron mainly blackish-brown with dispersed markings of brown to light brown; base of halter creamy black, knob creamy yellow; legs greyish-brown, apex of tarsi darker; abdomen blackish-brown; terminalia concolourous with abdomen.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 3–5 strong setae. Flagellomeres 1–3 subquadrate, 1 expanded; 3x as wide as

next segment, equal to length of 2 and 3 combined; flagellomeres 4–10 cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing three pronounced setae.

Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae. Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 1.8–2.5 mm. Dark, infusate throughout, apex somewhat narrowed; C fringed in small setulae, with few microtrichia scattered throughout; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely approximated, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated in basal third of $R_1(+R_{2+3})$; bend in R_{4+5} gentle; R_{4+5} and M_1 running parallel toward margin; M_1 straight; M_2 with gentle bend in apical third; M_4 with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, few setae restricted to laterad on posterior third and medially beneath sclerite; sternites 3–7 rectangular, lacking distinct sclerites, setae restricted to posterior two-thirds; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, lacking setae.

Male terminalia (Figs. 3.12A, 3.13A). Epandrium quadrate in ventral view, posterior margin rounded with large, medial indentation; long, extending well beyond gonostyli;

without lobes or projections. Hypandrium absent. Gonocoxites conical, one-third longer than wide, anterior margin rounded, expanded dorsally behind gonocoxal plate, nearly meeting medially, extending anteriorly toward sternite 8; posterior inner margin produced into rounded projection, outer margin not notched; inner margin densely setose.

Gonostylus short, three-quarters length of cercus, strongly curved laterally throughout; widest at base, tapered toward bifurcated apex; outer margin bearing laterally directed setae. Parameres fused at gonocoxal apodeme, widest at point of fusion; extended beyond cerci; forming canal-like structure, flattened apically, tapered to off-centre sharp point (occasionally specimens with broken apex). Gonocoxal plate well sclerotized; tongue-like plate extended anteroventrally; hollow medially, pitcher plant-like; gonocoxal apodeme with secondary structure comprising three arms fused medially, running along interior of paramere canal, flaring into 3–4 filaments, projected ventrally at apex, with pair of lateral flanges near point of fusion. Cercus large, prominent; subquadrate; projected posteriorly; situated within epandrial indentation.

Description of female. *n* = 15.

Similar to male except as follows: *Abdomen*: Tergite 9 noticeably more sclerotized than preceding tergites; sternite 8 well-sclerotized. *Female terminalia* (Figs. 3.14A,B).

Hypogynial valve not projecting beyond tergite 9; posterior margin deeply emarginate in ventral view, nearly dividing sternite in half, forming two subtriangular lobes; lobes densely setose. Tergite 9 subtriangular in lateral view, three times as wide as tergite 8, lacking lateral projections; posterior margin heavily sclerotized at base of cerci. Sternite 9 (genital fork) slender, Y-shaped anteriorly and posteriorly; lateral arms extending slightly beyond hypogynial valve, diverging toward apex. Hypoproct sclerotized, narrow. Cercus

rounded, projecting posteroventrally; bearing numerous setae.

Description of pupa. *n* = 8 (Figs. 3.21C, 3.22C, 3.23C, 3.33E).

Length 3.0–4.0 mm.

Colouration. Brown; with black spot above eyes in developing adult.

Head. Maxillary sheath short, posteromedially directed; gently tapered toward truncate apex; apices of palpi separated medially. Three short, slender setae above black spot over eye.

Thorax. Width nearly subequal to abdomen at widest point. Foreleg sheath projecting straight, slightly longer than wing sheaths, reaching posterior margin of sternite 2; anterior half of midleg visible anterior to wing sheath, then hidden behind foreleg, slightly shorter than foreleg; hind leg concealed behind wing sheath, only apex visible between apex of foreleg and wing sheath, shorter than foreleg. Wing sheaths not reaching posterior margin of abdominal sternite 2; large tubercle at base bearing pair of short, slender setae. Respiratory organ short and squat, much shorter than maxillary sheath, broadest subapically; bulbous; spiracular openings encircling apex; stalk wide, emerging from small tubercle. Tubercle situated posterodorsally to respiratory organ, rounded, projecting slightly laterally; apex nearly touching or touching respiratory organ. Tubercle situated posterolaterally to respiratory organ crenulate, projecting slightly anteriorly. Ridge located anteroventrally to respiratory organ with single, thin, midlateral seta; mesothorax with group of four short, slender dorsocentral setae near ridge; single seta on humeral lobe.

Abdomen. Subcylindrical, strongly tapered at caudal segment. Spiracles weakly developed, not projecting or distinctly visible. Tergites 1–8 rectangular, without ridges;

bearing pair of slender lateral setae above lateral margins and pair of slender dorsolateral setae. Tergite 9 rounded, posterior with dorsolateral ridges bearing pair of lateral setae and hind margin emarginated; projection pointing posteriorly in lateral view. Sternites 3–8 rectangular, without row of faint setulae along anterior margin; lateral margins crenulate, bearing few thin, short setae. Sternites 3 and 4 bearing pair of small lateral adhesive structures, sternite 5 bearing pair of large lateral adhesive structures on lateral margin. Caudal sternite subquadrate, with pair of posteriorly projecting medial lobes; posterior margin with pair of medial ridges, curving dorsally forming small, dorsally projecting tubercle in lateral view; without distinct caudal hooks.

Description of larva. $n = 8$ (Figs. 3.24C, 3.25C, 3.26C, 3.27A,B, 3.31, 3.33C–E).

Length of final instar 6.2–6.7 mm. *Colouration.* Head capsule usually black or dark brown, may be black with light brown markings. Body mottled with various shades of grey and brown. *Head capsule* (Figs. 3.27 A,B). Two large, circular eye spots, elevated on tubercle; antenna on largest tubercle, with three finger-like processes; with five pairs of tubercles outside of ecdysial lines (not including antennal and ocular tubercle), two outermost tubercles bifurcate; single tubercle between ecdysial line, bi- or trifurcate; 15 pairs of unbranched setae; five pairs of sensory pits (13, 14, 18, 20, 21), sensory pit 13 above antennal tubercle. *Thorax.* Prothorax with pair of anterodorsolateral protuberances bare; anterolateral protuberances with one long and two short setae; spiracular protuberance bearing two protuberances, inner protuberance with pair of setae, outer with single seta; pair of midlateral setae below anterolateral protuberance; three closely approximated setae near base of prothoracic leg (Keilin's organ). Mesothorax and metathorax with pair of dorsolateral protuberances bearing pair of closely approximated

setae, one thickened, one slender; mesothorax with additional seta beneath protuberance; lateral protuberance on both segments bearing four setae; one long seta slightly ventral to lateral protuberance; three mid-ventrolateral setae directed ventrally. Prothorax bearing proleg, posterior half with rectangular adhesive structure; meso- and metathoracic sternites with rectangular adhesive structure (Fig. 3.31). *Abdomen*. Abdominal sternites 1–7 modified into circular, suction cup-like adhesive structure; sternite 8 with quadrate adhesive structure, extending over sternite 9; sternite 9 smooth, bearing anal proleg. Tergites 1–7 with single anterolateral protuberance on each side with a single seta, and pair of posterodorsolateral protuberances, each bearing two closely approximated short, thin setae; lateral adhesive structure swelling bearing four setae, two lateral, two basal; additional single seta located anterior to lateral swelling. Segment 8 with dorsolateral protuberance on either side of posterior spiracular plate, each bearing pair of small setae; lateral protuberance with three setae; single short ventrolateral seta; ventral surface bearing pair of setae. Posterior spiracular plate with sclerite encircling procerci; procercus shorter than length of spiracular plate, bearing four setae, two thick, two slender; without cone-like protuberance on either side of cerci. Terminal segment with anterior dorsal protuberance bare; pair of posterior lateral protuberances with pair of setae; five lateral setae; two pairs of long setae on posterior margin, above pair of anal papillae; ventral sternite bearing single pair of setae.

Additional Material Examined. Known only from the type series.

Distribution. Known from the south-central Andes of Chile (Fig. 3.32A); this species includes the northern-most record for *Niphta* in South America.

Bionomics. This species was collected at higher elevations than any other species in this

publication. Larvae and pupae have ventral adhesive structures and were collected only from rocky substrates. All immatures were collected from the margin of waterfalls, where water flow was quite slow.

Etymology. *Niphtha acus* is from the Latin *acu* (needle, pin) in allusion to the needle-like tip of the parameres.

***Niphtha downesi* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: “CHILE: Region XIV (Los Ríos)/ Rte. T-85, 13.xii.2016/ 40°19'58.6"S 72°16'56.1"W/ elev. 95m, roadcut seep, J.K./ Moulton & R.J. Pivar”; “HOLOTYPE/ *Niphtha/ downesi/* Pivar” (CNC). **Paratypes:** CHILE. REGION X (Los Lagos): Ensenada, nr. Baños de Petrohué, 12.i.1985, J.A. Downes (1♂, CNC); REGION XIV (Los Ríos): same label data as holotype (1♂); Rte. T-85, 13.xii.2016, 40°19'58.7"S 72°16'54.8"W, elev. 145 m, foliage around waterfalls, J.K. Moulton & R.J. Pivar (12 ♂).

Recognition. This species is recognized by both filaments of the paramere and the aedeagal guide being easily visible in lateral view, giving it a tri-filamentous appearance. Also, the bend in the gonostylus is slightly stronger than that of *Ni. mapuche*.

Description of male. *n* = 3.

Length 1.9–2.4 mm.

Colouration (Fig. 3.9B). Head dull, black; pronotum and postpronotum black; postpronotal lobe, prescutum and mesoscutum shiny, blackish-brown, lateral face of postpronotal lobe brown, creamy around anterior spiracle; scutellum blackish-brown

dorsally, light brown ventrally; mediotergite shiny, blackish-brown; pteropleuron mainly brown with dispersed markings of black and dark brown; base of halter blackish, knob creamy yellow; legs greyish-brown, apex of tarsi darker; abdomen blackish-brown; terminalia concolourous with abdomen.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with two strong setae. Flagellomeres 1–3 subquadrate, expanded; 1.5× as wide as next segment, as long as 2 and 3 combined; flagellomeres 4–10 cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing single pronounced, medial seta flanked by two smaller setae. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long black marginal setae. Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 2.2–2.4 mm. Dark, infusate throughout, apex somewhat narrowed; C and posterior wing margin with fringe of microtrichia; Sc incomplete; R₁ and R₁(+R₂₊₃) with three weakenings or depigmented gaps, first slightly beyond R₂₊₃, second and third closely approximated, near C; microtrichia of R₁(+R₂₊₃) confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R₂₊₃ distinct, situated in basal third of R₁(+R₂₊₃) (sometimes absent); bend in R₄₊₅ gentle; R₄₊₅ and M₁ running parallel toward margin; M₁ straight; M₂ with gentle bend in apical third; M₄ with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender

median sclerite, few setae restricted to laterad on posterior third and medially below sclerite; sternites 3–7 rectangular, lacking distinct sclerites, setae restricted to posterior two-thirds and laterad; sternite 8 strongly reduced, anterior margin well sclerotized, strongly arched into preceding segment, lacking setae.

Male terminalia (Figs. 3.12B, 3.13B). Epandrium quadrate in ventral view, posterior margin rounded with large, medial indentation; long, extending well beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites subquadrate, one-third longer than wide, anterior margin rounded, expanded dorsally behind gonocoxal plate, nearly meeting medially, extended anteriorly toward sternite 8; posterior inner margin produced into pointed projection, outer margin notched; inner margin densely setose. Gonostylus short, slightly more than half-length of cercus, strongly curved lateroventrally throughout; apex pointed; outer margin bearing laterally directed setae. Parameres distally fused, widest medially, as wide as gonocoxite; extended past posterior margin of epandrium; lateral margins curved ventrally, canal-like medially in ventral view, then tapered rapidly to filamentous, pointed apex projected posterodorsally, extended beyond epandrium; medial structure cradled within canal, protruded ventrally; in lateral view ending at halfway point of longest filament. Gonocoxal plate broad, well sclerotized; tongue-like plate extended anteroventrally; gonocoxal apodeme with secondary structure comprising single filament, running along interior of paramere canal, projected ventrally at apex. Cercus large, prominent; trapezoidal; projected posteroventrally; situated within epandrial indentation.

Female. Unknown.

Immature Stages. Unknown.

Additional Material Examined. Known only from the type series.

Distribution. Known from the foothills of the southern Andes in Chile (Fig. 3.32A).

Etymology. *Niphta downesi* is named in honour of veterinary and medical entomologist J.A. Downes, who collected the first specimen of this species in 1985.

***Niphta halteralis* (Edwards)**

Austrothaumalea halteralis Edwards, 1930: 114.

Stuardo, 1946: 42 (catalogue); Stone, 1966: 1 (catalogue); Arnaud, 1977: 284 (distribution).

Niphta halteris (Edwards): Theischinger 1986: 316 (*lapis calami*, new combination); McLellan, 1988: 563 (moved to genus *Niphta* by Theischinger (1986)).

Type material examined. Holotype: ♂, minuten pinned with abdomen mounted in resin, labelled: “Casa Pangué./ 4–10.xii.1926.”; “Austrothaumalea/ halteralis Edw./ F.W. Edwards/ det. 1930.”; “S. Chile:/ Llanquihue prov./ F. & M. Edwards./ B.M. 1927 – 63.”; “HOLO-/ TYPE”; “NHMUK010210689” (BMNH).

Recognition. This species is recognized by its distinct hook-tipped paramere.

Redescription of male. *n* = 44.

Length 1.3–2.0 mm.

Colouration (Fig. 3.9C). Head dull, dark brown to black; pronotum and postpronotum dark brown; postpronotal lobe, prescutum and mesoscutum shiny, brown, middle of postscutum light brown; scutellum and mediotergite shiny, light brown; pteropleuron

light brown with dispersed dark brown markings; base of halter light brown, knob creamy yellow; legs pale brown, tarsi darker; abdomen dark brown, hind margins of tergites whitish; terminalia light brown.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with two strong setae. Flagellomeres 1–3 subquadrate, slightly expanded; 0.25× as wide as next segment, slightly shorter than 2 and 3 combined; flagellomeres 4–10 cylindrical, becoming progressively more thin and elongate. Vertex clothed in golden setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing single pronounced medial seta flanked by two smaller setae. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black, marginal setae. Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 2.0–2.9 mm. Infusate throughout, apex somewhat narrowed; C and posterior wing margin with fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely approximated, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated slightly before middle of $R_1(+R_{2+3})$; bend in R_{4+5} gentle; R_{4+5} and M_1 running parallel toward margin; M_1 and M_2 straight; M_4 with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, few setae restricted to laterad on posterior third and medially below sclerite; sternites 3–7 rectangular, lacking distinct sclerites, setae restricted to posterior

half; sternite 8 strongly reduced, anterior margin well sclerotized, strongly arched into preceding segment, lacking setae.

Male terminalia (Figs. 3.12C, 3.13C). Epandrium quadrate in ventral view, posterior margin rounded with broad, medial indentation; long, extended well beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites subquadrate, one-third longer than wide; anterior margin rounded, expanded dorsally behind gonocoxal plate, nearly meeting medially, extended anteriorly toward sternite 8; posterior inner margin produced into pointed projection; inner margin densely setose; outer margin without notch. Gonostylus short, about as long as cercus, widest at base, strongly tapered along outer margin with rounded apex; apex slightly flanged outward, appearing pointed in lateral view; distal half bearing setae along outer apical margin, a few setae on inner margin. Parameres fused at gonocoxal apodeme, widest medially, as wide as gonocoxite; extended past posterior margin of epandrium; lateral margins curved ventrally forming canal-like structure with median ‘wings’ projecting posteriorly, margins feathered; apex with pointed hook-like projection, recurved and projected anteriorly to left. Gonocoxal plate well sclerotized; tongue-like plate extended anteroventrally; gonocoxal apodeme with secondary bridge connected with base of paramere. Cercus large, prominent; trapezoidal; projected posteroventrally; situated within epandrial indentation.

Description of female. *n* = 6.

Similar to male except as follows: *Abdomen*. Tergite 9 noticeably more sclerotized than preceding tergites; sternite 8 well sclerotized. *Female terminalia* (Figs. 3.14 C, D).

Hypogynial valve not projected beyond tergite 9; posterior margin deeply emarginate in ventral view, forming two triangular lobes; lobes densely setose. Tergite 9 oblong in

lateral view, twice as wide as tergite 8, lacking lateral projections. Sternite 9 (genital fork) slender, T-shaped; lateral arms not extended beyond hypogynial valve, Y-shaped; with ventral sclerite in hypogynial valve cleft. Hypoproct sclerotized, narrow. Cercus rounded, projected posteroventrally; bearing numerous setae. Two sclerotized, tube-like structures dorsal to base of lateral arms in lateral view, spermathecal pumps perhaps; spermathecal receptacles and ducts not observed.

Immature Stages. Unknown.

Additional Material Examined. CHILE. REGION X (Los Lagos): Camino de Penetracion @ Hwy. 7 sign, 16.xii.2013, 42°07'57.5"S 72°27'45.3"W, seep, sweeping veg., G.R. Curler (1♂, 1♀*); Camino de Penetracion @ km post 125.600, 16.xii.2013, 42°03'33.3"S 72°27'07.4"W, rock seep, G.R. Curler (1♂); Casa Pangué, Llanquihue, xii.1926, R&E Shannon, USNMENT01115811 (1♂, USNM); Ensenada, nr. Baños de Petrohué, 12.i.1985, J.A. Downes (2♂, CNC; 1♂, USNM (USNMENT01115812)); Rte. 215, 12.xii.2016, 40°40'32.4"S 72°17'35.6"W, elev. 252 m, trickle falls, J.K. Moulton & R.J. Pivar (1♂); Rte. U-99, 10.xii.2016, 41°08'09.6"S 72°35'43.3"W, elev. 81 m, roadside falls, J.K. Moulton & R.J. Pivar (15♂; 4♀*); Rte. U-99, 10.xii.2016, 41°08'28.2"S 72°35'16.8"W, elev. 101 m, roadside seeps/creek, J.K. Moulton & R.J. Pivar (11♂, 1♀*); Rte. V-69, 12.xii.2016, 41°26'37.7"S 72°17'42.2"W, elev. 34 m, cascading stream, J.K. Moulton & R.J. Pivar (1♂); Rte. V-69, Puente El Salto, 12.xii.2016, 41°31'29.2"S 72°17'14.6"W, elev. 37 m, splash zone above falls, J.K. Moulton & R.J. Pivar (2♂); REGION XIV (Los Ríos): Antilhue, Rte. T-35, 9.xii.2016, 39°49'09.8"S 72°56'22.6"W, elev. 40 m, roadside creek, J.K. Moulton & R.J. Pivar (3♂); Valdivia, Los Ulmos Rd., 15.i.1985, J.A. Downes, (5♂, CNC).

Distribution. Known from both the Andes and Chilean Coastal Range in southern Chile (Fig. 3.32A).

Bionomics. This species appears restricted predominantly to low elevations in the Valdivian temperate rain forest.

***Niphta mapuche* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: “CHILE: Region IX (Araucanía)/ Rte. S-365, 14.xii.2016/ 38°46'27.0"S 71°36'51.0"W/ elev. 809 m, creek/small falls/ J.K. Moulton & R.J. Pivar”; “HOLOTYPE/ *Niphta/ mapuche/* Pivar” (CNC). **Allotype:** ♀, same label data as holotype (CNC). **Paratypes:** CHILE. REGION VIII (Bío Bío): Rte. Q-61, 8.xii.2016, 37°48'22.8"S 71°40'46.6"W, elev. 379 m, cascading creek, J.K. Moulton & R.J. Pivar (2♂, 1♀*); Rte. Q-689, 8.xii.2016, 37°54'55.6"S 71°35'43.2"W, elev. 552 m, cascading creek, J.K. Moulton & R.J. Pivar (2♀*); REGION IX (Araucanía): nr. Tolhuaca N.P., Rte. 71, 15.xii.2016, 38°13'23.5"S 71°49'07.8"W, elev. 934 m, stream, J.K. Moulton & R.J. Pivar (1♂); Rte. S-365, 14.xii.2016, 38°46'27.0"S 71°36'51.0"W, elev. 809 m, creek/small falls, J.K. Moulton & R.J. Pivar (4♂, 1♀*); Salto El León (spray zone at base), 2.xii.2013, 39°25'10.9"S 71°45'42.3"W, elev. 760 m, madicolous habitats, G.W. Courtney (CH13080) (3♀*, 4 pupal exuviae); same label data as previous except, 3.x.2007 (8 larvae); upper Rio Malleco @ Rd Xing, 25.xi.2013, 38°13'20.0"S 71°44'40.8"W, elev. 1040 m, from riffle rocks, G.W. Courtney (CH13-030) (7 larvae*); REGION X (Los Lagos): Parque Nacional Alerce Andino, culvert falls above Sargazo

GS, 1.xii.2008, 41°30'31.8"S 72°37'13.8"W, elev. 335 m, G.W. Courtney (CH08-23) (4 larvae*); REGION XIV (Los Ríos): Estero Altura Pazas on Cosh-Liq Rd., i.xii.2013, 39°44'43.8"S 71°56'22.14"W, elev. 385 m, on wetted rock, G.W. Courtney (CH13-079) (6 larvae*); Rte. T-29, Puente Altura Pazas, 14.xii.2016, 39°44'43.6"S 71°56'24.4"W, elev. 363 m, cascading creek, J.K. Moulton & R.J. Pivar (2♂, 2♀*).

Recognition. This species is recognized by the paramere being mostly hidden behind the epandrium in lateral view, giving the paramere a two-filament appearance. The gonostylus is less recurved than that of *Ni. downesi*.

Description of male. *n* = 10.

Length 1.4–2.5 mm.

Colouration (Fig. 3.9D). Head dull, black; pronotum and postpronotum brown; postpronotal lobe brown with dark brown markings, light brown surrounding anterior spiracle; prescutum and mesoscutum shiny, blackish-brown, pleura brown; scutellum and mediotergite shiny, blackish-brown; katapisternum brown with blackish-brown margins, remaining pteropleuron mainly blackish-brown with dispersed markings of brown to light brown; base of halter light brown turning black medially, knob creamy yellow; legs ranging from pale brown to blackish brown, apex of tarsi darker; abdomen blackish-brown, posterior margins creamy; terminalia variable in colour from blackish brown to grey.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 3–5 strong setae. Flagellomeres 1–3 subquadrate, expanded, 2× as wide as next segment, as long as 2 and 3 combined; flagellomeres 4–10, cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length,

with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing three pronounced setae.

Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae. Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 1.8–3.1 mm. Dark, infusate throughout, apex somewhat narrowed; C fringed in small setulae, with few microtrichia scattered throughout; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely approximated, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated in basal third of $R_1(+R_{2+3})$; bend in R_{4+5} gentle; R_{4+5} and M_1 running parallel toward margin; M_1 straight; M_2 with gentle bend in apical third; M_4 with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, a few setae restricted to laterad on posterior third; sternites 3–7 rectangular, lacking distinct sclerites, setae restricted to lateral margins and middle third; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, three or fewer setae medially and on lateral margins.

Male terminalia (Figs. 3.12D, 3.13D). Epandrium quadrate in ventral view, posterior margin rounded with broad, medial indentation; long, extended well beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites subquadrate, one-third longer than wide, anterior margin rounded, expanded dorsally behind gonocoxal plate,

nearly meeting medially, extended anteriorly toward sternite 8; posterior inner margin slightly produced into pointed projection, outer margin notched; inner margin densely setose. Gonostylus short, less than half-length of cercus, strongly curved lateroventrally throughout; apex pointed; outer margin bearing laterally directed setae. Parameres distally fused, widest at point of fusion; not reaching posterior margin of epandrium; dividing into two pointed, filamentous projections medially; shorter ventral filament not extended beyond gonostyli, apex directed posteriorly; longer dorsal filament not extended to posterior margin of epandrium, at most slightly beyond base of cercus, apex projected slightly posterodorsally. Gonocoxal plate broad, well sclerotized, tongue-like plate extending anteroventrally; gonocoxal apodeme with secondary structure comprising single short, indistinct filament, running along interior of paramere, ending before apical margin of gonostylus. Cercus large, prominent; trapezoidal; projected posteriorly; situated within epandrial indentation.

Description of female. *n* = 10.

Similar to male except as follows: *Abdomen*. Tergite 9 noticeably more sclerotized than preceding tergites; sternite 8 well sclerotized. *Female terminalia* (Figs. 3.14 E, F). Hypogynial valve not projected beyond tergite 9; posterior margin deeply emarginate in ventral view, forming two triangular lobes; lobes densely setose; lateral margin sclerotized at base. Tergite 9 triangular in lateral view, three times as wide as tergite 8, lacking lateral projections; posterior margin heavily sclerotized at base of cerci. Sternite 9 (genital fork) slender, Y-shaped; lateral arms not extended beyond hypogynial valve, Y-shaped, fused distally forming rounded posterior margin; ventral, lightly sclerotized plate at base of hypogynial valve. Hypoproct sclerotized, narrow. Cercus rounded, projected

posteroventrally; bearing numerous setae. Two sclerotized, tube-like structures dorsal to genital fork, spermathecal pumps perhaps; spermathecal receptacles and ducts not observed.

Description of pupa. *n* = 4 (not figured due to condition of specimens).

Length 3.5–4.0 mm.

Colouration. Brown; with black spot above eyes in developing adult.

Head. Maxillary sheath short, posteromedially directed, gently tapered toward truncate apex, apices of palpi separated medially. Setae not observed.

Thorax. Width nearly subequal to abdomen at widest point. Foreleg sheath projected straight, slightly longer than wing sheaths, nearly reaching posterior margin of sternite 2; anterior half of midleg visible anterior to wing sheath, then hidden behind foreleg, slightly shorter than foreleg; hindleg concealed behind wing sheath, only apex visible between apex of foreleg and wing sheath, slightly shorter than foreleg, but longer than wing sheath. Wing sheaths not reaching posterior margin of abdominal sternite 2; large tubercle at base, setae not visible. Respiratory organ short and squat, much shorter than maxillary sheath, broadest subapically; bulbous; spiracular openings encircling apex; stalk wide, emerging from small tubercle. Tubercle situated posterodorsally to respiratory organ, rounded, projected slightly laterally; apex nearly touching or touching respiratory organ. Tubercle situated posterolaterally to respiratory organ crenulate, projected slightly anteriorly. Setae not observed.

Abdomen. Subcylindrical, strongly tapered at caudal segment. Spiracles weakly developed, not projected or distinctly visible. Tergites 1–8 rectangular, without ridges; setae not visible. Tergite 9 rounded, posterior with dorsolateral ridges and hind margin

emarginated; projection pointed posteriorly in lateral view. Sternites 3–8 rectangular, without row of faint setulae along anterior margin; lateral margins crenulate, bearing a few thin, short setae. Sternites 3 and 4 bearing pair of small lateral adhesive structures, sternite 5 bearing pair of large lateral adhesive structures on lateral margin. Caudal sternite subquadrate, with pair of posteriorly projected medial lobes; posterior margin with pair of medial ridges, curved dorsally forming small, dorsally projected tubercle in lateral view; without distinct caudal hooks.

Description of larva. $n = 17$.

Length of final instar. 6.3–6.7 mm. *Colouration.* Head capsule variable, ranging from light brown to black. Body mottled brown and grey, possibly pale brown to creamy; cream coloured ventrally. *Head capsule* (Figs. 3.27 C, D). Two large, circular eye spots, elevated on tubercle; antenna with three finger-like process, on largest tubercle; with five pairs of tubercles outside of ecdysial lines (not including antennal and ocular tubercle), tubercles below and beside eye bifurcate; two tubercles between ecdysial line, upper tubercle bi- or trifurcate; 15 pairs of unbranched setae; five pairs of sensory pits (13, 14, 18, 20, 21), sensory pit 13 above antennal tubercle. *Thorax.* Prothorax with pair of anterodorsolateral protuberances bare; anterolateral protuberances with one long and two short setae; spiracular protuberance bearing two protuberances, inner protuberance with pair of setae, outer with single seta; pair of midlateral setae below anterolateral protuberance; three closely approximated setae near base of prothoracic leg (Keilin's organ). Mesothorax and metathorax with pair of dorsolateral protuberances bearing pair of closely approximated setae, one thickened, one slender, mesothorax with additional seta beneath protuberance; lateral protuberance on both segments bearing four setae;

single long seta slightly ventral to lateral protuberance; three mid-ventrolateral setae directed ventrally. Prothorax bearing proleg, posterior half with rectangular adhesive structure; meso- and metathoracic sternites with rectangular adhesive structure. *Abdomen.* Abdominal sternites 1–7 modified into circular, suction cup-like adhesive structure; sternite 8 with quadrate adhesive structure, extended over sternite 9; sternite 9 smooth, bearing anal proleg. Tergites 1–7 with single anterolateral protuberance on each side with a single seta, and pair of posterodorsolateral protuberances, each bearing two closely approximated short, thin setae; lateral adhesive structure swelling bearing five setae, two lateral, three basal; 1 seta located anterior to lateral swelling. Segment 8 with dorsolateral protuberance on either side of posterior spiracular plate, each bearing pair of small setae; lateral protuberance with three setae; single short ventrolateral seta; ventral sternite bearing pair of setae. Posterior spiracular plate with sclerite encircling procerci; procercus shorter than length of spiracular plate, bearing four setae, two thick, two slender; without cone-like protuberance on either side of cerci. Terminal segment with anterior dorsal protuberance bare; pair of posterior lateral protuberances with pair of setae; five lateral setae; two pairs of long setae on posterior margin, above pair of anal papillae; ventral sternite bearing single pair of setae.

Egg. Unknown.

Additional Material Examined. Known only from the type series.

Distribution. Known from the southern Andes of Chile (Fig. 3.32A).

Bionomics. This is a mid-elevation species. Adults were observed flying around and resting on leaf tips, roughly 2 m from the nearest splash zones. Larvae and pupae have ventral adhesive structures and were collected only from rocky substrates at the margin of

a waterfall.

Etymology. This species is named after the Mapuche (*mapu* ‘land’, *che* ‘people’) indigenous peoples, who since ~500 B.C., have inhabited the regions of southern Chile, where *Ni. mapuche* is known.

The *Ni. nudipennis* group

***Niphtha brunnea* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: “CHILE: Region IX (Araucanía)/ Rte. 71, 15.xii.2016/ 38°14'20.6"S 71°53'46.6"W,/ elev. 953m, roadside seeps,/ J.K. Moulton & R.J. Pivar”; “HOLOTYPE/*Niphtha/ brunnea/* Pivar” (CNC). **Paratypes:** CHILE: REGION VIII (Bío Bío): Rte. Q-61, 8.xii.2016, 37°49'14.2"S 71°40'34.1"W, elev. 366 m, creek, J.K. Moulton & R.J. Pivar (1♂); Rte. Q-61, Estero Agua Blanca 8.xii.2016, 37°46'30.8"S 71°42'03.9"W, elev. 552 m, vegetation near splash zones, J.K. Moulton & R.J. Pivar (1♂); REGION IX (Araucanía): same label data as holotype (3♂); same label data as holotype except, larvae/pupae on foliage in splash zone (6 larvae*, 5 pupae, 3 pupal exuviae).

Recognition. This species is recognized by the cheliform gonostylus bearing non-bifurcate apices and the gonocoxite with three projections: two that are nearly equal in size and the third, much smaller and inconspicuous, situated at the base of the anterior one. It is darker in colouration compared to the closely related *Ni. sinclairi*, and the darkest of the *Ni. nudipennis* group.

Description of male. $n = 5$.

Length 1.6–1.9 mm.

Colouration (Figs. 3.10A, 3.11A). Head dull, dark brown; pronotum and postpronotum brown; postpronotal lobe and lateral margins of prescutum light brown; remaining scutum shiny, brown, pleura light brown; scutellum shiny, brown; mediotergite shiny, anterior half light brown, posterior half dark brown; katepisternum mainly brown, lighter near anterior spiracle; paratergite brown; remaining pteropleuron mainly brown with dispersed markings of dark/light brown; base of halter creamy, distal half of stalk and knob light brown; legs pale brown, apex of tarsi darker; abdomen brown; terminalia light brown.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 2 strong setae. Flagellomeres 1–3 subquadrate, 1 expanded, 1.5× as wide as next segment, shorter in length than 2 and 3 combined; flagellomeres 4–10, cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing three setae, middle seta most pronounced. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutal setae long, black. Scutellum with row of long, black marginal setae.

Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 1.9–2.2 mm. Infusate throughout, apex somewhat narrowed; C fringed in small setulae, with widely spaced microtrichia; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely

approximated, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated in basal third of $R_1(+R_{2+3})$; bend in R_{4+5} strong; R_{4+5} and M_1 running parallel toward margin; M_1 straight; M_2 with gentle bend in apical third; M_4 with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, few setae restricted to posterior third; sternites 3–7 rectangular, lightly sclerotized, setae restricted to posterior half; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, a few setae restricted to laterad.

Male terminalia (Figs. 3.17D, 3.18D). Epandrium quadrate in ventral view, posterior margin rounded, with narrow medial cleft; long, extended beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites oblong, longer than wide; anterior margin rounded, somewhat expanded dorsally, not closely approximated; with three spine-like projections; anterior large projection nearly equal in length to posterior projection, gradually tapered toward apex; smaller anterior projection positioned somewhat anterior to large tooth, may be small and inconspicuous in some specimens; posterior projection strongly tapered toward apex, some specimens with second basal tooth on outer margin of projection; margin around anterior projection with long, thin setae. Gonostylus cheliform, dorsoventrally flattened, margins concave causing bowl-like appearance; anterior apex with a few indistinct setae, posterior margin with strong setae. Parameres medially fused, attached basally to arms of gonocoxal plate; divided distally into dorsal parameral apodeme and ventral arm; ventral arm projected anteroventrally toward gonocoxal plate, carving knife-shaped, ventral margin serrate; ventral arm extends posteroventrally presumably to aid in copulation; when retracted, rests ventrally between

dorsal arm of gonocoxal plate and inner margin of gonocoxite, apex reaching base of anterior gonocoxal projection. Gonocoxal plate broad, well sclerotized; anterior margin triangular, basal margin cleft; pair of dorsal arms connect to parameres; medial aedeagal guide projected ventrally between gonostyli, well sclerotized, consisting of two parts, anterior structure with five projections and posterior rounded plate bearing minute setulae. Cercus ovoid, only slightly visible in lateral view; projected anteroventrally; situated within epandrial indentation.

Female. Unknown.

Description of pupa. *n* = 6 (Figs. 3.21A, 3.22A, 3.23A, 3.29).

Length 2.7–2.9 mm.

Colouration. Brown; sometimes with black spot above eyes in developing adult.

Head. Maxillary sheath short, posteromedially directed, gently tapered toward truncate apex, apices of palpi separated medially; devoid of tubercles and setae.

Thorax. 1.5× wider than abdomen at widest point. Foreleg sheath projecting straight and slightly beyond wing sheaths, reaching hind margin of sternite 2; anterior half of midleg visible anterior to wing sheath, then hidden behind foreleg, not projected beyond wing sheath; hindleg concealed behind wing sheath, only small triangular apex visible between apex of foreleg and wing sheath, not extended beyond wing sheath. Wing sheaths extended to posterior margin of abdominal sternite 2. Respiratory organ slightly shorter than maxillary sheath, broadest subapically; ovate, slightly arched medially, tapered toward apex; spiracular openings encircling apex; stalk thin, emerging from small tubercle. Tubercle situated posterodorsally to respiratory organ, rounded, projected laterally; apex nearly touching or touching respiratory organ. Thorax devoid of setae.

Abdomen. Subcylindrical, evenly tapered toward caudal segment. Spiracles well developed, distinct on segments 3–7, projected anterodorsolaterally; spiracle on segment 8 short, less than half length of preceding spiracles, projected dorsolaterally; all bearing minute spine-like setulae. Tergites 1–8 quadrate, devoid of setae, with pair of dorsolateral ridges and faint medial transverse groove, groove not meeting dorsolateral ridges. Tergite 9 rounded, posterior margin emarginated, laterally compressed compared to preceding segments; small, rounded projection pointing posteriorly in lateral view. Sternites 3–8 rectangular, with row of faint setulae along anterior margin, not connecting to lateral margin; lateral margins crenulate, lacking setae. Sternites 3 and 4 bearing pair of small lateral adhesive structures, sternite 5 bearing pair of large lateral adhesive structures on lateral margin. Caudal sternite triangular, with medial sclerotized groove and pair of medial rounded, posteromedially projected lobes; posterior margin with medial longitudinal ridge; without distinct caudal hooks.

Description of larva. *n* = 6 (Figs. 3.24A, 3.25A, 3.26A, 3.30, 3.34B, C).

Length of final instar 4.8–5.1 mm. *Colouration.* Head capsule pale brown, anterolateral margin of ecdysial line black. Body creamy brown. *Head capsule* (Fig. 3.28A, B). Two large, circular eye spots, elevated on tubercle; antenna with three finger-like processes, elevated on largest tubercle; with five pairs of smaller tubercles outside of ecdysial lines (not including antennal and ocular tubercle); single tubercle between ecdysial lines, about same size as ocular tubercle; 15 pairs of unbranched setae; six sensory pits (13, 14, 18, 19, 20, 21), sensory pit 13 above antennal tubercle. *Thorax.* Prothorax with single pair of protuberances bearing single spiracle; spiracular protuberance bearing one pair of dorsal setae anterior to spiracle and single dorsolateral seta; three mid-lateral setae, two long,

one short and fine; two closely approximated setae near base of prothoracic leg (Keilin's organ). Mesothorax and metathorax with pair of small dorsolateral protuberances and pair of large lateral protuberances; mesothoracic dorsolateral protuberance bearing single thickened seta, metathoracic lateral protuberance bearing pair of closely approximated setae, anterior seta thickened and longer than posterior seta; lateral protuberance on both segments bearing three setae, two short, one long; single long seta slightly ventral to lateral protuberance; one pair of mid-ventrolateral setae. Prothorax bearing proleg, posterior half with rectangular adhesive structure; meso- and metathoracic sternites with rectangular adhesive structure. *Abdomen*. Abdominal sternites 1–7 modified into circular, suction cup-like adhesive structure; sternite 8 with quadrate adhesive structure; sternite 9 smooth, bearing anal proleg. Segments 1–7 lacking distinct protuberances, at most, pair of dorsal lateral swellings bearing single or paired short, thin setae; single seta situated anterolaterally; lateral adhesive structure swelling bearing numerous setae, two anterolateral, two midlateral, four basalateral. Segment 8 with small dorsolateral protuberance on either side of posterior spiracular plate, each bearing pair of small setae; lateral protuberance with single seta; single short ventrolateral seta; ventral sternite bearing pair of setae. Posterior spiracular plate with sclerite encircling procerci; procercus shorter than length of spiracular plate, bearing four setae, two thick, two slender; without cone-like protuberance on either side of cerci. Terminal segment with pair of protuberances, no setae; pair of dorsolateral setae; five lateral setae; two pairs of long setae on posterior margin, above pair of anal papillae; ventral sternite lacking setae.

Egg. Unknown.

Additional Material Examined. Known only from the type series.

Distribution. Known only from two localities in the Andes of southern Chile (Fig. 3.32B).

Bionomics. The larvae and pupae both possess the ventral adhesive structures seen in other known immature stages of South American *Niphta*. Immatures were collected from wetted vegetation in the splash zones, never from rocks. Vegetation included both living and dead plant material, including smooth leaves and herbaceous stems. The vegetation was not in the direct flow of water, but rather being lightly splashed by water droplets that maintained enough moisture for the immatures to survive.

Etymology. *Niphta brunnea* is from the Latin *brunneus* (brown) in allusion to its brown colouration, the darkest of the *Ni. nudipennis* group.

***Niphta courtneyi* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: “CHILE, Region X (Los Lagos)/ East side Lago Llanquihue/ small falls on road (nr PN/ VPR) 41°08.47’S 72°35.28’W/ ≈100m 2.xii.2008 GW/ Courtney (CH08-30)””; “HOLOTYPE/ *Niphta/ courtneyi*/ Pivar” (CNC). **Allotype:** ♀*, same label data as holotype (CNC). **Paratypes:** CHILE: REGION X (Los Lagos): Rte. U-99, 10.xii.2016, 41°08’28.2”S 72°35’16.8”W, elev. 101 m, roadside seeps/creek, J.K. Moulton & R.J. Pivar (1♀*).

Recognition. This species is recognized by the bifurcate posterior apex of the cheliform gonostylus and the presence of three individual gonocoxal projections. It is lighter in colouration than the closely related *Ni. moultoni*.

Description of male. $n = 1$.

Length 2.1–2.3 mm.

Colouration (Figs. 3.10B, 3.11B). Head dull, brown; pronotum and postpronotum brown; postpronotal lobe and lateral margins of prescutum yellow; scutum shiny with three distinct dark brown bands, pleura yellow; postscutum yellow, two lateral brown spots above scutoscutellar suture; scutellum shiny, yellow; mediotergite shiny, anterior half yellow, posterior half brown; katapisternum mainly pale brown, yellow near fore coxa; paratergite brown; remaining pteropleuron mainly yellow with dispersed brown markings; halter creamy yellow; legs pale yellow, apex of tarsi darker; abdomen brown; terminalia yellow.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 3 strong setae. Flagellomeres 1–3 subquadrate, 1 expanded, 1.5× as wide as next segment, shorter in length than 2 and 3 combined; flagellomeres 4–10, cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing three setae, middle seta most pronounced. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae.

Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 1.9–2.2 mm. Lightly infusate throughout, apex somewhat narrowed; C fringed in small setulae, with widely spaced microtrichia; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely

approximated, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated in basal third of $R_1(+R_{2+3})$; bend in R_{4+5} strong; R_{4+5} and M_1 running parallel toward margin; M_1 straight; M_2 with gentle bend in apical third; M_4 with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, a few setae restricted to posterior third; sternites 3–7 rectangular, lightly sclerotized, setae restricted to posterior half; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, a few setae restricted to laterad.

Male terminalia (Figs. 3.17B, 3.18B). Epandrium quadrate in ventral view, posterior margin rounded, with narrow medial cleft; long, extended beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites oblong, longer than wide; anterior margin rounded, somewhat expanded dorsally, not closely approximated; with three spine-like projections; two anterior projections, inner projection shorter than outer one; posterior projection long, slender, tapered to single apex, 3.5x longer than shortest projection; margin around gonostylus with dense long, thin setae. Gonostylus cheliform, dorsoventrally flattened anteriorly, swollen posteriorly; anterior apex with a few setae; posterior apex bifurcate, setose. Parameres medially fused, attached basally to arms of gonocoxal plate; divided distally into dorsal parameral apodeme and ventral arm; ventral arm projected anteroventrally toward gonocoxal plate, strongly curved anteriorly, sickle-shaped, surface textured with tiny bumps, except for smooth apex; ventral arm extends posteroventrally presumably to aid in copulation; when retracted, rests ventrally between dorsal arm of gonocoxal plate and inner margin of gonocoxite. Gonocoxal plate broad, well sclerotized; anterior margin subquadrate, basal margin cleft; pair of dorsal arms that

connect to parameres; medial aedeagal guide projected ventrally between posterior margins of gonocoxites, well sclerotized, consisting of two parts, anterior Y-shaped structure and posterior triangular plate. Cercus ovoid, only slightly visible in lateral view; projected anteroventrally; situated within epandrial indentation.

Description of Female. *n* = 2.

Similar to male except as follows: *Abdomen*. Tergite 9 noticeably more sclerotized than preceding tergites; sternite 8 well sclerotized, with distinct blunt projection between hypogynial valves. *Female terminalia* (Figs. 3.19B, 3.20B). Hypogynial valve not projected beyond tergite 9; posterior margin deeply cleft in ventral view, forming two triangular lobes; lobes densely setose, with both stout, thickened setae and thinner, long setae with slight apical bend; hypogynial protuberance between valves. Tergite 9 subquadrate in lateral view, 2x as wide as tergite 8, lacking lateral projections. Sternite 9 (genital fork) slender, Y-shaped at both ends; lateral arms forming complex of highly modified structures: medial heavily sclerotized circular opening, dorsal to posterior cleft of hypogynial valve, expanded dorsally into pair of circular plates, which further expand anteriorly into pair of heavily sclerotized plates, strongly recurved posteroventrally toward circular opening, remaining dorsal to genital fork; dorsal surface of recurved plates with tiny grooves and indentations. Hypoproct lightly sclerotized, narrow. Cercus quadrate, projected posteroventrally; bearing numerous setae. Spermathecae not observed; two spermathecal ducts visible in center of lateral arm circle.

Immature stages. Unknown.

Additional Material Examined. Known only from the type series.

Distribution. Known only from the type locality, the East side of Lago Llanquihue in

Southern Chile (Fig. 3.32B).

Etymology. *Niphta courtneyi* is named in honour of its collector, Gregory W. Courtney (Iowa State University). Dr. Courtney collected three new species of Thaumaleidae from Chile (*A. fredericki*, *Ni. courtneyi* and *Ni. mapuche*) and was part of the expedition that collected the first South American *Niphta* larvae. These collections were one of the major reasons for the author's interest in Chilean thaumaleids.

***Niphta daniellae* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: "CHILE: Region IX (Araucanía)/ Rte. 71, 15.xii.2016/ 38°14'20.6"S 71°53'46.6"W/ elev. 953m, roadside seeps/ J.K. Moulton & R.J. Pivar"; "HOLOTYPE/ *Niphta/ daniellae/* Pivar" (CNC). **Paratypes:** CHILE. REGION VIII (Bío Bío): Rte. Q-61, 8.xii.2016, 37°48'22.8"S 71°40'46.6"W, elev. 379 m, cascading creek, J.K. Moulton & R.J. Pivar (1♂).

Recognition. This species is recognized by the gonostylus tapering to a sharp point with no projections and not cheliform, unlike all remaining species in the *Ni. nudipennis* group. The gonocoxal plate also has a pair of lateral arms projected anteriorly.

Description of male. *n* = 2.

Length 1.7–2.0 mm.

Colouration (Figs. 3.10C, 3.11C). Head dull, dark brown; pronotum and postpronotum dark brown; postpronotal lobe and lateral margins of prescutum yellow; scutum shiny with three distinct brown bands, pleura yellow; postscutum yellow, two lateral brown

spots above scutoscutellar suture; scutellum shiny, yellowish; mediotergite shiny, anterior half yellow, posterior half brown; katapisternum dark brown, except yellow at base of fore coxa; anepisternum and paratergite brown; remaining pteropleuron yellow; halter entirely creamy yellow; legs yellowish brown, tarsi dark brown; abdominal tergites brown, posterior margin pale brown, sternites mainly yellow with scattered brown markings; terminalia yellowish-brown.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 2–3 strong setae. Flagellomeres 1–3 subquadrate, 1 expanded, 1.5× as wide as next segment, subequal in length to 2 and 3 combined; flagellomeres 4–10, cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing three setae, middle seta most pronounced. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae.

Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 2.2–2.3 mm. Lightly infusate throughout, apex somewhat narrowed; C fringed in small setulae, with widely spaced microtrichia; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely approximated, smaller than first, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated in basal third of $R_1(+R_{2+3})$; bend in R_{4+5} gentle; R_{4+5} and M_1 running parallel toward margin; M_1 straight; M_2 with gentle bend in apical third; M_4 with slight apical bend; CuA

without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, lacking setae; sternites 3–7 rectangular, lightly sclerotized, setae restricted to posterior two-thirds; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, lacking setae.

Male terminalia (Figs. 3.15A, 3.16A). Epandrium quadrate in ventral view, posterior margin rounded, with narrow medial cleft; long, extended beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites oblong, longer than wide, inner margin setose, expanded anterodorsally above gonocoxal plate, closely approximated but not fused; two posteromedially directed spine-like projections, one anterior to gonostylus, one posterior; anterior projection pointed, bare; posterior projection blunt, setose.

Gonostylus longer than wide, distal half strongly tapered to pointed apex; dorsoventrally compressed, margins curled slightly ventrally, scoopula-like, heavily sclerotized; a few setae scattered throughout, apex bare. Parameres medially fused, attached basally to arms of gonocoxal plate; surface textured with tiny bumps, except for smooth apex; divided medially into dorsal parameral apodeme and ventral arm; ventral arm projected anteroventrally, expanded medially, apical third quickly tapered to pointed apex, sharply curved anterodorsally; ventral arm extends posteroventrally presumably to aid in copulation, when retracted, rests ventrally between lateral margin of gonocoxal plate and gonocoxite. Gonocoxal plate broad, well sclerotized; anteroventral margin subtriangular, basal margin cleft forming two ventrally directed projections; pair of dorsal arms connect to parameres; with median expansion projected ventrally between posterior margins of gonocoxites. Cercus ovoid, visible in lateral view; projected anteroventrally; situated

within epandrial indentation.

Female. Unknown.

Immature Stages. Unknown.

Additional Material Examined. Known only from the type series.

Distribution. Known from two localities in the Andes of south-central Chile (Fig. 3.32B).

Etymology. This species is named in honour of RJP's wife, Danielle Lombardi, for her support during Pivar's graduate research and for playing an important role in organizing the Chilean expedition. Having grown up in northern Chile (Arica), her Spanish skills were critical for translating all communications with government and national park contacts, as well as translating our requests for collecting permits.

***Niphta eurydactylus* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: "CHILE: Region X (Los Lagos)/Rte. U-99, 10.xii.2016/ 41°08'28.2"S 72°35'16.8"W/ elev. 101m, roadside seeps/ and creek, J.K. Moulton & R.J./ Pivar"; "HOLOTYPE/ *Niphta/ eurydactylus/* Pivar" (CNC). **Paratypes:** CHILE. REGION X (Los Lagos): Rte. 215, 12.xii.2016, 40°40'32.4"S 72°17'35.6"W, elev. 252 m, trickle falls, J.K. Moulton & R.J. Pivar (1♂); Rte. U-99, 10.xii.2016, 41°08'09.6"S 72°35'43.3"W, elev. 81 m, roadside falls, J.K. Moulton & R.J. Pivar (1♂); Rte. U-99, 10.xii.2016, 41°08'28.2"S 72°35'16.8"W, elev. 101 m, roadside seeps/creek, J.K. Moulton & R.J. Pivar (9♂); REGION XIV (Los Ríos): Antilhue, Rte. T-35, 9.xii.2016,

39°49'09.8"S 72°56'22.6"W, elev. 40 m, roadside creek, J.K. Moulton & R.J. Pivar (1♂); Rte. T-29, 14.xii.2016, 39°43'03.4"S 71°55'31.6"W, elev. 340 m, seepage, J.K. Moulton & R.J. Pivar (1♂); Rte. T-85, 13.xii.2016, 40°19'58.7"S 72°16'54.8"W, elev. 145 m, foliage around waterfalls, J.K. Moulton & R.J. Pivar (3♂).

Recognition. This species is recognized by a broad, straight, finger-like projection on the gonostylus.

Description of male. *n* = 17.

Length 1.5–1.8 mm.

Colouration (Figs. 3.10D, 3.11D). Head dull, dark brown; pronotum and postpronotum dark brown. Variable colouration, even among specimens from same population; base colouration of thorax either yellow or orange as follows: postpronotal lobe and lateral margins of prescutum orangey/yellowish-brown; scutum shiny with three distinct brown bands, pleura yellow to yellowish-brown; postscutum orangey/yellowish-brown, two lateral brown spots above scutoscuteellar suture; scutellum shiny, orangey/yellowish-brown; mediotergite shiny, anterior half orangey/yellowish-brown, posterior half brown; katepisternum mainly pale brown with scattered orange/yellow and black markings, or mostly yellowish-brown with brown lateral markings above mid coxae; paratergite brown; remaining pteropleuron mainly orangey-brown to yellowish-brown with dispersed markings of brown and black; base of halter creamy grey, knob pale yellow; legs pale brown, apex of tarsi darker; abdomen brown; terminalia pale brown.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 2 strong setae. Flagellomeres 1–3 subquadrate, 1 expanded, 2× as wide as next segment, shorter in length than 2 and 3 combined; flagellomeres 4–10, cylindrical,

becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing 3–4 setae, middle seta most pronounced. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae.

Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 1.8–2.2 mm. Infusate throughout, apex somewhat narrowed; C fringed in small setulae, with widely spaced microtrichia; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely approximated, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated in basal third of $R_1(+R_{2+3})$; bend in R_{4+5} strong; R_{4+5} and M_1 running parallel toward margin; M_1 straight; M_2 with gentle bend in apical third; M_4 with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, a few setae restricted to posterior third; sternites 3–7 rectangular, lightly sclerotized, setae restricted to posterior two-thirds; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, lacking setae.

Male terminalia (Figs. 3.15B, 3.16B). Epandrium quadrate in ventral view, posterior margin rounded, with narrow medial cleft; long, extended beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites oblong, longer than wide, large C-shaped scallop where gonostyli inserted; anterior margin rounded, somewhat expanded dorsally, not closely approximated; anterior inner margin with stout spine-like projection;

margin around gonostylus setose. Gonostylus subquadrate; with finger-like projection emerging from posterolateral corner, gently tapered toward apex, bearing a few setae; inner anterior margin with small, rounded projection bearing a few fine setae. Parameres medially fused, attached basally to arms of gonocoxal plate; divided distally into dorsal parameral apodeme and ventral arm; ventral arm projected anteroventrally toward gonocoxal plate, strongly curved anteriorly, sickle-shaped, surface textured with tiny bumps, except for smooth apex; ventral arm extends posteroventrally presumably to aid in copulation; when retracted, rests ventrally between lateral margin of gonocoxal plate and gonostylus. Gonocoxal plate broad, well sclerotized; anterior margin rounded; pair of dorsal arms connect to parameres; median aedeagal guide trident-shaped, weakly sclerotized. Cercus prominent; ovoid; projected anteroventrally; situated within epandrial indentation.

Female. Unknown.

Immature Stages. Unknown.

Additional Material Examined. Known only from the type series.

Distribution. Known from both the Chilean Coastal Range and Andes of southern Chile (Fig. 3.32B).

Etymology. *Eurydactylus* is from the Greek *eury* (broad, wide) and *daktylos* (finger), in allusion to the broad, finger-like projection on the gonostylus.

***Niphta moultoni* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: “CHILE: Region XIV (Los Ríos)/ Antilhue, Rte. T-35, 9.xii.2016/ 39°49'09.8"S 72°56'22.6"W/ elev. 40 m, roadside creek,/ J.K. Moulton & R.J. Pivar”; “HOLOTYPE/ *Niphta/ moultoni/* Pivar” (CNC). **Allotype:** ♀*, same label data as holotype (CNC). **Paratypes:** REGION XIV (Los Ríos): same label data as holotype (2♂).

Recognition. This species is recognized by the bifurcate, posterior apex of the cheliform gonostylus and the bifurcate anterior projection of the gonocoxite. It is darker in colouration compared to the closely related *Ni. courtneyi*.

Description of male. *n* = 3.

Length 1.7–1.9 mm.

Colouration (Figs. 3.10E, 3.11E). Head dull, dark brown; pronotum and postpronotum dark brown; postpronotal lobe and lateral margins of prescutum light brown; scutum shiny with three distinct dark brown bands, pleura light brown; postscutum brown; scutellum shiny, light brown; mediotergite shiny, anterior half light brown, posterior half brown; katepisternum mainly dark brown, light brown near coxa 1; paratergite brown; remaining pteropleuron mainly brown with dispersed light brown and black markings; base of halter pale brown, knob pale yellow; legs pale brown, apex of tarsi darker; abdomen brown; terminalia pale brown.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 2 strong setae. Flagellomeres 1–3 subquadrate, 1 expanded, 2× as wide as next

segment, shorter in length than 2 and 3 combined; flagellomeres 4–10, cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing three setae, middle seta most pronounced. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae.

Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 2.1–2.4 mm. Infusate throughout, apex somewhat narrowed; C fringed in small setulae, with widely spaced microtrichia; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely approximated, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated in basal third of $R_1(+R_{2+3})$; bend in R_{4+5} strong; R_{4+5} and M_1 running parallel toward margin; M_1 straight; M_2 with gentle bend in apical third; M_4 with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, few setae restricted to posterior third; sternites 3–7 rectangular, lightly sclerotized, setae restricted to posterior half; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, few setae restricted to laterad.

Male terminalia (Figs. 3.17A, 3.18A). Epandrium quadrate in ventral view, posterior margin rounded, with medial cleft; long, extended beyond gonostyli; without lobes, projections. Hypandrium absent. Gonocoxites oblong, longer than wide; anterior margin rounded, somewhat expanded dorsally behind gonocoxal plate, not closely approximated;

with two spine-like projections; anterior projection wide, bifurcate; posterior projection long, slender, slightly sinuous, tapered to single apex, nearly twice as long as anterior projection; inner margin with numerous long, thin setae. Gonostylus cheliform, dorsoventrally flattened anteriorly, swollen posteriorly; anterior apex with a few setae; posterior apex bifurcate, setose. Parameres medially fused, attached basally to arms of gonocoxal plate; divided distally into dorsal parameral apodeme and ventral arm; ventral arm projected anteroventrally toward gonocoxal plate, strongly curved anteriorly, sickle-shaped, surface textured with tiny bumps, except for smooth apex; ventral arm extends posteroventrally presumably to aid in copulation; when retracted, rests ventrally between dorsal arm of gonocoxal plate and dorsal to anterior gonocoxal projection. Gonocoxal plate broad, well sclerotized; anterior margin subquadrate, basal margin cleft; pair of dorsal arms connected to parameres; medial aedeagal guide projected ventrally between posterior margins of gonocoxites, well sclerotized, comprising two parts: anterior Y-shaped structure with five finger-like projections protruding from posterior margin and dorsal triangular plate. Cercus ovoid, only slightly visible in lateral view; projected anteroventrally; situated within epandrial indentation.

Description of Female. $n = 1$.

Similar to male except as follows: *Abdomen*. Tergite 9 noticeably more sclerotized than preceding tergites; sternite 8 well sclerotized, with distinct blunt projection at base of hypogynial valve. *Female terminalia* (Figs. 3.19A, 3.20A). Hypogynial valve not projected beyond tergite 9; posterior margin deeply cleft in ventral view, forming two triangular lobes; lobes densely setose, with both stout, thickened setae and thinner, long setae with slight apical bend; hypogynial protuberance between valves. Tergite 9

subquadrate in lateral view, 1.5x as wide as tergite 8, lacking lateral projections. Sternite 9 (genital fork) slender, Y-shaped at both ends; lateral arms forming complex of highly modified structures: medial heavily sclerotized circular opening, dorsal to posterior cleft of hypogynial valve, with pair of lateral sclerotized triangular expansions; triangular expansions expanded dorsally into pair of circular plates, those further expanded anteriorly into pair of heavily sclerotized plates that strongly recurve posteroventrally toward circular opening, remaining dorsal to genital fork; dorsal surface of recurved plates tiny grooves and indentations. Hypoproct lightly sclerotized, narrow. Cercus quadrate, projecting posteroventrally; bearing numerous setae. Spermathecae not observed; two spermathecal ducts visible in center of lateral arm circle.

Immature Stages. Unknown.

Additional Material Examined. Known only from the type series.

Distribution. Known only from the type locality in the Chilean Coastal Range (Fig. 3.32B).

Etymology. *Niphta moultoni* is named in honour of Dr. John K. Moulton (The University of Tennessee) for his mentorship during Pivar's graduate research. As his major advisor, Moulton sparked Pivar's interest in thaumaleids and played an important role in his development as an entomologist.

***Niphta nudipennis* (Edwards)**

Austrothaumalea nudipennis Edwards, 1930: 113.

Stuardo, 1946: 42 (catalogue); Stone, 1966: 1 (catalogue); Arnaud, 1977: 284

(distribution).

Niphta nudipennis (Edwards): Theischinger 1986: 316 (new combination); McLellan.

1988: 563 (moved to genus *Niphta* by Theischinger (1986)).

Type material examined. Holotype: ♂, minuten pinned with abdomen mounted in resin, labelled: “Ancud./ 17–19.xii.1926.”; “Austrothaumalea/ nudipennis Edw./ F.W. Edwards/ det. 1930.”; “S. Chile:/ Chiloe I./ F. & M. Edwards./ B.M. 1927 – 63.”; “HOLO-/ TYPE”; “NHMUK010210690” (BMNH). **Allotype:** ♀, same label data as holotype (BMNH). **Paratypes:** same label data as holotype (4♂, 4♀, BMNH).

Recognition. This species is recognized by a long, narrow, finger-like projection on the gonostylus that has a medial bend.

Redescription of male. *n* = 22.

Length 1.5–2.3 mm.

Colouration (Figs. 3.10F, 3.11F). Head dull, dark brown; pronotum and postpronotum dark brown; postpronotal lobe and lateral margins of prescutum orangey-brown; scutum shiny with three distinct brown bands, pleura orangey-brown (sometimes scutal bands concolourous with pleura); postscutum orangey-brown, two lateral brown spots above scutoscutellar suture; scutellum shiny, yellowish-brown; mediotergite shiny, anterior half yellowish-brown, posterior half brown; katapisternum variable in colour, may be mostly brown with orange markings, or mostly yellowish-brown with brown lateral markings above mid coxa; paratergite brown; remaining pteropleuron mainly orangey-brown to yellowish brown with dispersed brown to light brown markings; base of halter pale yellow, turning brown, knob yellowish-orange; legs pale brown, apex of tarsi darker;

abdomen brown; terminalia light brown.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 2 strong setae. Flagellomeres 1–3 subquadrate, 1 expanded, 2× as wide as next segment, subequal in length to 2 and 3 combined; flagellomeres 4–10, cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing three setae, middle seta most pronounced. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae.

Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing (Fig. 3.2B). Wing length: 2.0–2.9 mm. Infusate throughout, apex somewhat narrowed; C fringed in small setulae, with widely spaced microtrichia; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R₁ and R₁(+R₂₊₃) with three weakenings or depigmented gaps, first slightly beyond R₂₊₃, second and third closely approximated, near C; microtrichia of R₁(+R₂₊₃) confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R₂₊₃ distinct, situated in basal third of R₁(+R₂₊₃); bend in R₄₊₅ strong; R₄₊₅ and M₁ running parallel toward margin; M₁ straight; M₂ with gentle bend in apical third; M₄ with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, a few setae restricted to laterad on posterior third; sternites 3–7 rectangular, lightly sclerotized, setae restricted to posterior two-thirds; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, lacking

setae.

Male terminalia (Figs. 3.15C, 3.16C). Epandrium quadrate in ventral view, posterior margin rounded, with narrow medial cleft; long, extended beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites oblong, wider than long, large C-shaped scallop where gonostyli inserted; anterior margin rounded, somewhat expanded dorsally, not closely approximated, extended anteriorly toward sternite 8; anterior margin with stout spine-like projection; inner margin anterior to spine setose with short, fine setulae; margin around gonostylus setose. Gonostylus quadrate basally; with pointed, finger-like projection emerging from posterolateral corner, bent 45° at midpoint, bearing a few setae at base; inner anterior margin with small, rounded projection bearing a few fine setae; posterior margin with fringe of setae. Parameres medially fused, attached basally to gonocoxal plate; divided medially into dorsal parameral apodeme and ventral arm; ventral arm projected anteroventrally toward gonocoxal plate, strongly curved anteriorly, sickle-shaped, surface textured with tiny bumps, except for smooth apex; ventral arm extends posteroventrally presumably to aid in copulation (Figs. 3.15D, 3.16D); when retracted, rests ventrally between lateral margin of gonocoxal plate and gonostylus; when extended, gonostyli move inward, finger-like projections crossing and forming 'X' allowing parameres to extend ventrally. Gonocoxal plate broad, well sclerotized; anterior margin rounded, basal margin cleft; pair of dorsal arms connected to parameres; with median expansion projecting ventrally between gonostyli, weakly sclerotized. Cercus ovoid, slightly visible in lateral view; projecting anteroventrally; situated within epandrial indentation.

Redescription of female. *n* = 6.

Similar to male except as follows: *Abdomen*. Tergite 9 noticeably more sclerotized than preceding tergites; sternite 8 well sclerotized, with distinct blunt projection at base of hypogynial valve. *Female terminalia* (Figs. 3.19D, 3.20D). Hypogynial valve not projected beyond tergite 9; posterior margin deeply cleft in ventral view, forming two subquadrate lobes; lobes densely setose, with both stout, thickened setae and thinner, long setae with slight apical bend; elongate hypogynial protuberance between valves. Tergite 9 subquadrate in lateral view, 2x as wide as tergite 8, lacking lateral projections. Sternite 9 (genital fork) slender, Y-shaped at both ends; lateral arms forming complex of highly modified structures: medial heavily sclerotized circular opening, dorsal to posterior cleft of hypogynial valve, expanded dorsally into pair of circular plates, further expanded anteriorly into pair of heavily sclerotized plates that strongly recurve posteroventrally toward circular opening, remaining dorsal to genital fork; dorsal surface of recurved plates tiny grooves and indentations. Hypoproct lightly sclerotized, narrow. Cercus quadrate, projected posteroventrally; bearing numerous setae. Spermathecae not observed; two spermathecal ducts visible in center of lateral arm circle.

Description of pupa. *n* = 4 (Figs. 3.21B, 3.22B, 3.23B, 3.34E).

Length 3.0–3.1 mm.

Colouration. Light brown; with black spot above eyes in developing adult.

Head. Maxillary sheath short, posteromedially directed, gently tapered toward truncate apex, apices of palpi separated medially; devoid of tubercles and setae.

Thorax. 1.25× wider than abdomen at widest point. Foreleg sheath projected straight, slightly shorter than wing sheaths; anterior half of midleg visible anterior to wing sheath, then hidden behind foreleg, apices visible, slightly longer than foreleg, not projected

beyond wing sheath; hindleg concealed behind wing sheath, only apex visible between apex of foreleg and wing sheath, longer than foreleg, extended slightly beyond wing sheath but not reaching hind margin of sternite 2. Wing sheaths not reaching posterior margin of abdominal sternite 2. Respiratory organ slightly longer than maxillary sheath, broadest subapically; ovate, slightly arched medially, tapered toward apex; spiracular openings encircling apex; stalk thin, emerging from small tubercle. Tubercle situated posterodorsally to respiratory organ, rounded, projected slightly posterolaterally; apex well separated from respiratory organ. Thorax devoid of setae.

Abdomen. Subcylindrical, evenly tapered toward caudal segment. Spiracles well developed, distinct on segments 3–7, projected anterodorsolaterally; spiracle on sternite 8 small, indistinct, projected slightly anterolaterally; all bearing minute spine-like setulae. Tergites 1–8 quadrate, devoid of setae, with pair of dorsolateral ridges (indistinct on tergites 1–6) and faint medial transverse groove, not meeting dorsolateral ridges. Tergite 9 rounded, posterior margin emarginated, laterally compressed compared to preceding segments; small, rounded projection pointed posteriorly in lateral view. Sternites 3–8 rectangular, with row of faint setulae along anterior margin, not connecting to lateral margin; lateral margins crenulate, lacking setae. Sternites 3 and 4 bearing pair of small lateral adhesive structures, sternite 5 bearing pair of large lateral adhesive structures on lateral margin. Caudal sternite subquadrate, lacking medial lobes; posterior margin with medial longitudinal groove; without distinct caudal hooks.

Description of larva. *n* = 27 (Figs. 3.24B, 3.25B, 3.26B).

Length of final instar 4.7–5.4 mm. *Colouration.* Head capsule pale brown (sometimes dark brown), anterolateral margin of ecdysial line black. Body creamy brown. *Head*

capsule (Figs. 3.28C, D). Two large, circular eye spots, elevated on tubercle; antenna with three finger-like processes, on largest tubercle; with five pairs of tubercles outside of ecdysial lines (not including antennal and ocular tubercle), all smaller than ocular tubercle; two tubercles between ecdysial lines, upper tubercle larger than lower; 15 pairs of unbranched setae; six sensory pits (13, 14, 18, 19, 20, 21), sensory pit 13 above antennal tubercle. *Thorax*. Prothorax with single pair of protuberances bearing single spiracle; spiracular protuberance bearing one pair of dorsal setae anterior to spiracle and single lateral seta; three mid-lateral setae, two long, one short and fine; two closely approximated setae near base of prothoracic leg (Keilin's organ). Mesothorax and metathorax with pair of small dorsolateral protuberances and pair of large lateral protuberances; dorsolateral protuberance bearing pair of closely approximated setae, anterior seta thickened and longer than posterior seta; lateral protuberance on both segments bearing three setae, two short, one long; single long seta slightly ventral to lateral protuberance; one pair of mid-ventrolateral setae. Prothorax bearing proleg, posterior half with rectangular adhesive structure; meso- and metathoracic sternites with rectangular adhesive structure. *Abdomen*. Abdominal sternites 1–7 modified into circular, suction cup-like adhesive structure; sternite 8 with subquadrate adhesive structure; sternite 9 smooth, bearing anal proleg. Segments 1–7 lacking distinct protuberances, or with pair of dorsolateral swellings bearing single or paired short, thin setae; single seta situated anterolaterally; lateral adhesive structure inflated bearing four setae, two midlateral, two basalateral. Segment 8 with small dorsolateral protuberance on either side of posterior spiracular plate, each bearing pair of small setae; lateral protuberance with single seta; single short ventrolateral seta; ventral sternite bearing pair of setae. Posterior

spiracular plate with sclerite encircling procerci; procercus shorter than length of spiracular plate, bearing four setae, two thick, two slender; without cone-like protuberance on either side of cerci. Terminal segment with pair of protuberances bearing pair of setae; four lateral setae, two long, two short and fine; two pairs of long setae on posterior margin, above pair of anal papillae; ventral sternite without setae.

Additional Material Examined. CHILE. REGION X (Los Lagos): Chiloé, Cascada Tocoihue, 10.xii.2016, 42°18'20.3"S 73°26'08.9"W, elev. 32 m, smaller falls, J.K. Moulton & R.J. Pivar (2♂, 4♀*); Ensenada, nr. Baños de Petrohué, 12.i.1985, J.A. Downes (13♂, 2♀, CNC); Isla Chiloé, Ancud, xii.1926, R. & E. Shannon, USNMENT01115824 (1♂, USNM) [Note: There is an additional USNM specimen identified as *Ni. nudipennis* from this same collection event; however, the abdomen is missing and species identification cannot be confirmed (USNMENT01115825)]; Rte. V-69, 12.xii.2016, 41°19'51.5"S 72°24'40.0"W, elev. 129 m, roadside seep, J.K. Moulton & R.J. Pivar (2♂); Rte. V-69, 12.xii.2016, 41°31'48.8"S 72°17'31.2"W, elev. 39 m, trickling creek, J.K. Moulton & R.J. Pivar (5♂); same label data as previous except, larvae/pupae on foliage in splash zone (27 larvae*, 4 pupae, 3 pupal exuviae); REGION XIV (Los Ríos): Rte. T-85, 13.xii.2016, 40°19'58.7"S 72°16'54.8"W, elev. 145 m, foliage around waterfalls, J.K. Moulton & R.J. Pivar (1♂).

Distribution. Known from both the Chilean Coastal Range and the Andes of southern Chile (Fig. 3.32B).

Bionomics. *Niphta nudipennis* is a low-elevation species inhabiting the Valdivian temperate rainforest. Adults were collected mainly from foliage around splash zones. The larvae possess the ventral adhesive structures found in the *Ni. halteralis* group; however,

they were only collected from overhanging vegetation in the splash zone. Vegetation included both living and dead plant tissue, on textures spanning smooth leaf surfaces, to more textured fern fronds and herbaceous stems. Pupae were collected from the same habitats and also possess adhesive structures. The amount of water splashing on the vegetation appeared to be just enough to keep it damp enough to keep the immatures alive.

***Niphta sinclairi* Pivar sp.n.**

Type material. Holotype: ♂, glued to point with abdomen in glycerine microvial pinned beneath, labelled: “CHILE: Region VII (Maule)/ Los Queñes, Rte. J-25,/ 6.xii.2016, 34°59'46.7"S 70°49'19.2"W/ elev. 679m, cascading creek,/ J.K. Moulton & R.J. Pivar”; “HOLOTYPE/ *Niphta/ sinclairi/* Pivar” (CNC). **Allotype:** ♀*, same label data as holotype (CNC). **Paratypes:** CHILE: REGION VII (Maule): Los Queñes, Rte. J-25, 6.xii.2016, 34°59'48.8"S 70°48'37.0"W, elev. 684 m, seep, J.K. Moulton & R.J. Pivar (1♀*).

Recognition. This species is recognized by the cheliform gonostylus with non-bifurcate apices and the gonocoxite with two projections, the anterior one long and bifurcate, the posterior one small, tooth-like. It is lighter in colouration than the closely related *Ni. brunnea*.

Description of male. *n* = 1.

Length 1.9–2.4 mm.

Colouration (Figs. 3.10G, 3.11G). Head dull, dark brown; pronotum and postpronotum

brown; postpronotal lobe and lateral margins of prescutum pale yellow; remaining scutum shiny, brown, pleura yellow; postscutum with medial brown spot above scutoscutellar suture, encircled by yellowish-brown margin; scutellum shiny, yellow; mediotergite shiny, dark brown except anterior margin yellow; katepisternum mainly brown, lighter near anterior spiracle; paratergite brown; remaining pteropleuron mainly pale yellow with dispersed brown markings; base of halter yellow, distal half of stalk and knob grey; legs pale yellow, apex of tarsi darker; abdomen brown; terminalia light brown.

Head. Eyes above antennae broadly joined, small triangular frons visible above antennae; frons with 3 strong setae. Flagellomeres 1–3 subquadrate, 1 expanded, 1.5× as wide as next segment, equal in length to 2 and 3 combined; flagellomeres 4–10, cylindrical, becoming progressively more thin and elongate. Vertex clothed in black setae of uniform length, with long, black orbital setae.

Thorax. Mesoscutum with prominent antealar ridge, bearing three setae, middle seta most pronounced. Scutum clothed dorsally in short, black setulae; notopleural, supra-alar and postscutum setae long, black. Scutellum with row of long, black marginal setae.

Proepisternal setae absent. Pteropleuron bare. All legs with tarsi simple.

Wing. Wing length: 2.0–2.4 mm. Lightly infusate throughout, apex somewhat narrowed; C fringed in small setulae, with widely spaced microtrichia; posterior wing margin with closely spaced fringe of microtrichia; Sc incomplete; R_1 and $R_1(+R_{2+3})$ with three weakenings or depigmented gaps, first slightly beyond R_{2+3} , second and third closely approximated, near C; microtrichia of $R_1(+R_{2+3})$ confined to base near humeral crossvein, remaining veins bare; R flexed into cell br; R_{2+3} distinct, situated in basal third of

R₁(+R₂₊₃); bend in R₄₊₅ gentle; R₄₊₅ and M₁ running parallel toward margin; M₁ straight; M₂ with gentle bend in apical third; M₄ with slight bend; CuA without basal appendage.

Abdomen. Abdominal sternite 1 narrow, spectacle-shaped; sternite 2 reduced to slender median sclerite, few setae restricted to posterior third; sternites 3–7 rectangular, lightly sclerotized, setae restricted to posterior half; sternite 8 strongly reduced, anterior margin well sclerotized, arched slightly into preceding segment, a few setae restricted to laterad.

Male terminalia (Figs. 3.17C, 3.18C). Epandrium quadrate in ventral view, posterior margin rounded, with narrow medial cleft; long, extended beyond gonostyli; without lobes or projections. Hypandrium absent. Gonocoxites oblong, wider than long; anterior margin rounded, somewhat expanded dorsally behind gonocoxal plate, not closely approximated; with two spine-like projections; anterior projection bifurcate, projected posteriorly, 3x longer than posterior projection; posterior projection short, tooth-like; margin around gonostylus setose. Gonostylus cheliform, dorsoventrally flattened, margins concave causing bowl-like appearance; anterior apex with a few indistinct setae, posterior apex with strong setae. Parameres medially fused, attached basally to arms of gonocoxal plate; divided distally into dorsal parameral apodeme and ventral arm; ventral arm projected anteroventrally toward gonocoxal plate, strongly curved anteriorly, blade-like, ventral margin serrate; ventral arm extends posteroventrally presumably to aid in copulation; when retracted, rests ventrally between dorsal arm of gonocoxal plate and inner margin of gonocoxite, apex reaching base of anterior gonocoxal projection.

Gonocoxal plate broad, well sclerotized; anterior margin subquadrate, basal margin cleft; pair of dorsal arms connected to parameres; medial aedeagal guide projected ventrally between gonostyli, well sclerotized, comprising two parts: anterior Y-shaped structure

and posterior triangular plate. Cercus prominent, ovoid, projected anteroventrally, situated within epandrial indentation.

Description of Female. *n* = 2.

Similar to male except as follows: *Abdomen*. Tergite 9 noticeably more sclerotized than preceding tergites; sternite 8 well sclerotized, with distinct blunt projection at base of hypogynial valve. *Female terminalia* (Figs. 3.19C, 3.20C). Hypogynial valve not projecting beyond tergite 9; posterior margin deeply cleft in ventral view, forming two quadrate lobes; lobes densely setose, with both stout, thickened setae and thinner, long setae with slight apical bend; hypogynial protuberance between valves. Tergite 9 subquadrate in lateral view, 2x as wide as tergite 8, lacking lateral projections. Sternite 9 (genital fork) slender, Y-shaped at both ends; lateral arms forming complex of highly modified structures: medial heavily sclerotized circular opening, dorsal to posterior opening of hypogynial valve, with pair of lateral sclerotized triangular expansions; triangular expansions expanded dorsally into pair of circular plates, those further expanded anteriorly into pair of heavily sclerotized plates, then strongly recurve posteroventrally toward circular opening, remaining dorsal to genital fork; dorsal surface of recurved plates with tiny grooves and indentations. Hypoproct lightly sclerotized, narrow. Cercus quadrate, projected posteroventrally; bearing numerous setae. Spermathecae not observed.

Immature Stages. Unknown.

Additional Material Examined. Known only from the type series.

Distribution. Known only from the type locality in central Chile (Fig. 3.32B).

Etymology. *Niphta sinclairi* is named in honour of Dr. Bradley J. Sinclair (Canadian

Food Inspection Agency and Canadian National Collection of Insects), not only in recognition of his many contributions to the taxonomy and knowledge of Thaumaleidae, but also for his mentorship during Pivar's graduate school research.

DISCUSSION AND CONCLUSIONS

Species-groups

Austrothaumalea

McLellan (1988) and Sinclair (2008b) proposed six species groups for *Austrothaumalea*. Sinclair (2008b) hypothesized that the South American *Austrothaumalea* spp. belonged to two species groups: (1) *A. chilensis* and *A. setipennis* within the *A. capricornis* Theischinger group, and (2) *A. apicalis* and *A. spatulata* as possible members of the *A. fusca* Theischinger group. The *A. apicalis* group assignment was initially based on the holotype female, but now that the male is described herein, it is possible to confirm its placement in the *A. fusca* group on the basis of the truncate gonocoxal plate. *Austrothaumalea fredericki* is somewhat of an outlier compared to other members of the genus, in that the gonocoxal plate is U-shaped and broad. The parameres, however, are similar to those of *A. apicalis* and *A. spatulata*: spatulate apically, but with the addition of mid-lateral arms in *A. fredericki*. Perhaps *A. fredericki* is a more derived member of the *A. fusca* species group, with a reduction in the gonocoxal plate to allow the lateral arms of the parameres to be used during mating. Sinclair (2008b) suggests an *A. fusca* subgroup characterized by forked gonostyli consisting of Australian species, while the Chilean species have simple gonostyli; both *A. apicalis* and *A. fredericki* support this hypothesis. The Australian *A. fusca* appears to be an intermediate species

between the two subgroups, as it has simple gonostyli like the Chilean species, but a more conical/rod shaped paramere, as seen in the Australian group members.

Niphta

Until now, only five species of *Niphta* were described and few phylogenetic affinities have been discussed; Theischinger (1986) suggested that the Australian *Ni. farecta* Theischinger was more closely allied to the Chilean *Ni. nudipennis* than the other Australian species. With the additional nine species described herein, it is possible to better assess relationships based upon morphology. Species groups are proposed below for the genus *Niphta*. The only Australian species studied firsthand for this study was *Ni. farecta*, while conclusions regarding the placement of *Ni. bickeli* Theischinger and *Ni. collessi* Theischinger were based on original descriptions by Theischinger (1986). Firsthand study of these two additional species, and additional undiscovered ones, will assist in future evaluation and interpretation of these groups.

The *Ni. bickeli* group: This group is defined by broad gonocoxites extending to the posterior epandrial margin and lacking projections; long gonostyli; parameres fused medially, then separating into two arms that do not project anteriorly; cerci not discernible. This group is greyish-blackish in colouration. Additionally, immatures of *Ni. collessi* lack ventral adhesive structures and protuberances on the larval head capsule; these are tentatively seen as important characters uniting this species group until further Australian immatures are discovered and accurately associated with the adults (see discussion below comparing *Niphta* immatures). The *Ni. bickeli* group is found in Australia and includes the following species: *Ni. bickeli*, *Ni. collessi* and *Ni. farecta*.

The *Ni. halteralis* group: This group possesses the following characters: gonocoxites extending to midpoint of epandrium, lacking pointed projections; short and narrow gonostyli; parameres fused throughout; prominent cerci projecting anteriorly, extending well beyond the posterior epandrial margin. This group is darkly coloured, mostly black and grey. Immature stages have ventral adhesive structures, and larval head capsules have many protuberances. The *Ni. halteralis* group is known from Chile and includes the following species: *Ni. acus*, *Ni. downsei*, *Ni. halteralis* and *Ni. mapuche*.

The *Ni. nudipennis* group: This group is characterized by the gonocoxites extending, at most, to the midpoint of the epandrium, and bearing pointed projections; broad, short gonostyli that are cheliform or finger-like; parameres fused medially that separate into two arms that project anteriorly and may be flexed or extended; cerci small, projecting anteroventrally. Females possess a distinct blunt or pointed projection at the base of the hypogynial valves, as well as a highly modified sternite 9 (genital fork and lateral arms). Sternite 9 is greatly expanded and heavily sclerotized, presumably to be able to handle the highly modified male genitalia. This group tends to be lighter in colouration and is known from Chile. Immature stages have ventral adhesive structures, and larval head capsules have many protuberances. It includes: *Ni. brunnea*, *Ni. courtneyi*, *Ni. daniellae*, *Ni. eurydactylus*, *Ni. moultoni*, *Ni. nudipennis* and *Ni. sinclairi*.

Immature stages of South American Niphta

Pivar *et al.* (2018b) described the first immatures for South American thaumaleids, where the larva and pupa of *Ne. atlantica* were described. Sinclair (2000) described the larva and pupa of *Ni. collessi*, an Australian species and the first for the

genus. The remarkable immatures described herein are the first for South American *Niphta* and reveal unique evolutionary adaptations for the family. Unlike other described thaumaleid species, including *Ni. collessi*, immature South American *Niphta* are equipped with ventral adhesive structures, resembling suction cups. They are present on nearly all segments of the larva and on segments 3–5 of the pupa, and presumably aid in maintaining their position within the flow of water. The known immatures of the *Ni. halteralis* group species (*Ni. acus* and *Ni. mapuche*) were both collected from rocky substrates (Fig. 3.33), whereas the known immatures of the *Ni. nudipennis* group species (*Ni. brunnea* and *N. nudipennis*) were collected from adjacent vegetation in the splash zones, the first such observation for the family (Fig. 3.34).

Each group's morphology is well adapted to their microhabitat. On a rock face, immatures have to contend with debris being washed down from substrate above. Pupae of the *Ni. halteralis* group were collected from exposed microhabitats on the rock face, with no protection from flowing debris. Their stout and stocky body shape, hidden spiracles and small respiratory organs that do not extend far from the body likely help in withstanding any potential debris impact and reduce breakage of exposed appendages. Both pupae and larvae are mottled and much darker in colouration than the pupae of the *Ni. nudipennis* group, offering greater camouflage from predators on the exposed rock face. Larvae have the added vestiture of dorsal tubercles, which help to break up the outline of the body. Immatures of the *Ni. halteralis* group also bear more setae than species of the *Ni. nudipennis* group, perhaps for sensing any debris or predators that may be nearby.

Immatures of the *Ni. nudipennis* group were all collected from plant material,

either living or dead, but always directly in the splash zone. They were collected from both smooth leaf surfaces and more textured vegetation, such as ferns and herbaceous plant stems. Contrary to a rock face, the amount of debris flowing down a leaf is likely minimal. Pupae of the *Ni. nudipennis* group are conoid, with a broad head and thorax and a narrow abdomen, protruding spiracles, and large, laterally projecting respiratory organs. All of these features are indicative of life in a habitat where debris does not pose a problem of displacement, breakage, or even blockage of the respiratory organ. The leaf habitats were generally shaded and hidden from direct view, and coupled with constant splashing, individuals may experience less predation than on a rock face, thereby reducing the need for the dark, mottled colouration and tubercles. Eggs on vegetation were not observed, nor was oviposition by the adults, but they are presumably laid on the vegetation surface.

The presence of the adhesive structures has resulted in behavioural traits that differ from those of other Thaumaleidae. The typical thaumaleid larva will exhibit a characteristic quick, sidewinding motion to evade predation (Sinclair 2000; Pivar *et al.* 2018a). Chilean *Niphta* are much slower when trying to escape, almost caterpillar-like in movement, with slow, undulating motions. The thoracic adhesive structures are rectangular in shape and are more mobile than those of the abdominal segments, which are circular.

Despite the discovery of this new larval morphotype, specimens were never collected in areas of extremely high flow or aggressive splash zones; rather, they were collected in typical thaumaleid habitat that may be found anywhere else in the world (Fig. 3.35). Perhaps ancestral relatives originated in environments with higher water flow, such

as in a river, and the extant species are a result of having adapted to the more familiar recent habitats. The continued presence of the adhesive structures suggests a continuing evolutionary advantage in their present-day habitat.

Comparison of Niphta immatures

Immatures of the Australian *Ni. collessi* (Sinclair 2000) differ notably from Chilean *Niphta* most notably in the presence of the ventral adhesive structures. Several other characters that are also worth comparing.

The pupa of *Ni. collessi* has caudal hooks, much like in *Ne. atlantica*; however, the Chilean species lack caudal hooks or any other projection. There is also a significant reduction in setae, both in number and length. *Niphta collessi* has numerous setae, many of which are long, while members of the *Ni. halteralis* group have very few, short setae. Pupae of the *Ni. nudipennis* group lacks abdominal setae altogether. The respiratory organs of *Ni. collessi* are similar in appearance to those of the *Ni. nudipennis* group, where they are broadest subapically and projected laterally; whereas, those in the *Ni. halteralis* group are shorter and barely projecting laterally. The spiracles of *Ni. collessi* and the *Ni. nudipennis* group are also similar; well developed on sternites 3–7 and mounted on long, narrow, lateral tubercles. The spiracles are barely visible in the *Ni. halteralis* group.

The larvae also exhibit some significant differences and similarities. The biggest difference, aside from the adhesive structures, is in the sculpture of the head capsule. All described Chilean *Niphta* have protuberances and large antennal tubercles, similar to those of *Ne. atlantica*; *Niphta collessi* lacks protuberances and the antennae are on a short

tubercle. Also, much as in the pupae, Chilean *Niphta* larvae exhibit a reduction in setae compared to *Ni. collessi*. Another significant difference is the presence of cauliflower-like protuberances on both thoracic and abdominal tergites. These protuberances are especially prominent in the *Ni. halteralis* group, and are reduced but still present in *Ni. nudipennis* group. Based on Sinclair's (2000) description, it appears that *Ni. collessi* lacks all protuberances and is more reminiscent of *Austrothaumalea* larvae. Similarities between the two fauna are: larval head-capsule with sensory pit 13 near dorsal margin of antenna, head-capsule only with simple setae and caudal lobes flanking posterior spiracular plate absent.

Larvae with ventral adhesive structures have been collected in Australia. Though they have not been reared nor identification confirmed via DNA fingerprinting (attempts to fingerprint were made by the author, but with no success), morphology indicates these larvae are likely a member of *Niphta*. They appear very similar to members of the *Ni. halteralis* group: presence of distinct thoracic and abdominal protuberances, darker colouration, long abdominal setae (longer and more abundant than *Ni. halteralis* group) and pronounced head-capsule protuberances (not as large as *Ni. halteralis* group, but larger than *Ni. nudipennis* group). The adhesive structures appear very similar between all species: thoracic segments rectangular, adhesive structures felt-like; abdominal segments 1–7 circular, margins felt-like with smooth interior; abdominal segment 8 quadrate, felt-like (Figs. 3.30, 3.31, 3.36).

The range of immature morphology within the genus gives insight into the origins of *Niphta*. The Australian fauna exhibits both the thaumaleid ground plan of no ventral modifications and the presence of ventral adhesive structures. This suggests that *Niphta*

originated in the Australian region of Gondwanaland and moved westward toward South America, where all known species exhibit the derived state. Continued sampling on both continents will provide further insight into the evolution of *Niphta*.

Comparison of larval cuticle

During comparisons of the larval stages of *Niphta*, a series of SEM images were taken to view the larval dorsal cuticular sculpture. Specimens from each genus were studied and each revealed unique sculpturing. *Androprosopa* Mik and *Thaumalea* Ruthe exhibit a honeycomb-like reticulated pattern, which was first noted by Boussy *et al.* (1994, fig. 3) in *An. buckae* (Arnaud and Boussy). It is present on all thoracic and abdominal segments to varying degrees. In *An. americana* (Bezzi) (Fig. 3.37), the prothorax exhibits reduced patterning, while the meso-, metathorax and abdominal segments are fully patterned. Meanwhile, *An. becca* (Arnaud and Boussy) (Fig. 3.38), *An. striata* (Okada) (Fig. 3.39) and *Th. verralli* Edwards (Fig. 3.40) all have more pronounced honeycomb patterning on the anterior half of all thoracic segments, while the posterior halves are less intensely patterned; the abdominal segments are fully patterned, as in *An. americana*. The conical lobes flanking the posterior spiracular plate in all of the above species are also reticulated.

Austrothaumalea (Fig. 3.41) shares a similar honeycomb-like pattern; except the sculpture appears to be superficial, as opposed to the porous appearance of the latter two genera. *Austrothaumalea* also exhibits a series of raised lines on the dorsum of each segment. This line is reduced on the thoracic segments and comes in close proximity to the inner margin of the anterior spiracles. The raised line is much more prominent on the

abdomen, forming an almost squid-like pattern on each segment.

The remaining genera do not exhibit the honeycomb reticulated pattern. The cuticle of both *Afrothaumalea* (Fig. 3.42) and *Trichothaumalea* Edwards (Fig. 3.43) lack any kind of discernible pattern; rather, they are covered in pimple-like bumps.

Neothaumalea has a similar pimpled cuticular sculpture, except they appear to be connected by a web-like matrix (Fig. 3.44). It also has elevated protuberances, where the web-like appearance is lost at the base and is instead covered in individual bumps. On the apex of the protuberances and the area anterior to the spiracular plate, the cuticle appears felt-like.

The cuticle of *Niphta* appears to be quite variable in its sculpture. The Australian specimen is densely pimpled throughout, with only some instances of webbing, such as near the anterior spiracle (Fig. 3.36). *Niphta nudipennis* appears to have a more wart-like cuticle (Fig. 3.45), where the bumps are not as well defined as the Australian species. *Niphta acus* has the most complex cuticular structure, ranging from porous regions, to pimpled areas and cauliflower-like protuberances (Fig. 3.46). The protuberances on both Chilean *Niphta* species appear to have central openings, but its function is unknown.

In all genera, the larval cuticle is mainly sculptured dorsally, and somewhat laterally depending on the genus; the ventral cuticle always appears smooth, unless adhesive structures are present, as in most species of *Niphta*. The larval thaumaleid dorsum is likely hydrophobic, as observed when larvae are washed off the substrate and float on the meniscus layer (Sinclair, pers. comm.), and likely enables it to survive in madicolous environments (Vaillant 1956; Hinton 1958; Boussy *et al.* 1994). The larvae of the sawfly *Rhadinoceraea micans* Klug (Hymenoptera: Tenthredinidae) have been

shown to have a superhydrophobic cuticle, due in large part to its cuticular sculpture and the waxy secretions it produces (Voigt 2011; Boevé *et al.* 2013). The thaumaleid cuticle is similar in appearance to the sawfly, and although it has never been tested, perhaps the various sculptures discussed above are enhanced by a waxy coating. Future studies of the diverse larval cuticle of thaumaleids will likely yield interesting results.

Faunal patterns and habitat

South American thaumaleid diversity has now increased to 17 described species (Table 3.1). Additionally, there is the likely new species to be discovered in Ecuador, plus three more new species from Chile that were collected by the authors, but not yet described because they are females. Since males are the easier sex to identify, the authors have decided to wait until further material is collected before describing these females. Both morphological and molecular data (Pivar, Chapter 4) support the presence of these new species. Of the described species, only one is not from the Andes Range; *Neothaumalea atlantica* is recorded from the Atlantic Forest of southeastern Brazil, in the Serra Geral mountains (Pivar *et al.* 2018b). The remainder of the species are from a roughly 2000 km stretch of the south-central Chilean Andes, with only three records from the Argentinian side, all from Bariloche. The Andes are the longest mountain range in the world at roughly 7000 km long, running from the southern tip of Chile, north to Venezuela. There are only 16 described species from ~20% of sampled mountain range. The South American fauna is undoubtedly more diverse than is presently known and current numbers are reflective of under sampling.

In Chile, diversity increases as one moves south. Beginning in central Chile and

progressing south, the following are the known diversity of species per region (including the three undescribed females): Valparaíso (1 sp.), Santiago (2 spp.), O'Higgins (1 sp.), Maule (2 spp.), Bío Bío (5 spp.), Araucanía (7 spp.), Los Ríos (6 spp.), Los Lagos (8 spp.) and Aysén (1 sp.). These numbers are reflective of both the amount of time spent collecting in certain regions and regions sampled; some regions have likely never been sampled (particularly in northern Chile). Available habitat is also a large contributor to diversity. From Bío Bío to Los Lagos, specimens were collected in Valdivian temperate rainforests, characterized by high rainfall and cooler temperatures. Vegetation types include southern beech, laurel and broadleaved forest, bamboo and ferns. With an abundance of mountain streams and waterfalls, similar to the Nearctic Pacific Northwest, there is ample habitat for thaumaleids. From Valparaíso to Maule, habitat availability begins to drop off drastically, in particular from Valparaíso to O'Higgins. These regions are part of the Chilean Matorral ecoregion of central Chile, characterized by a temperate Mediterranean climate and sclerophyllous shrubs and trees, and cacti. Summers are dry and hot, and madicolous habitats scarce, as suggested by the diversity listed above. The western slopes of the Andes, from Santiago in central Chile to northern Peru, become extremely dry as they transition into the Atacama Desert. Suitable habitat is scant in these areas, though the eastern slopes of Argentina, Bolivia and Peru may contain more suitable habitat as they receive more moisture.

Most Chilean species are found in temperate rainforest regions, but some are found in both wet and dry climates (*A. chilensis*, *Ni. acus*). The type of madicolous habitat (*ie.* creek, rock face seep, stream, waterfall, *etc.*) does not seem to dictate where a particular species may be found; oftentimes, species were collected in multiple habitat

types. Multiple species and genera were frequently collected together, both within the same species group and mixed groups.

Future collections should focus on all areas of the Andes, with an emphasis on the northern sections to provide insight into the northern limits of the family and genera. Currently, the southern-most Nearctic species is *Androprosopa zempoala* Sinclair and Huerta from central Mexico (Sinclair and Huerta 2010) and the northern-most South American species is the undescribed specimen from Ecuador. Do Nearctic and South American genera overlap, or is there a point in which thaumaleids no longer occur, thus separating the fauna of both continents? Does one genus become more abundant than the other at higher/lower latitudes? Other mountain ranges on the continent should be explored, such as the Sierras de Córdoba (central Argentina), the Sierra Nevada de Santa Marta (northern Colombia), and continued studies in Brazil will surely lead to new discoveries. Focused collecting efforts in these regions will answer these questions, as well as divulge the true breadth of South American thaumaleid diversity.

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APPENDIX

Table 3.1. Checklist of South American Thaumaleidae with distribution and described life stages.

Genus	species	Country	Life Stages Known
<i>Austrothaumalea</i>	<i>apicalis</i> Edwards, 1930	Argentina, Chile	Adult (♂/♀)
	<i>chilensis</i> Edwards, 1930	Chile	Adult (♂/♀)
	<i>fredericki</i> sp.n.	Chile	Adult (♂)
	<i>setipennis</i> Edwards, 1930	Argentina, Chile	Adult (♂/♀)
	<i>spatulata</i> Schmid, 1970	Chile	Adult (♂/♀)
<i>Neothaumalea</i>	<i>atlantica</i> Pivar & Pinho, 2018	Brazil	All life stages
<i>Niphta</i>	<i>acus</i> Pivar sp.n.	Chile	All life stages
	<i>brunnea</i> Pivar sp.n.	Chile	Adult (♂), larva, pupa
	<i>courtneyi</i> Pivar sp.n.	Chile	Adult (♂/♀)
	<i>daniellae</i> Pivar sp.n.	Chile	Adult (♂)
	<i>downesi</i> Pivar sp.n.	Chile	Adult (♂)
	<i>eurydactylus</i> Pivar sp.n.	Chile	Adult (♂)
	<i>halteralis</i> (Edwards), 1930	Chile	Adult (♂/♀)
	<i>mapuche</i> Pivar sp.n.	Chile	All life stages
	<i>moultoni</i> Pivar sp.n.	Chile	Adult (♂/♀)
	<i>nudipennis</i> (Edwards), 1930	Chile	All life stages
	<i>sinclairi</i> Pivar sp.n.	Chile	Adult (♂/♀)

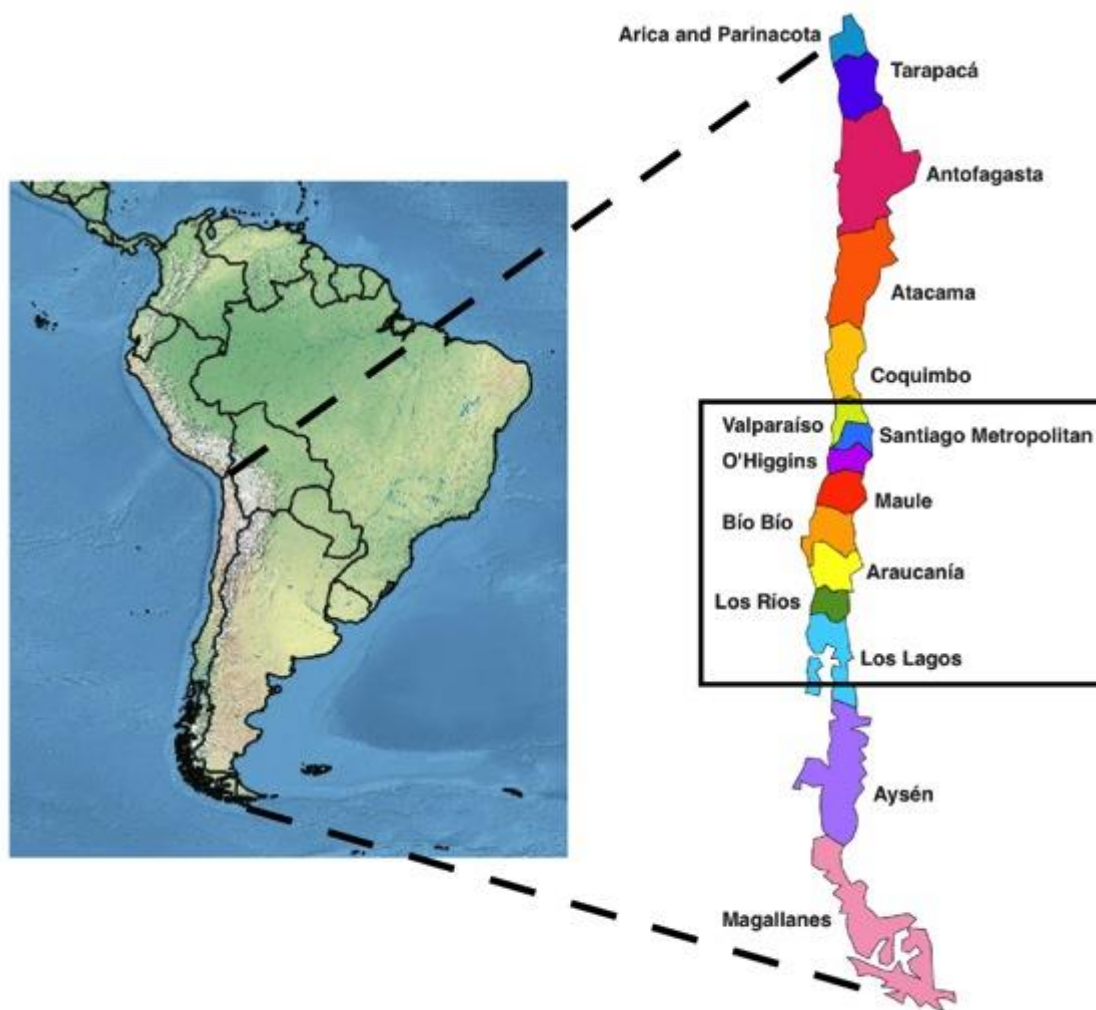


Figure 3.1. Map of South America and Chile. Enlarged map of Chile labeled with regions; box indicates focus of the authors' 2016 collecting trip. Map of Chile modified from <http://www.geocurrents.info/gc-maps/geocurrents-maps-by-country/geocurrents-maps-of-chile>

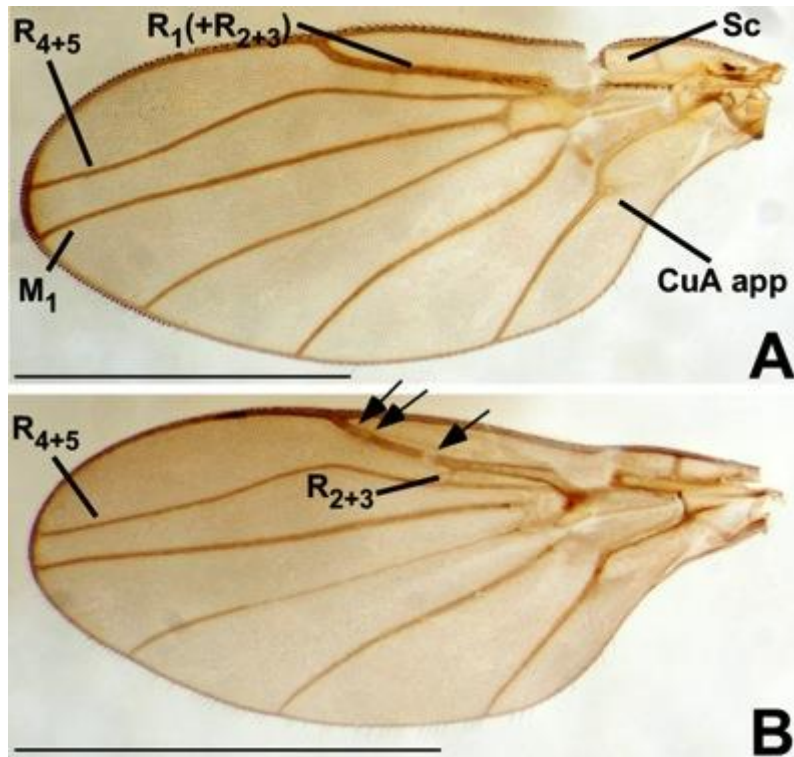


Figure 3.2. Wing exemplars: A, left wing of *Austrothaumalea spatulata*; B, left wing of *Niphta nudipennis* with arrows indicating three depigmented gaps. Abbreviations: CuA app, cubital vein with appendage; M, medial vein; R, radial vein; Sc, subcosta. Scale bars = 1.0 mm.

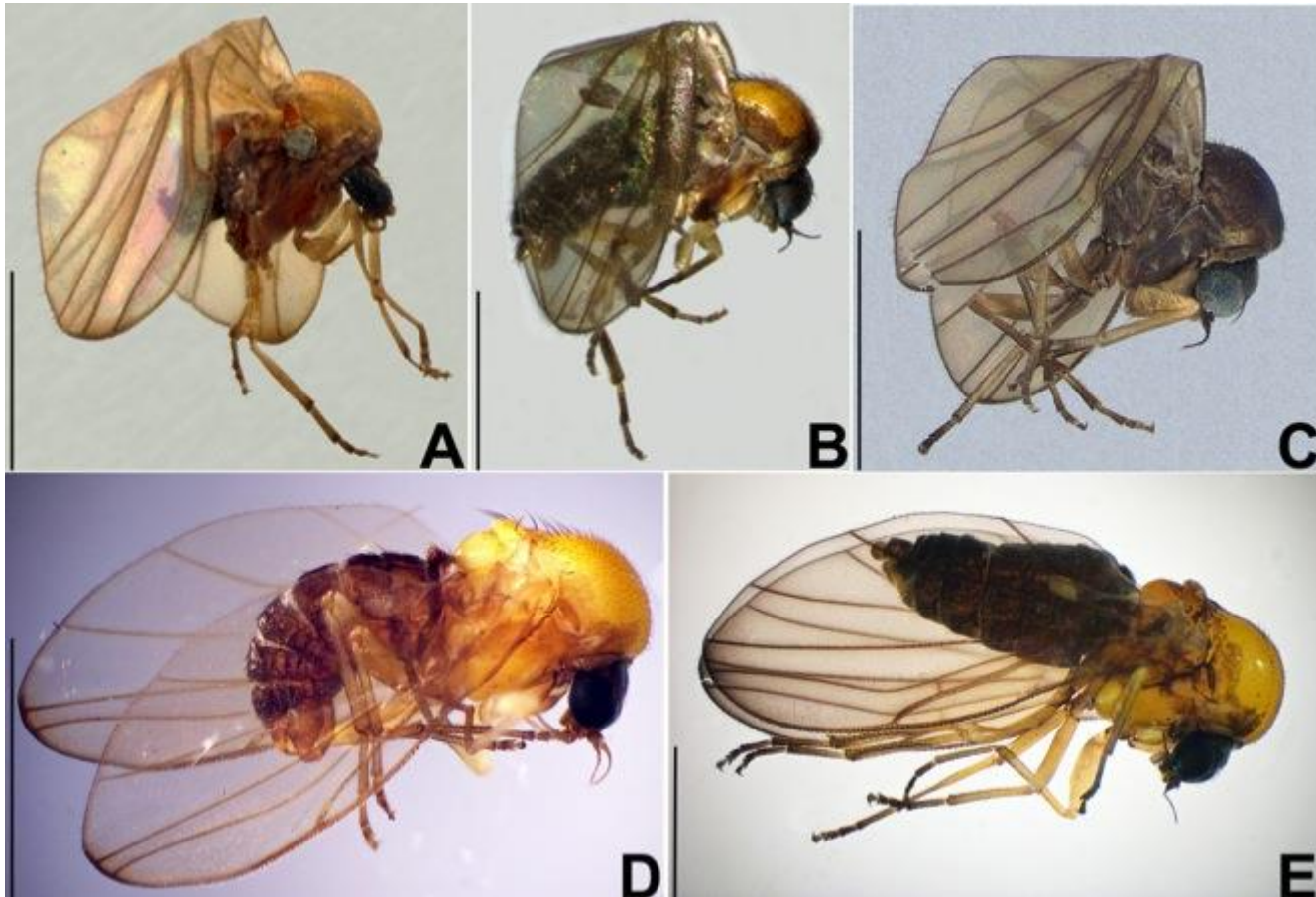


Figure 3.3. Adult lateral habitus micrographs of *Austrothaumalea*: A, *A. apicalis* (♂); B, *A. spatulata* (♂); C, *A. chilensis* (♂); D, *A. fredericki* sp.n. (♂); E, *A. setipennis* (♀). Scale bars = 1.0 mm.

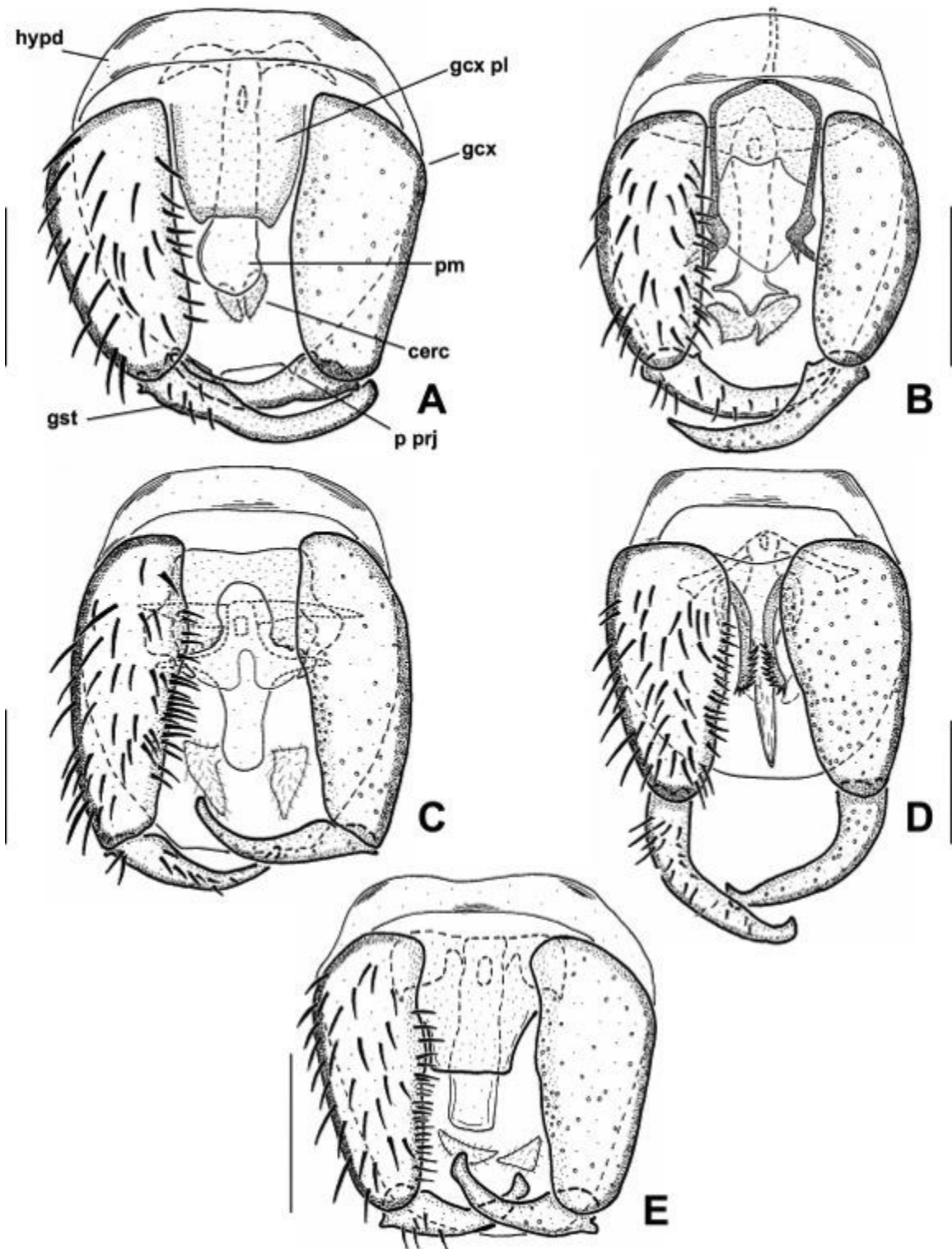


Figure 3.4. Ventral views of male *Austrothaumalea* terminalia: A, *A. apicalis*; B, *A. chilensis*; C, *A. fredericki* **sp.n.**; D, *A. setipennis*; E, *A. spatulata*. Abbreviations: cerc, cercus; gcx, gonocoxite; gcx pl, gonocoxal plate; gst, gonostylus; hypd, hypandrium; p prj, posterolateral projection; pm, paramere. Scale bars = 0.1 mm.

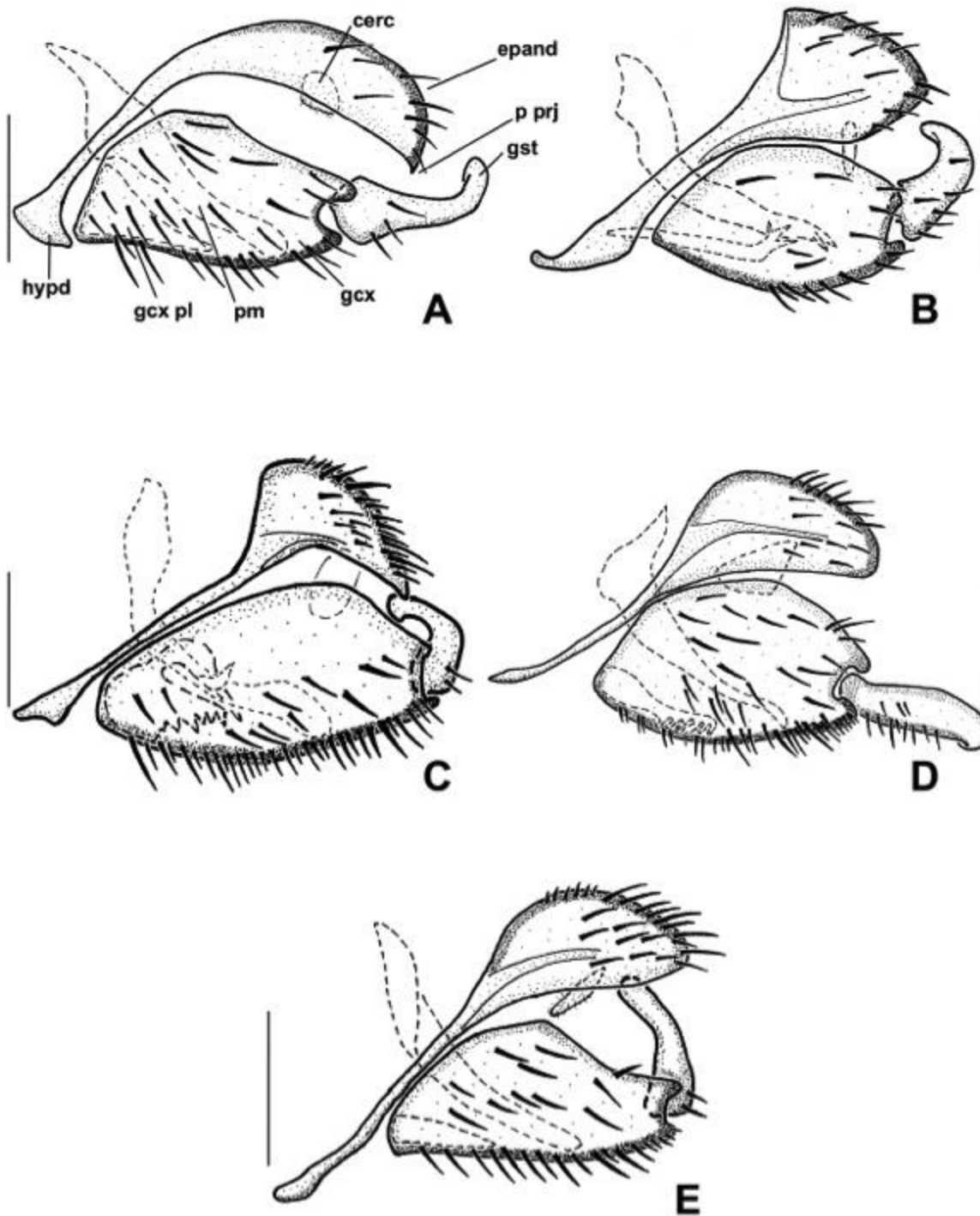


Figure 3.5. Lateral views of male *Austrothaumalea* terminalia: A, *A. apicalis*; B, *A. chilensis*; C, *A. fredericki* **sp.n.**; D, *A. setipennis*; E, *A. spatulata*. Abbreviations: cerc, cercus; epand, epandrium; gcx, gonocoxite; gcx pl, gonocoxal plate; gst, gonostylus; hypd, hypandrium; p prj, posterolateral projection; pm, paramere. Scale bars = 0.1 mm.

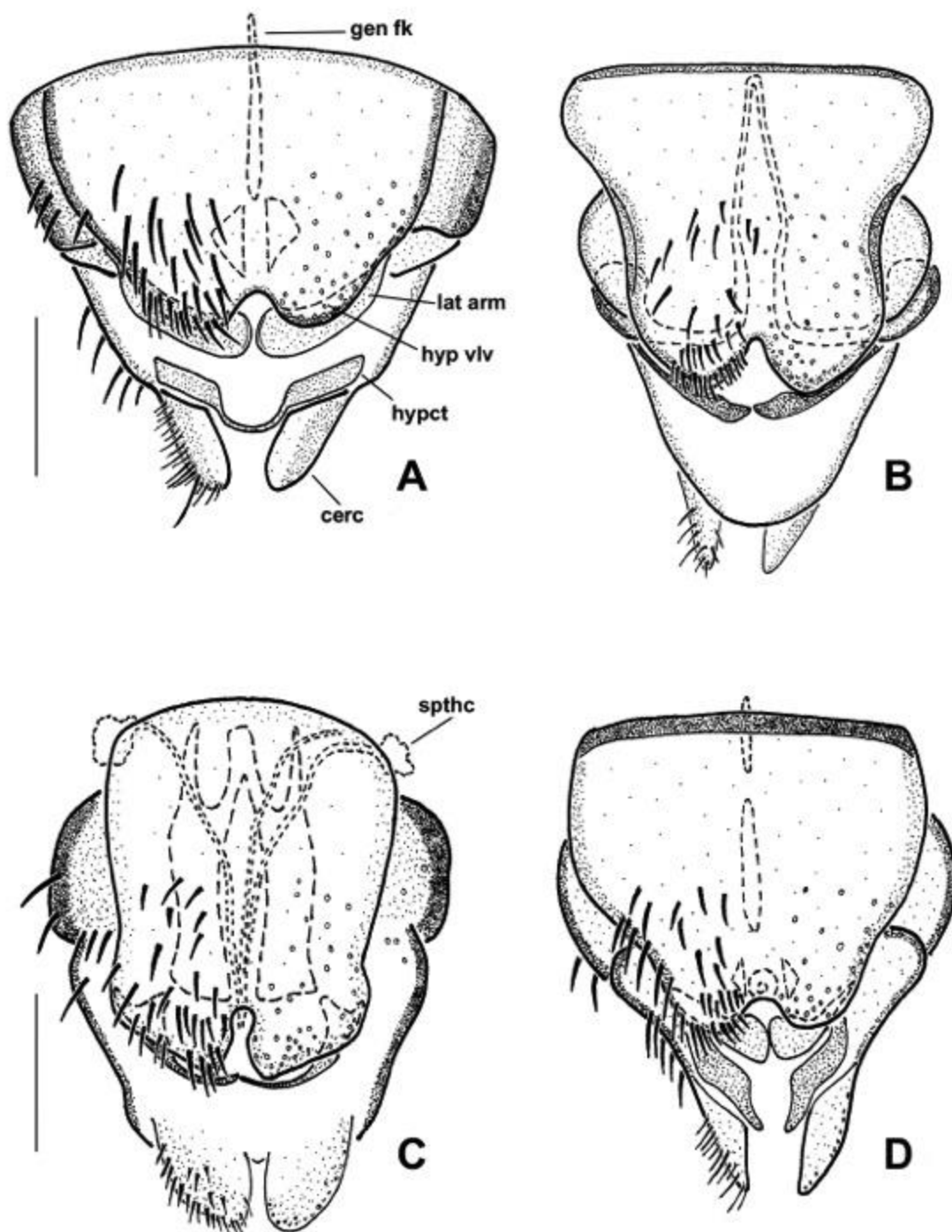


Figure 3.6. Ventral views of female *Austrothaumalea* terminalia: A, *A. apicalis*; B, *A. chilensis*; C, *A. setipennis*; D, *A. spatulata*. Abbreviations: gen fk, genital fork; hypct, hypoproct; hyp vlv, hypogynial valve; lat arm, lateral arms; spthc, spermatheca; T, tergite. Scale bars = 0.1 mm.

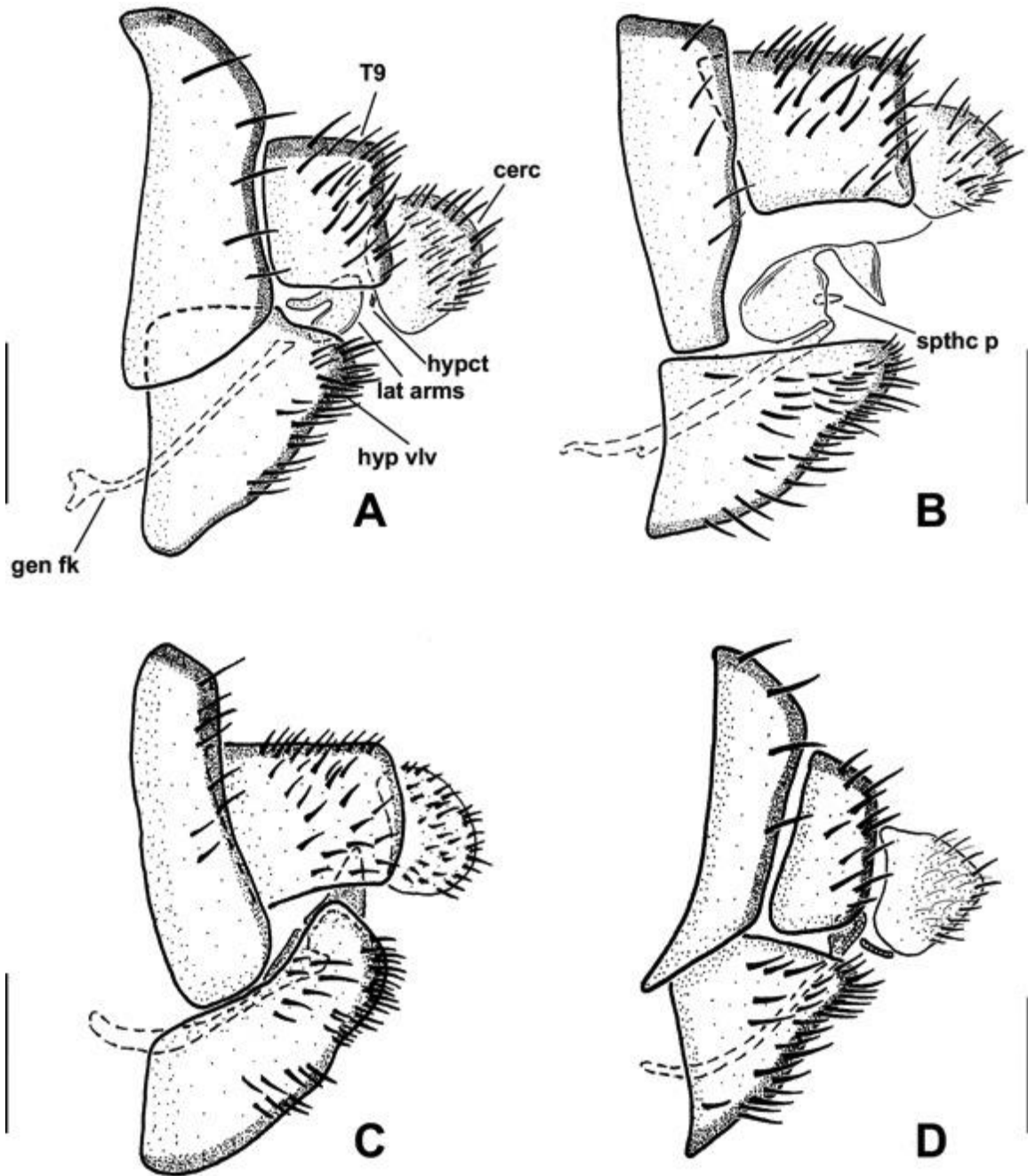


Figure 3.7. Lateral views of female *Austrothaumalea* terminalia: A, *A. apicalis*; B, *A. chilensis*; C, *A. setipennis*; D, *A. spatulata*. Abbreviations: gen fk, genital fork; hypct, hypoproct; hyp vlv, hypogynial valve; lat arm, lateral arms; spthc p, spermathecal pump; T, tergite. Scale bars = 0.1 mm.

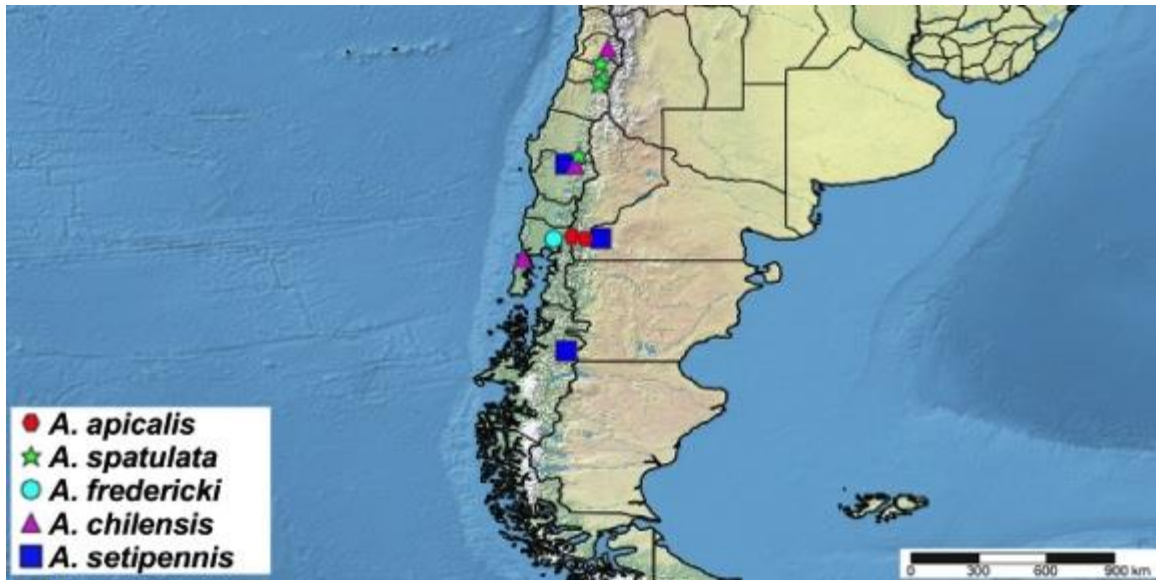


Figure 3.8. Known distribution of South American *Austrothaumalea*.

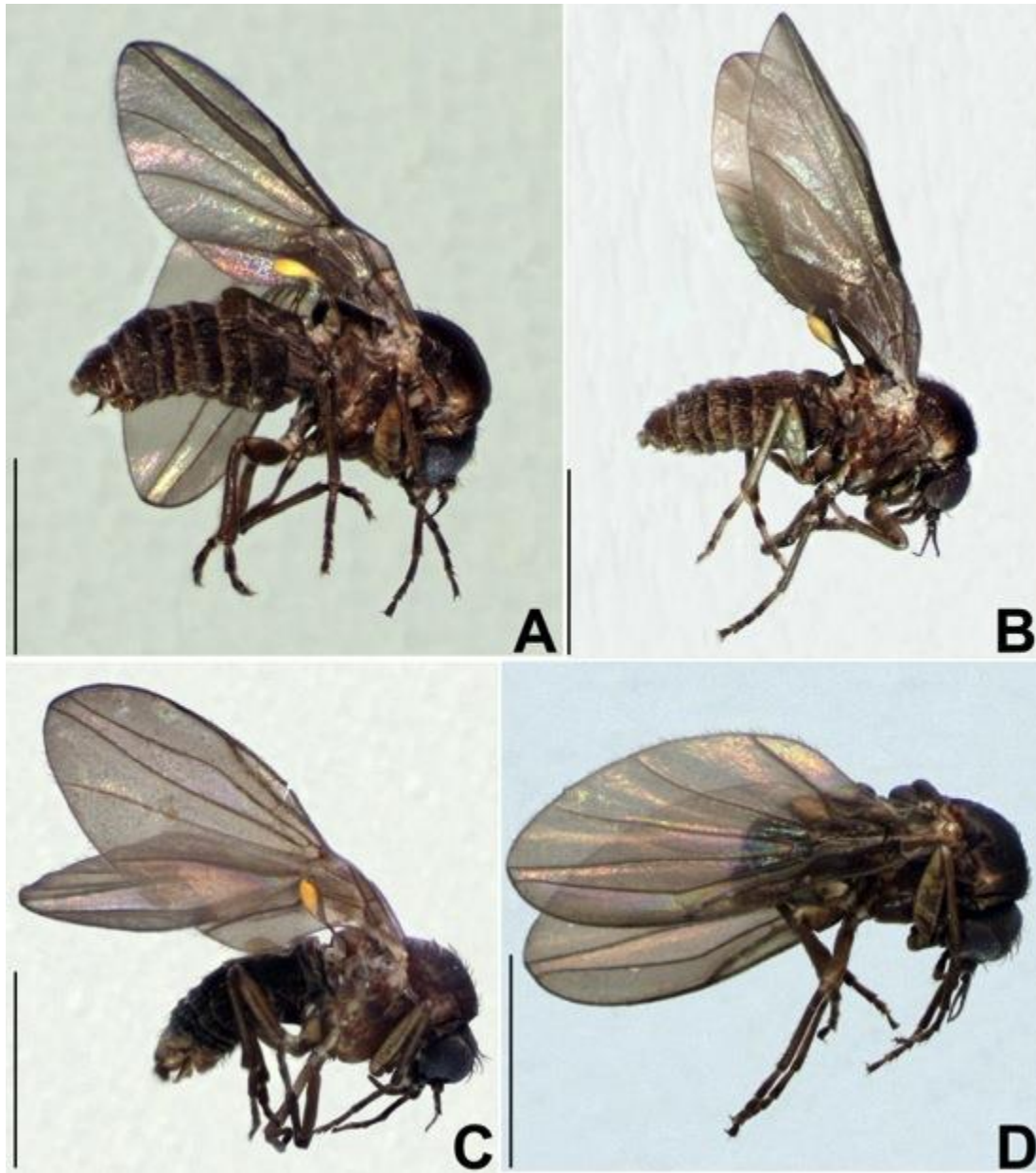


Figure 3.9. Adult male lateral habitus micrographs of the *Niphta halteralis* group: A, *N. acus* **sp.n.**; B, *N. downesi* **sp.n.**; C, *N. halteralis*; D, *N. mapuche* **sp.n.** Scale bars = 1.0 mm.

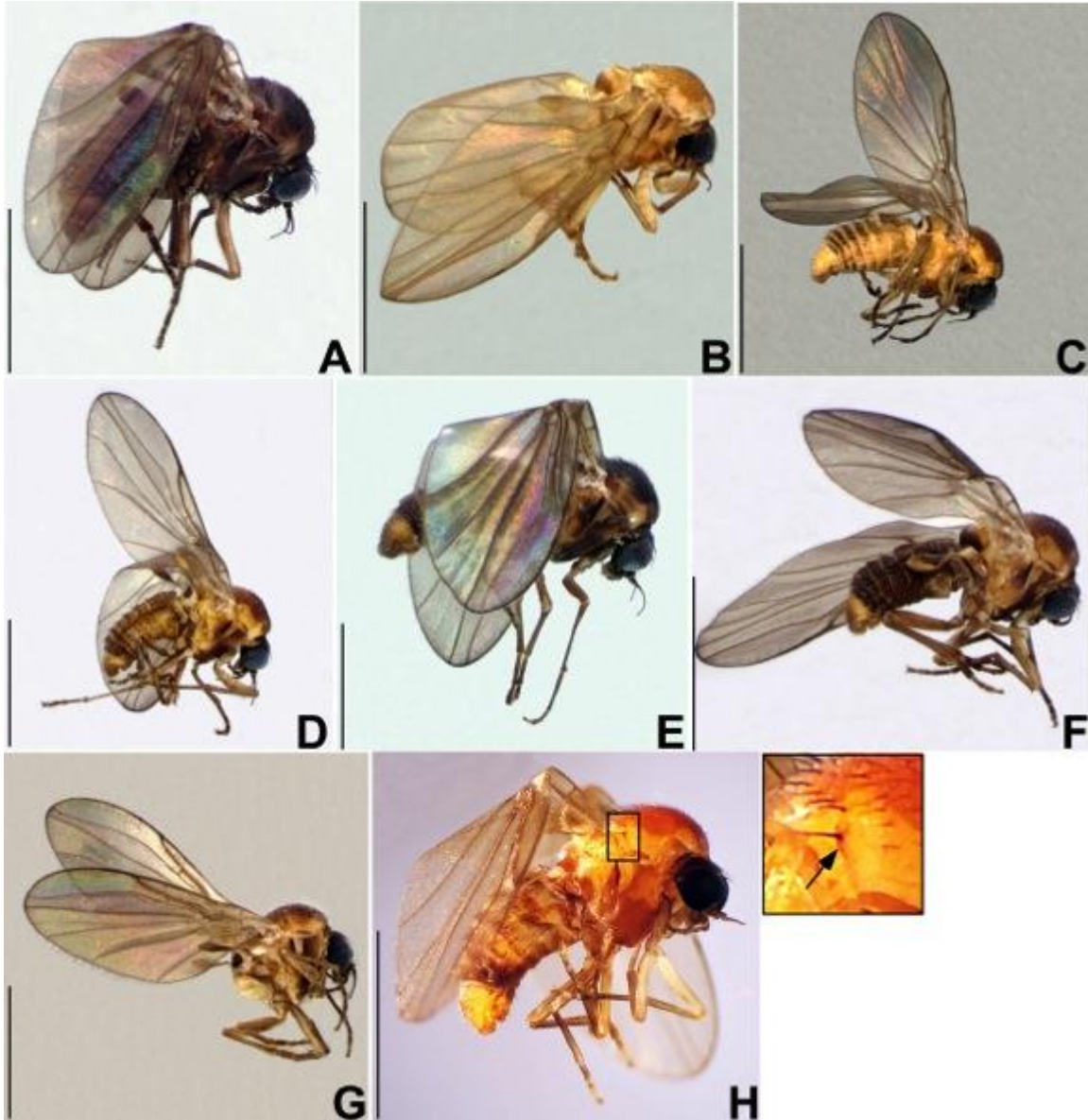


Figure 3.10. Lateral habitus micrographs of the *Niphta nudipennis* group: A, *N. brunnea* **sp. n.** (♂); B, *N. courtneyi* **sp.n.** (♀); C, *N. daniellae* **sp.n.** (♂); D, *N. eurydactylus* **sp.n.** (♂); E, *N. moultoni* **sp.n.** (♂); F, *N. nudipennis* (♂); G, *N. sinclairi* **sp.n.** (♂); H, *N. courtneyi* **sp.n.** (♂), arrow indicating antalar ridge. Scale bars = 1.0 mm.

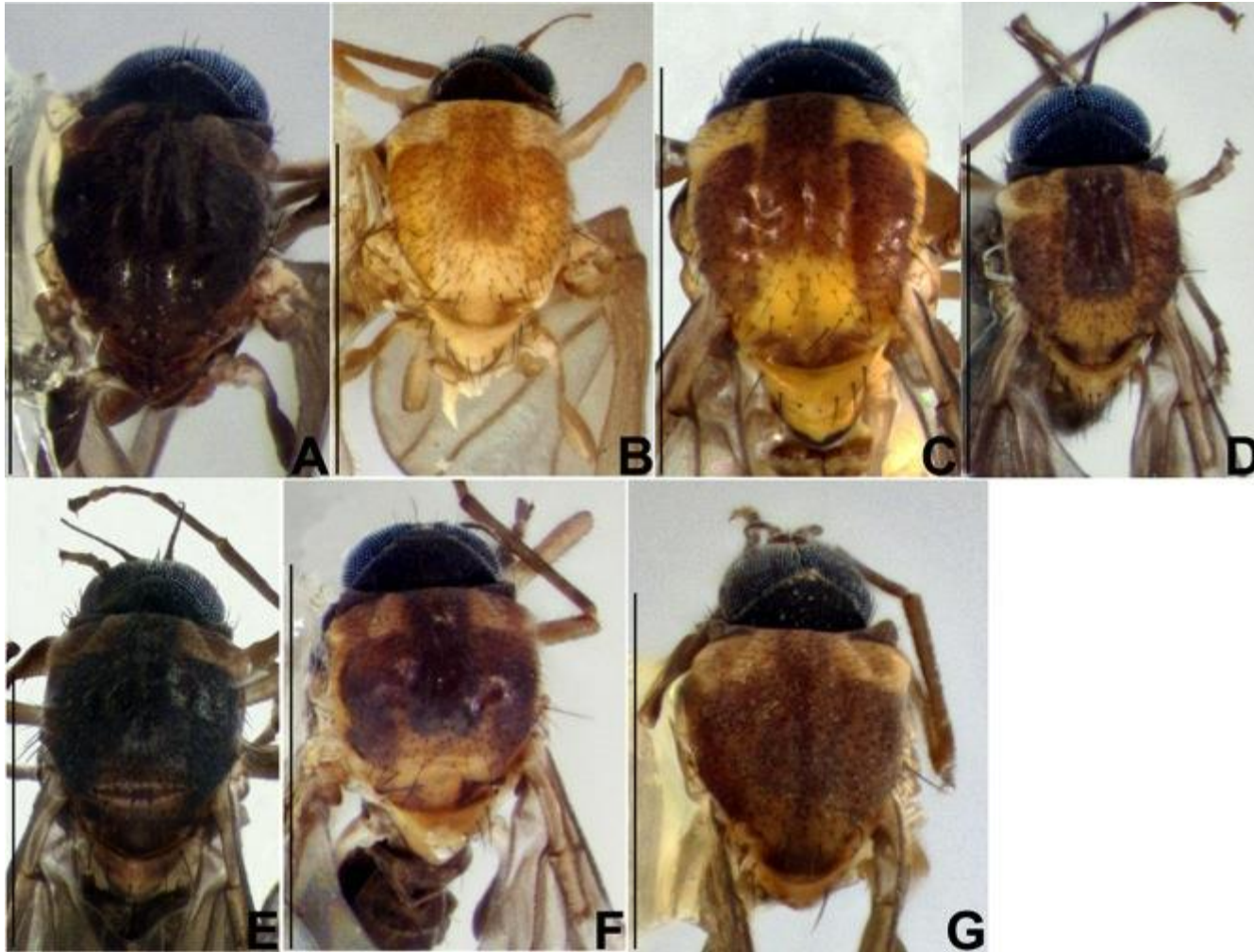


Figure 3.11. Adult dorsal habitus micrographs of the *Niphta nudipennis* group: A, *N. brunnea* **sp. n.** (♂); B, *N. courtneyi* **sp.n.** (♀); C, *N. daniellae* **sp.n.** (♂); D, *N. eurydactylus* **sp.n.** (♂); E, *N. moultoni* **sp.n.** (♂); F, *N. nudipennis* (♂); G, *N. sinclairi* **sp.n.** (♂) Scale bars = 1.0 mm.

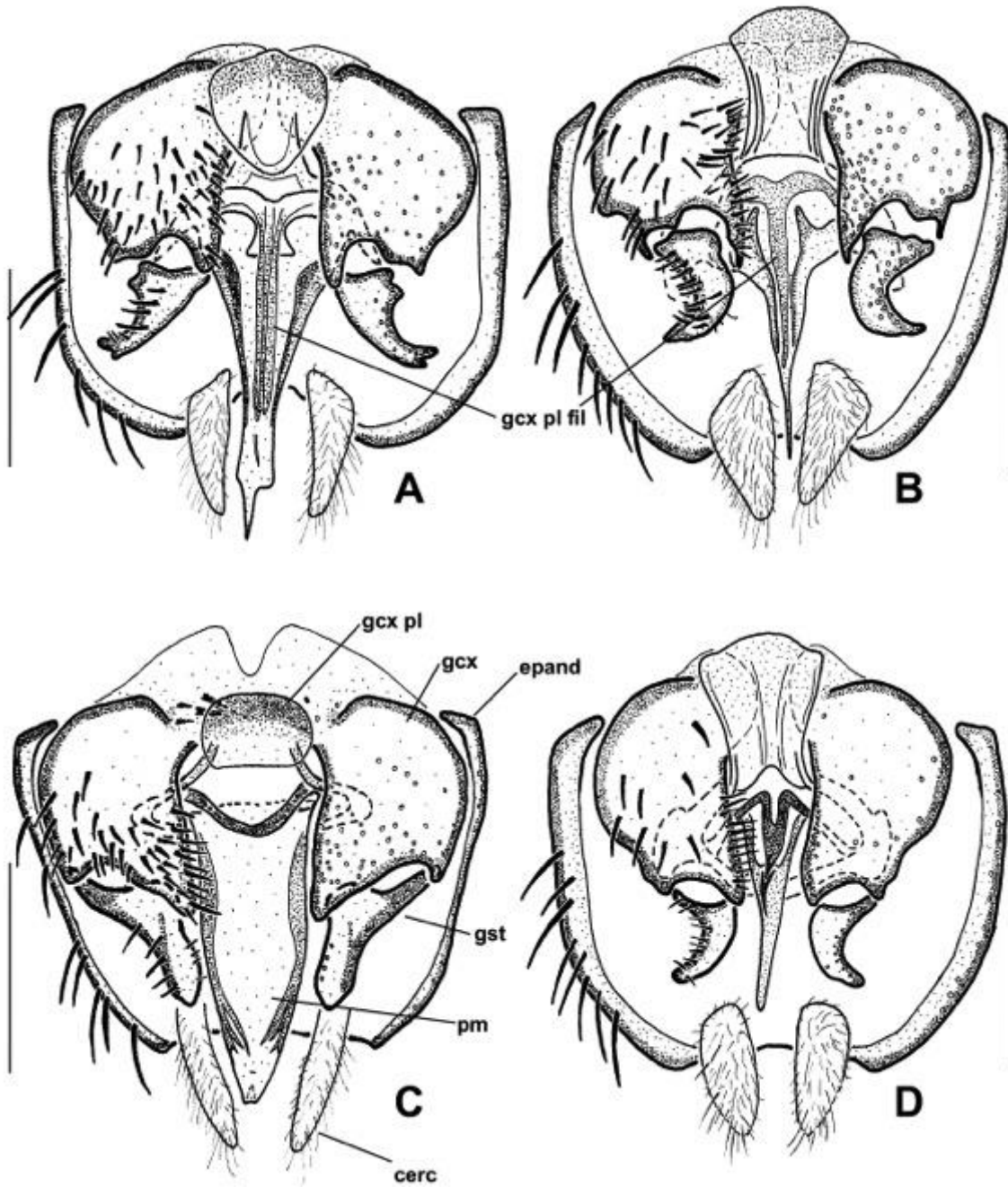


Figure 3.12. Ventral views of male *Niphta halteralis* group terminalia: A, *N. acus* **sp.n.**; B, *N. downesi* **sp. n.**; C, *N. halteralis*; D, *N. mapuche* **sp.n.** Abbreviations: cerc, cercus; epand, epandrium; gcn, gonocoxite; gcn pl, gonocoxal plate; gcn pl fl, gonocoxal plate filament; gst, gonostylus; pm, paramere. Scale bars = 0.1 mm.

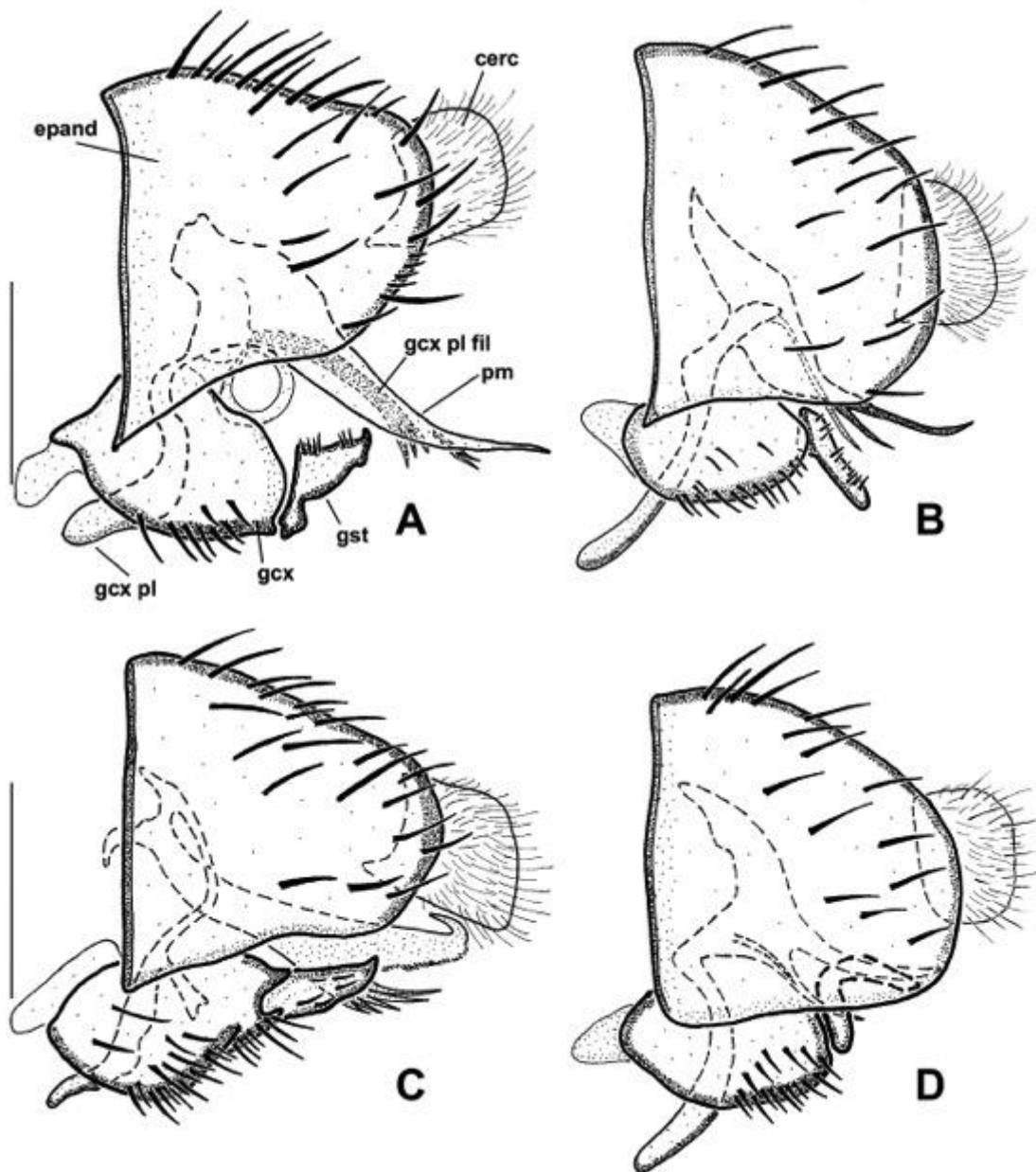


Figure 3.13. Lateral views of male *Niphta halteralis* group terminalia: A, *N. acus* **sp.n.**; B, *N. downesi* **sp.n.**; C, *N. halteralis*; D, *N. mapuche* **sp.n.** Abbreviations: cerc, cercus; epand, epandrium; gcx, gonocoxite; gcx pl, gonocoxal plate; gcx pl fl, gonocoxal plate filament; gst, gonostylus; pm, paramere. Scale bars = 0.1 mm.

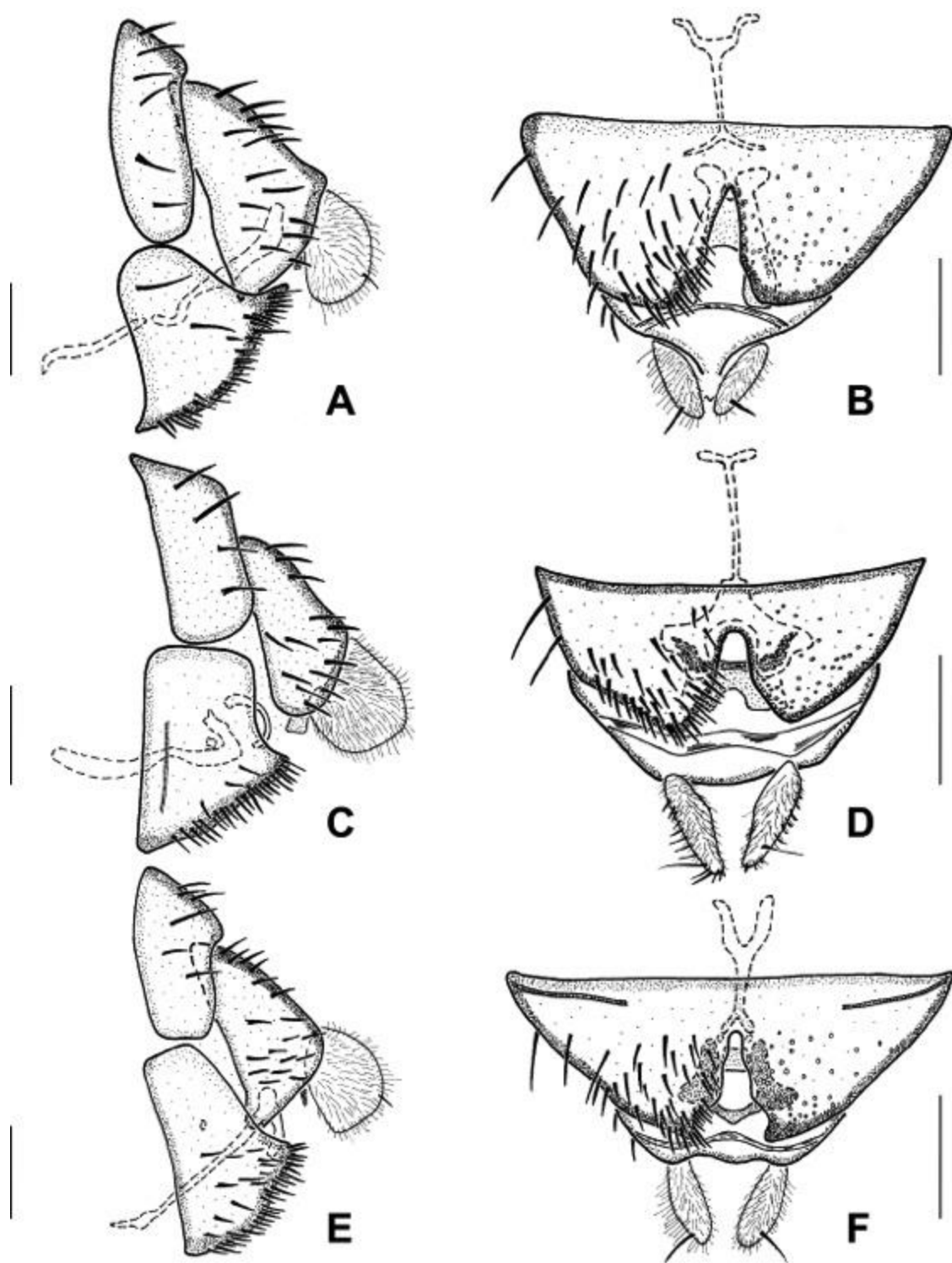


Figure 3.14. Female terminalia of the *Niphta halteralis* group: A, lateral *N. acus* **sp.n.**; B, ventral *N. acus* **sp.n.**; C, lateral *N. halteralis*; D, ventral *N. halteralis*; E, lateral *N. mapuche* **sp.n.**; F, ventral *N. mapuche* **sp.n.** Scale bars = 0.1 mm.

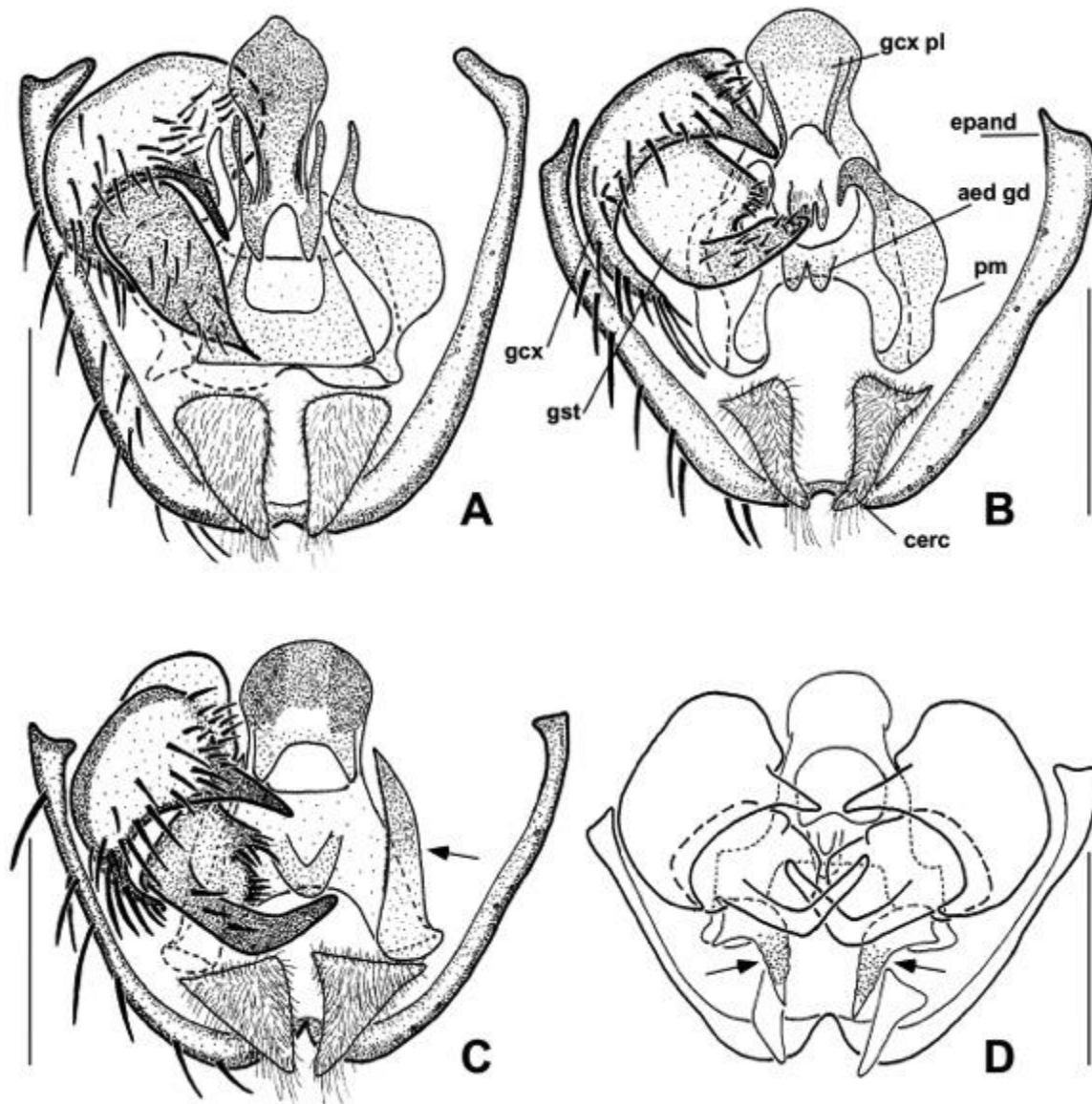


Figure 3.15. Ventral views of male *Niphta nudipennis* group terminalia: A, *N. daniellae* sp.n.; B, *N. eurydactylus* sp.n.; C, *N. nudipennis* with parameres retracted; D, *N. nudipennis* with parameres extended. Abbreviations: aed gd, aedeagal guide; cerc, cercus; epand, epandrium; gcx, gonocoxite; gcx pl, gonocoxal plate; gst, gonostylus; pm, paramere. Scale bars = 0.1 mm.

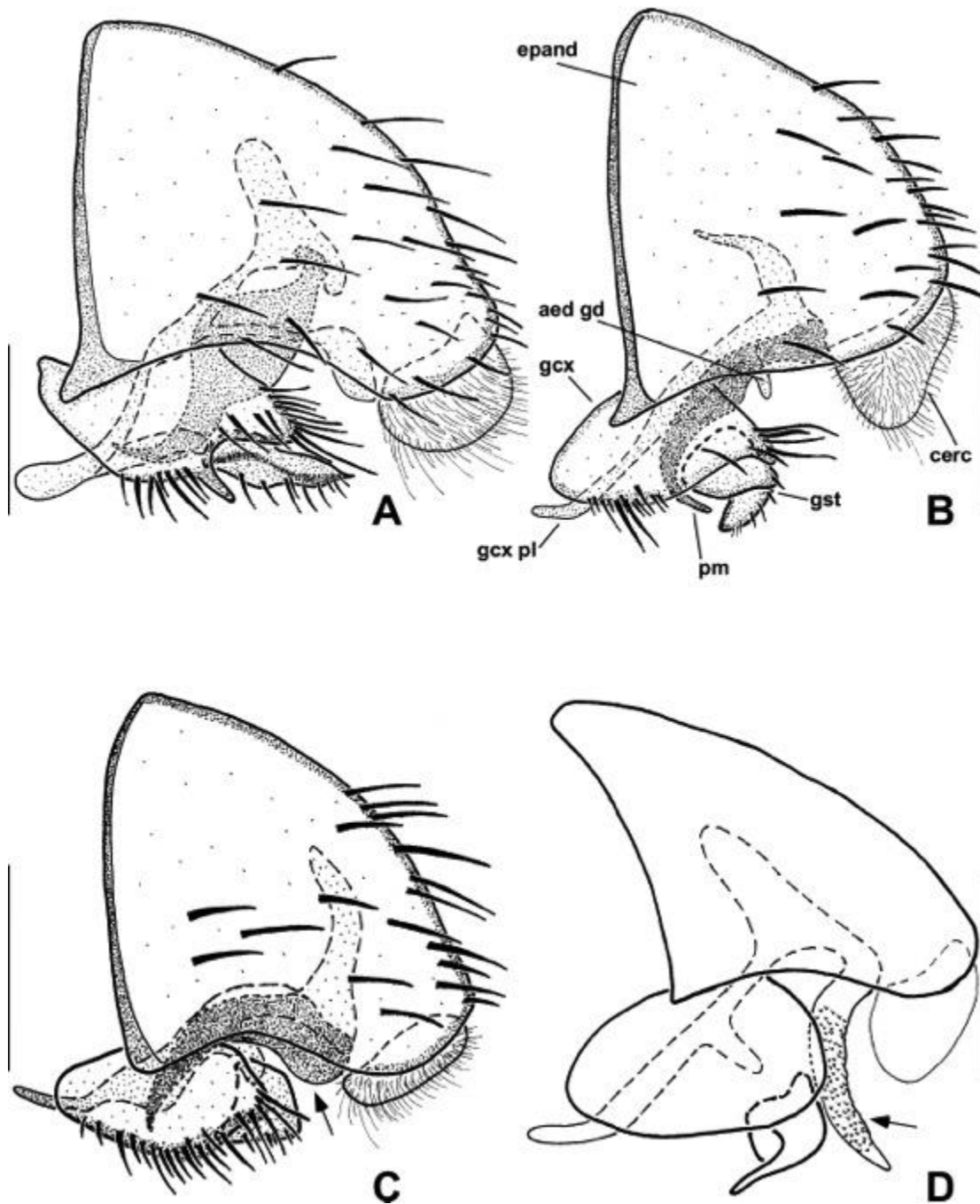


Figure 3.16. Lateral views of male *Niphta nudipennis* group terminalia: A, *N. daniellae* sp.n.; B, *N. eurydactylus* sp.n.; C, *N. nudipennis* with parameres retracted; D, *N. nudipennis* with parameres extended Abbreviations: aed gd, aedeagal guide; cerc, cercus; epand, epandrium; gcx, gonocoxite; gcx pl, gonocoxal plate; gst, gonostylus; pm, paramere. Scale bars = 0.1 mm.

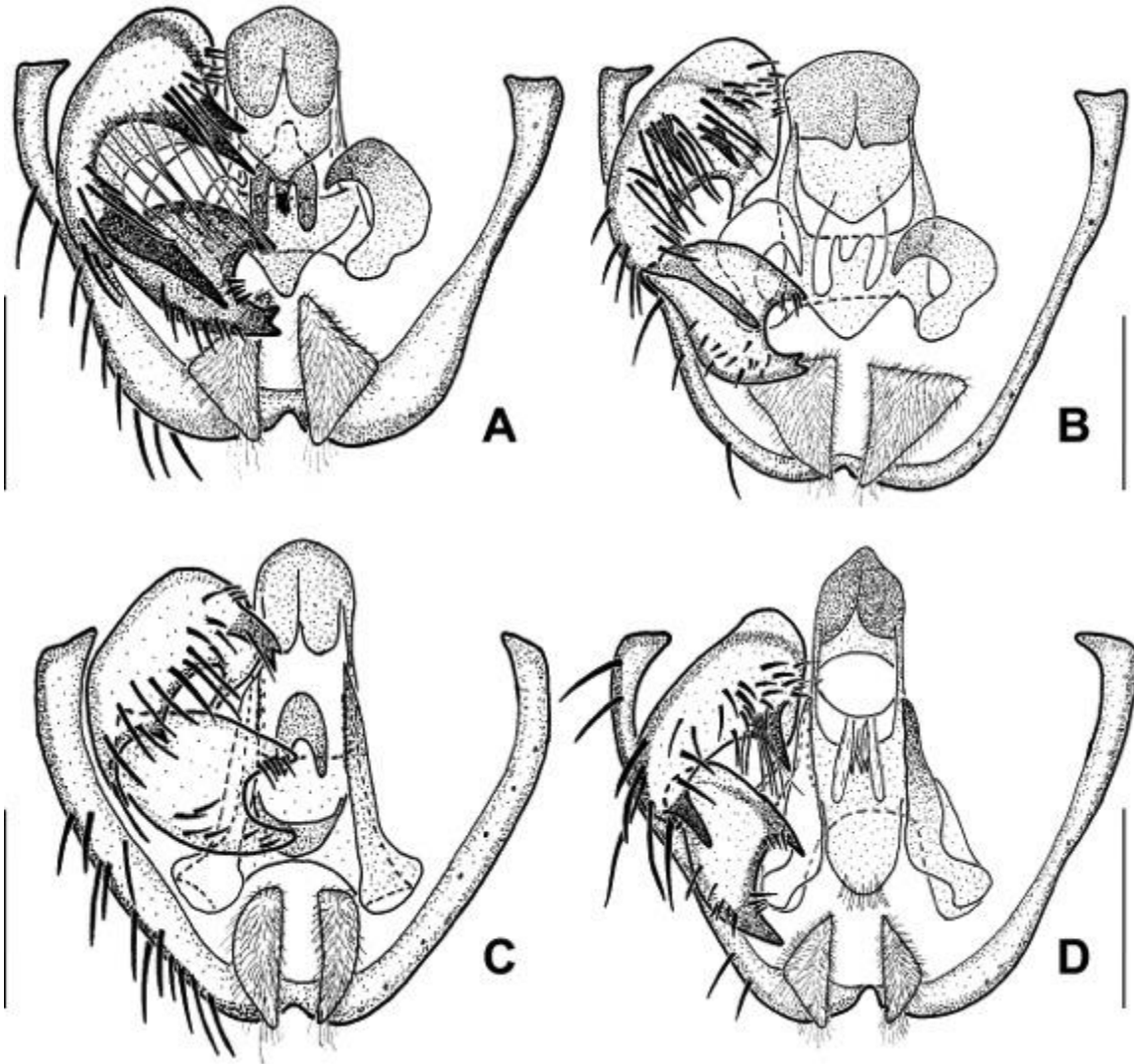


Figure 3.17. Ventral views of male *Niphta nudipennis* group terminalia: A, *N. moultoni* sp.n.; B, *N. courtneyi* sp.n.; C, *N. sinclairi* sp.n.; D, *N. brunnea* sp.n. Scale bars = 0.1 mm.

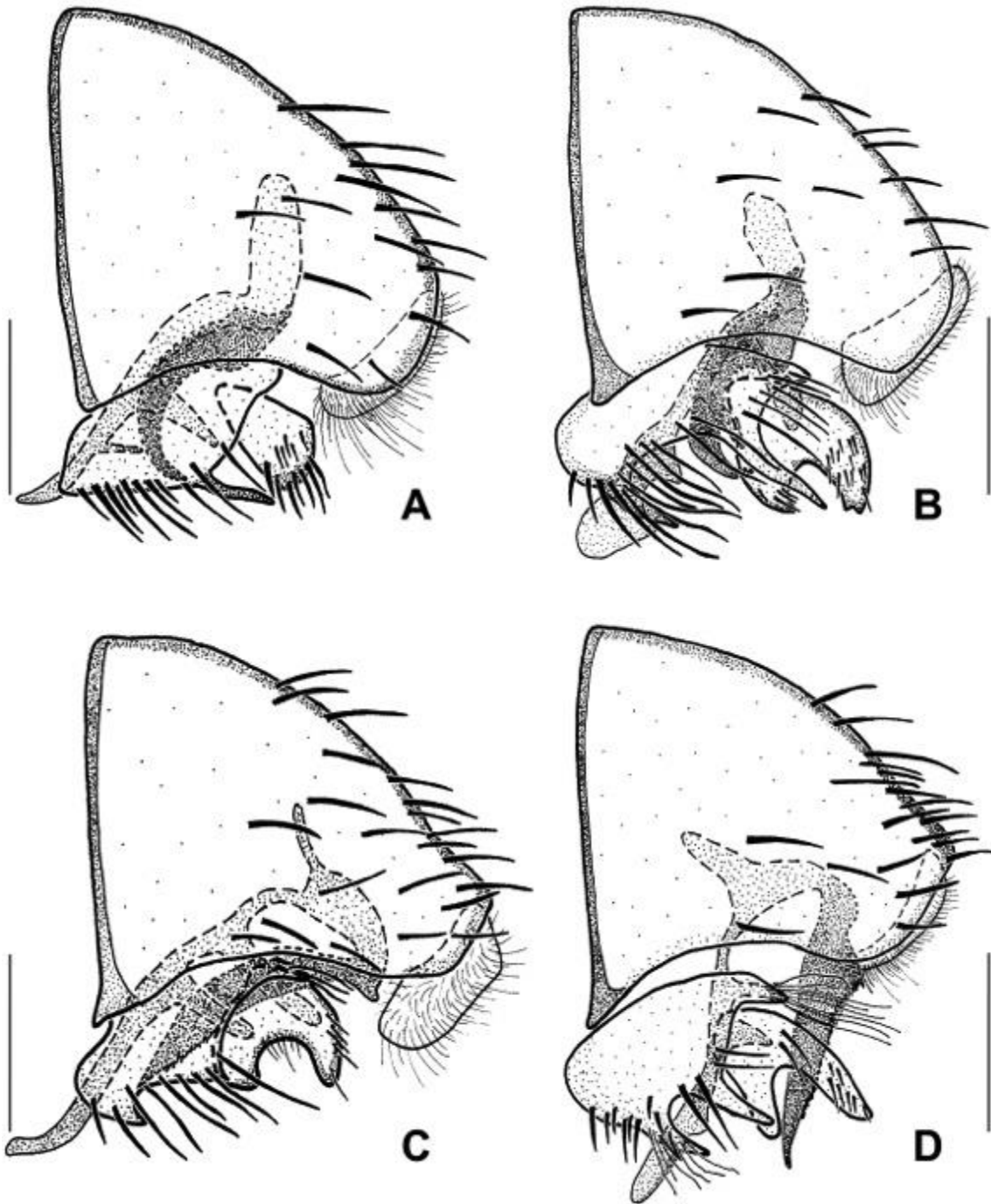


Figure 3.18. Lateral views of male *Niphta nudipennis* group terminalia: A, *N. moultoni* **sp.n.**; B, *N. courtneyi* **sp.n.**; C, *N. sinclairi* **sp.n.**; D, *N. brunnea* **sp.n.** Scale bars = 0.1 mm.

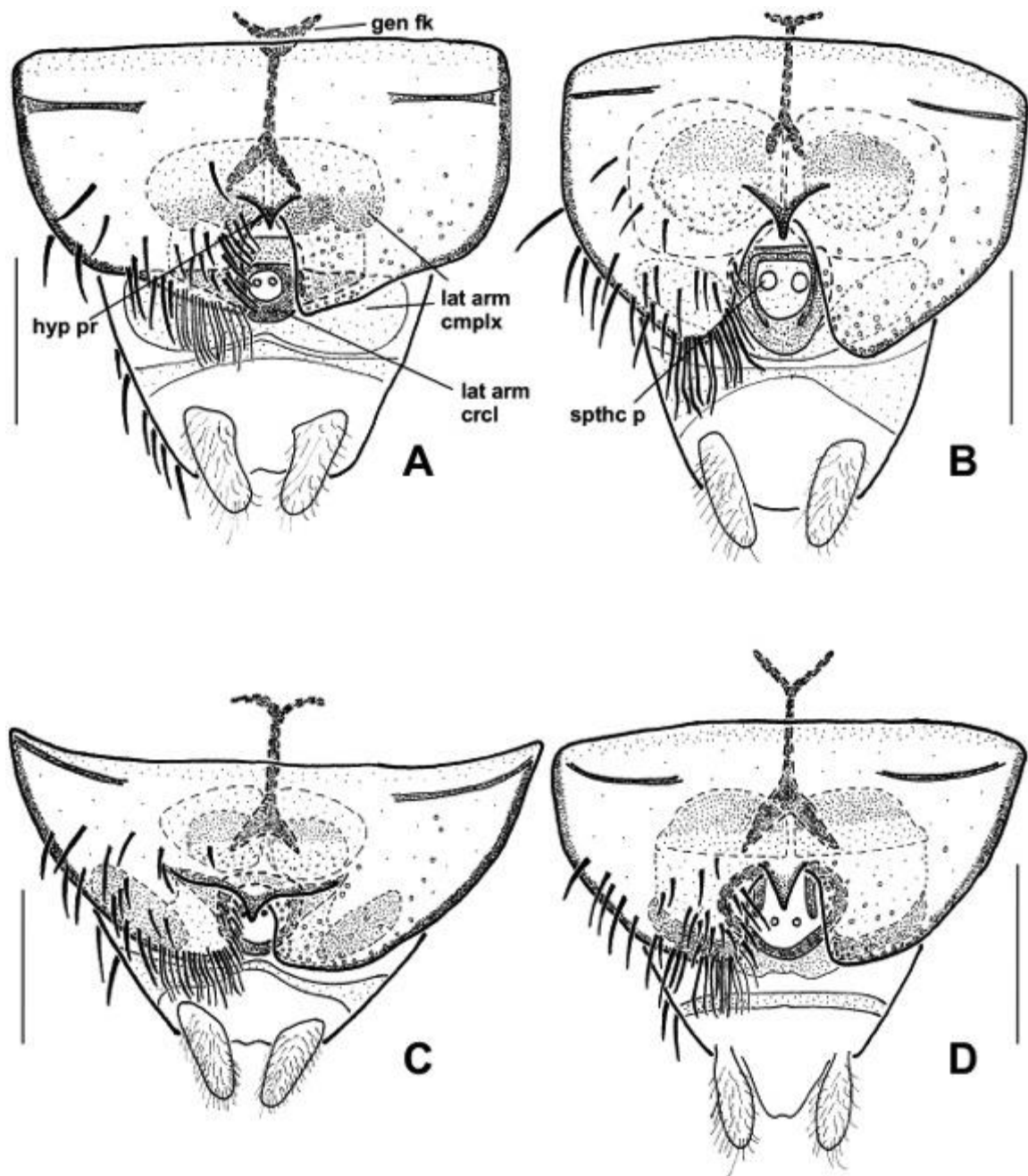


Figure 3.19. Ventral views of female *Niphta nudipennis* group terminalia: A, *N. moultoni* sp.n.; B, *N. courtneyi* sp.n.; C, *N. sinclairi* sp.n.; D, *N. nudipennis*. Abbreviations: gen fk, genital fork; hyp pr, hypogynial protuberance; lat arm crcl, lateral arm circle; lat arm cmplx, lateral arm complex; spthc p, spermathecal pump. Scale bars = 0.1 mm.

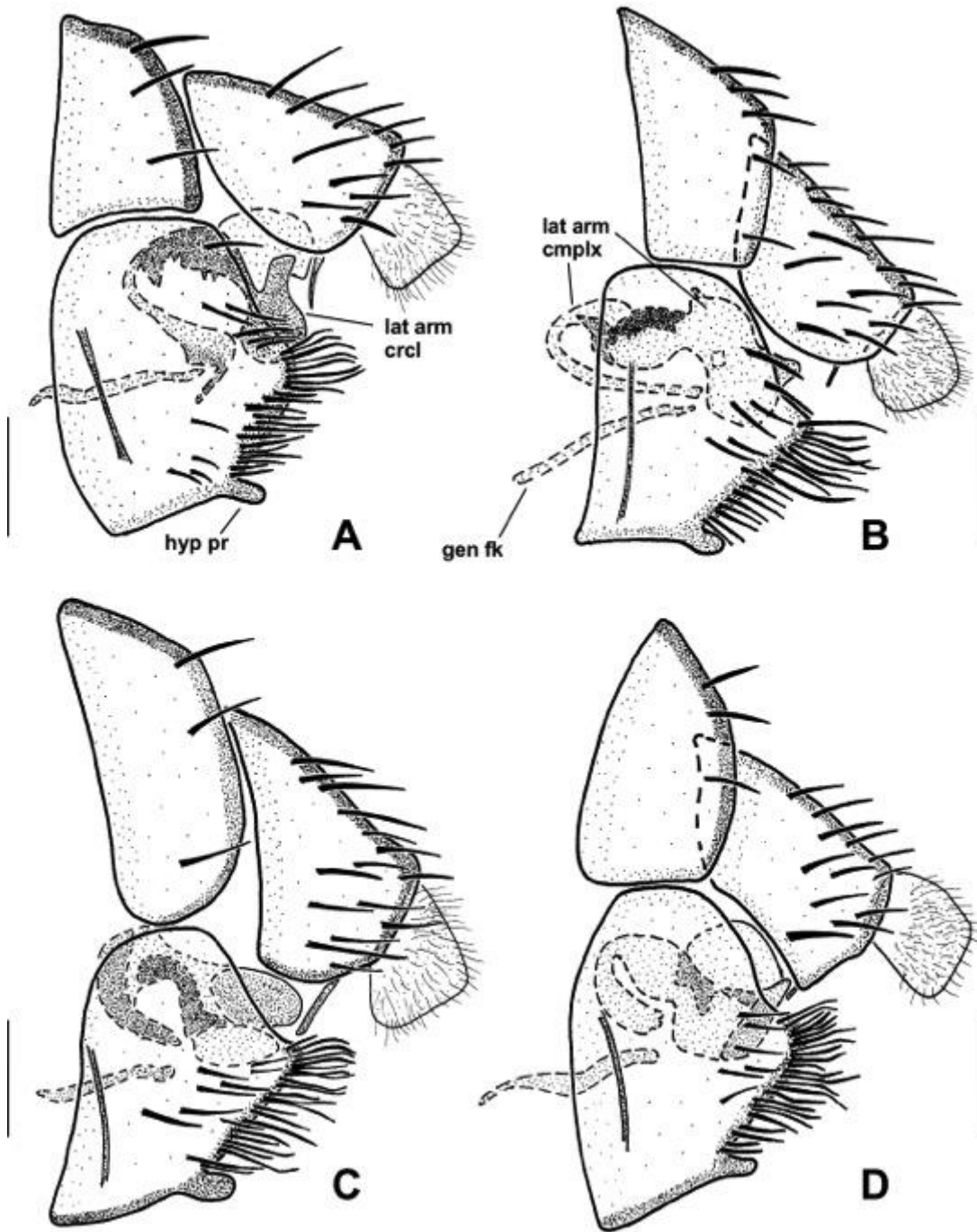


Figure 3.20. Lateral views of female *Niphta nudipennis* group terminalia: A, *N. moultoni* sp.n.; B, *N. courtneyi* sp.n.; C, *N. sinclairi* sp.n.; D, *N. nudipennis*. Abbreviations: gen fk, genital fork; hyp pr, hypogynial protuberance; lat arm crcl, lateral arm circle; lat arm cmplx, lateral arm complex. Scale bars = 0.1 mm.

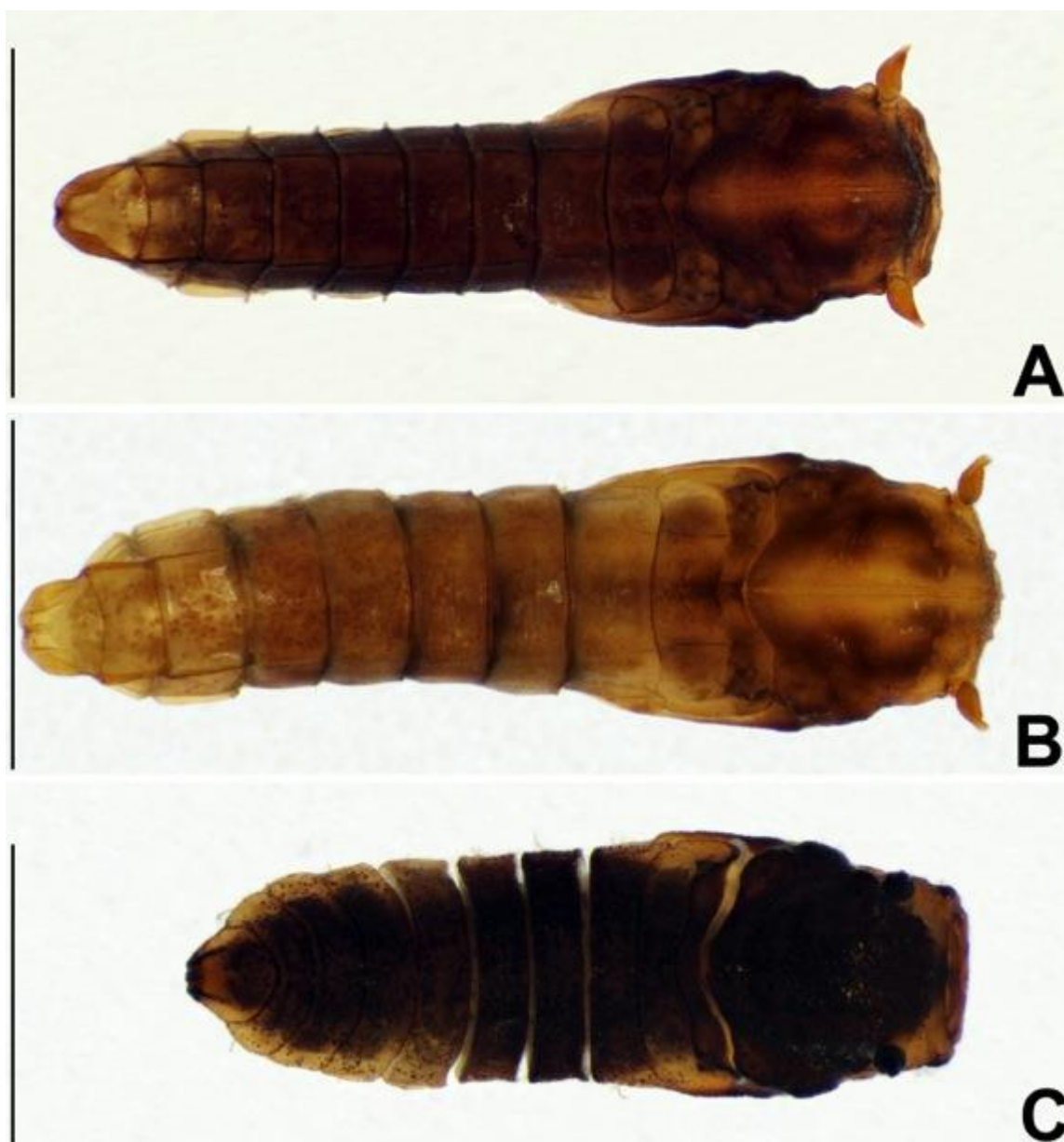


Figure 3.21. Dorsal views of *Niphta* pupae: A, *N. brunnea* **sp.n.**; B, *N. nudipennis*; C, *N. acus* **sp.n.** Scale bars = 1.0 mm.



Figure 3.22. Lateral views of *Niphta* pupae: A, *N. brunnea* **sp.n.**; B, *N. nudipennis*; C, *N. acus* **sp.n.** Scale bars = 1.0 mm.



Figure 3.23. Ventral views of *Niphta* pupae: A, *N. brunnea* **sp.n.**; B, *N. nudipennis*; C, *N. acus* **sp.n.** Scale bars = 1.0 mm.

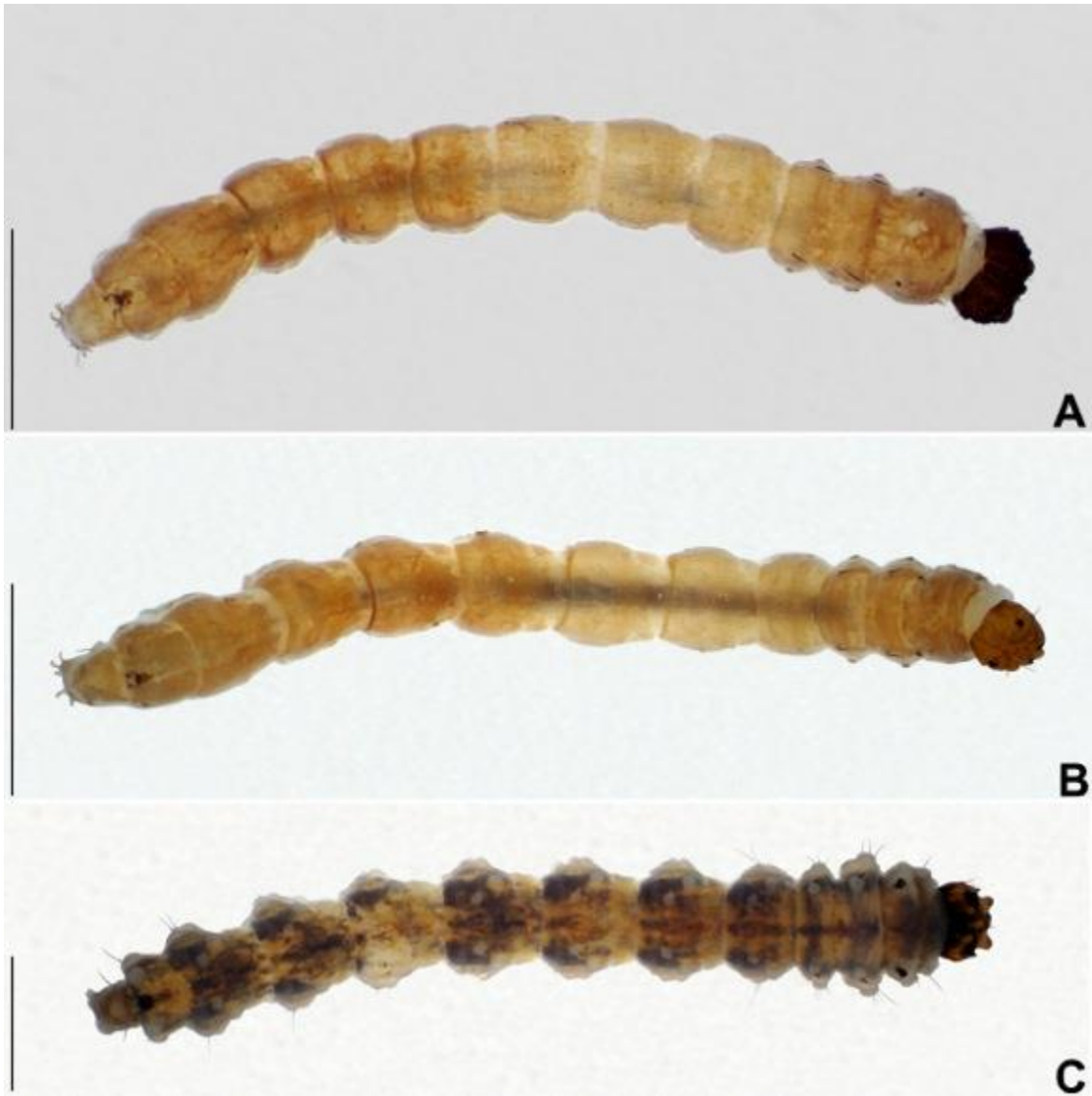


Figure 3.24. Dorsal views of *Niphta* larvae: A, *N. brunnea* **sp.n.**; B, *N. nudipennis*; C, *N. acus* **sp.n.** Scale bars = 1.0 mm.



Figure 3.25. Lateral views of *Niphta* larvae: A, *N. brunnea* **sp.n.**; B, *N. nudipennis*; C, *N. acus* **sp.n.** Scale bars = 1.0 mm.



Figure 3.26. Ventral views of *Niphta* larvae: A, *N. brunnea* **sp.n.**; B, *N. nudipennis*; C, *N. acus* **sp.n.** Scale bars = 1.0 mm.

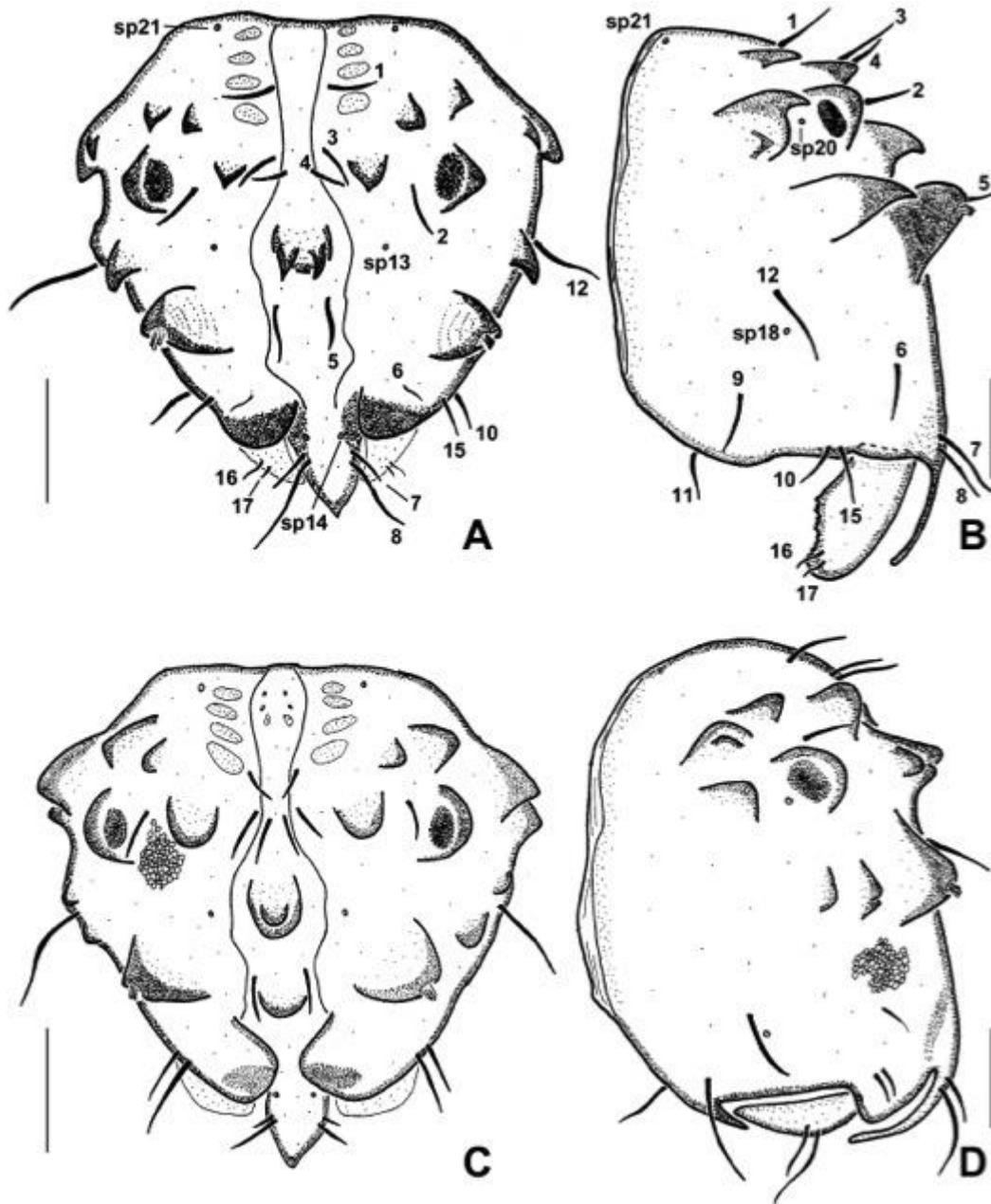


Figure 3.27. *Niphta halteralis* group head capsules with setae and sensory pits numbered: A, anterior *N. acus* sp.n.; B, lateral *N. acus* sp.n.; C, anterior *N. mapuche* sp.n.; D, lateral *N. mapuche* sp.n. Abbreviations: sp, sensory pit. Scale bars = 0.1 mm.

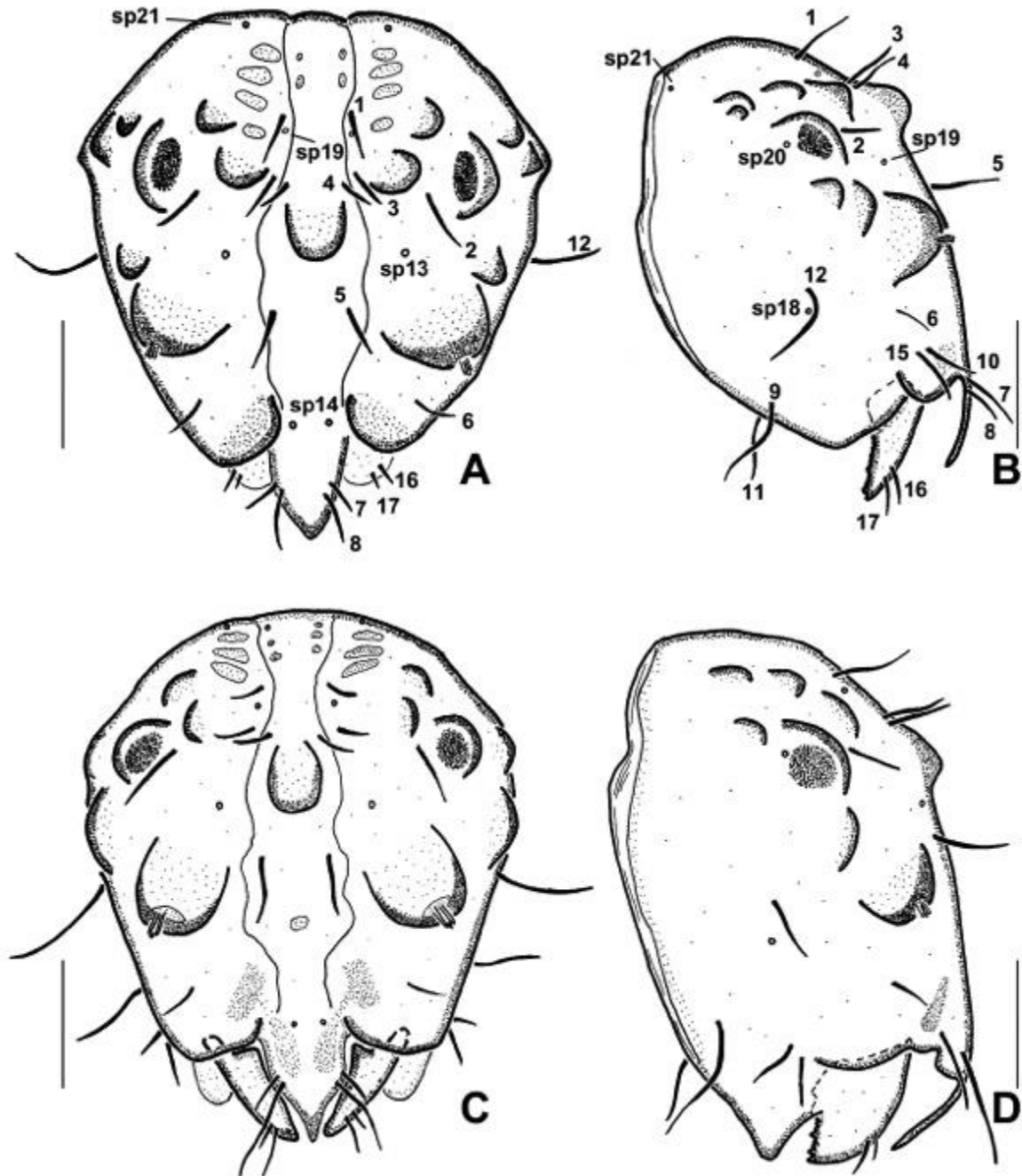


Figure 3.28. *Niphta nudipennis* group head capsules with setae and sensory pits numbered: A, anterior *N. brunnea* sp.n.; B, lateral *N. brunnea* sp.n.; C, anterior *N. nudipennis*; D, lateral *N. nudipennis*. Abbreviations: sp, sensory pit. Scale bars = 0.1 mm.

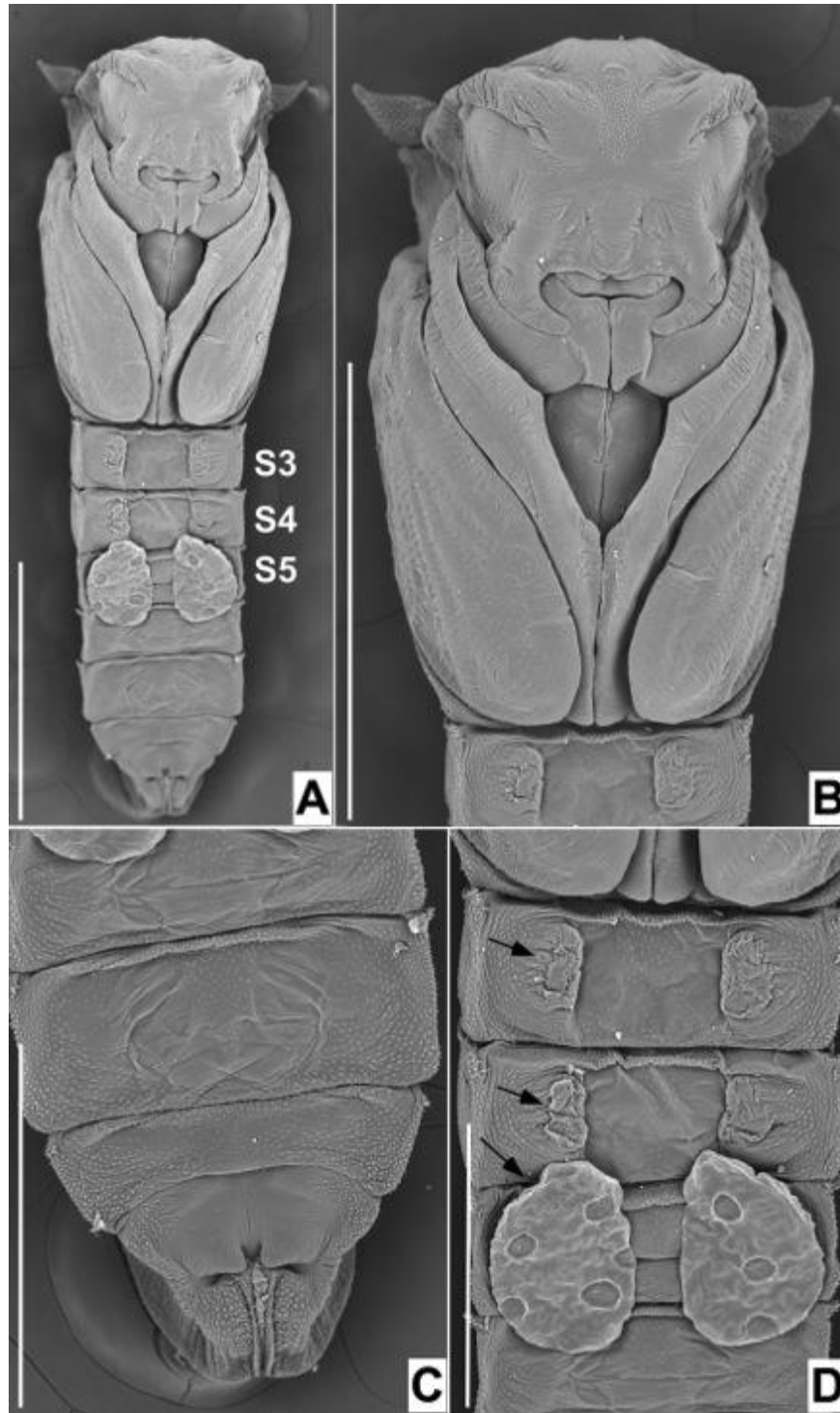


Figure 3.29. Scanning electron micrographs of ventral view of *Niphta brunnea* **sp.n.** pupa: A, habitus (scale bar = 1.0 mm); B, head and thorax (scale bar = 1.0 mm); C, posterior segments (scale bar = 0.5 mm); D, adhesive structures (scale bar = 0.5 mm). Abbreviations: S, sternite.

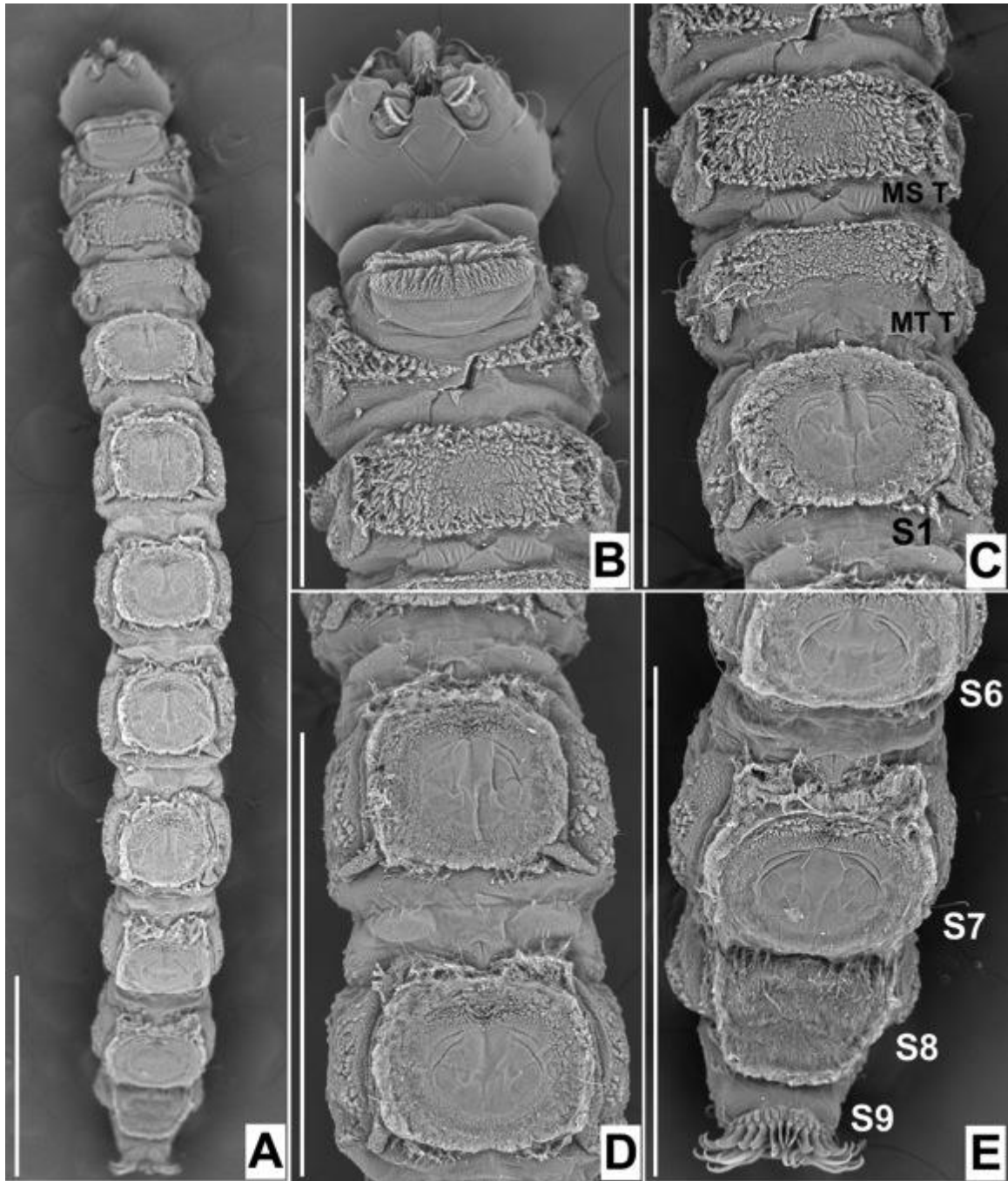


Figure 3.30. Scanning electron micrographs of ventral view of *Niphta brunnea* sp.n. larva: A, habitus; B, head and thoracic segments 1 & 2; C, thoracic segments 2 & 3 and abdominal segment 1; D, abdominal segments 2 & 3; E, abdominal segments 6, 7, 8 & 9. Abbreviations: MS T, mesothoracic segment; MT T, metathoracic segment; S, abdominal segment. Scale bars = 1.0 mm.

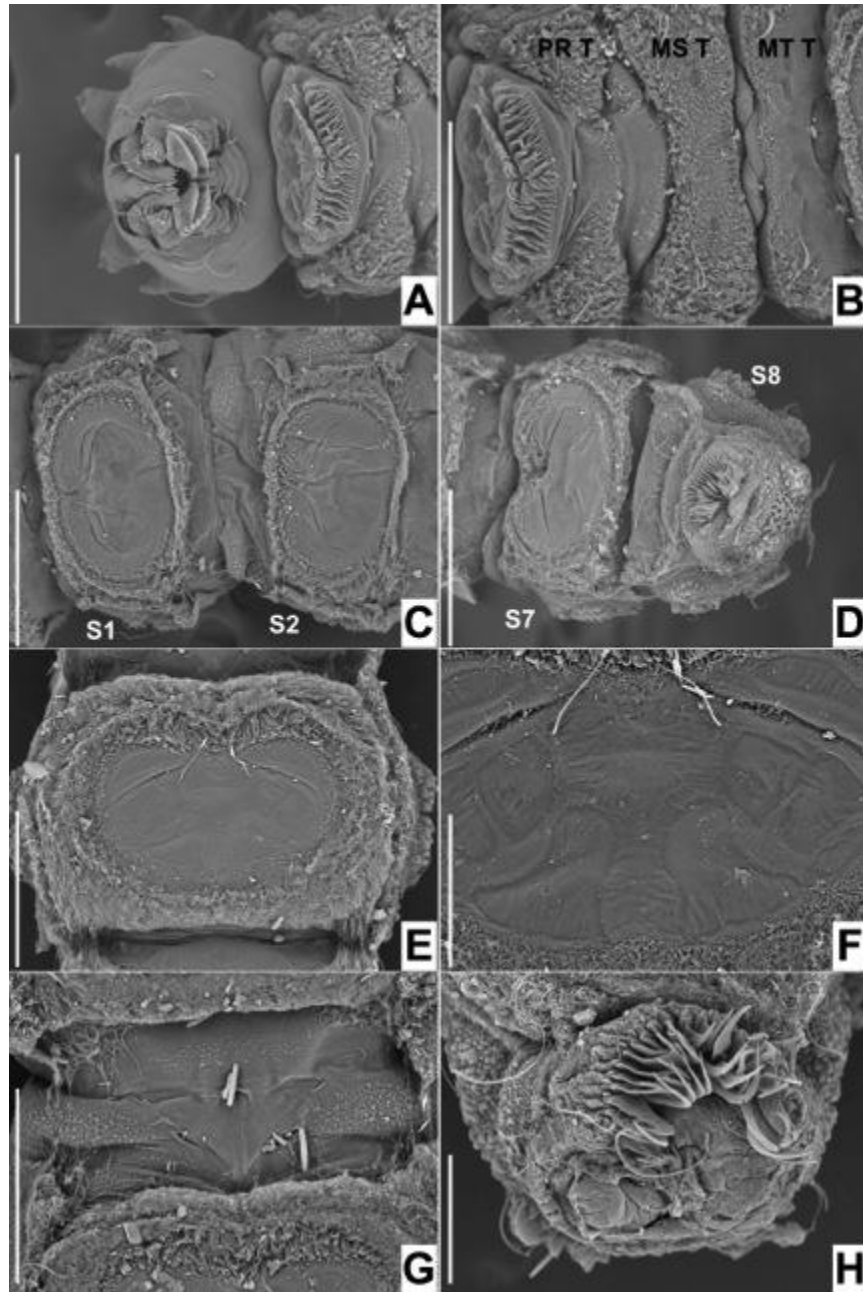


Figure 3.31. Scanning electron micrographs of ventral view of *Niphta acus* sp.n. larva: A, head and anterior thoracic proleg (scale bar = 0.25 mm); B, thoracic segments 1–3 (scale bar = 0.25 mm); C, abdominal segments 1 & 2 (scale bar = 0.25 mm); D, abdominal segments 7–9 (scale bar = 0.25 mm); E, abdominal segment 6 (scale bar = 0.25 mm); F, close-up of abdominal segment 6 (scale bar = 0.1 mm); G, intersegmental membrane of abdominal segments 4 & 5 (scale bar = 0.25 mm); H, close-up of anal proleg (scale bar = 0.1 mm). Abbreviations: MS T, mesothoracic segment; MT T, metathoracic segment; PR T, prothoracic segment; S, abdominal segment.

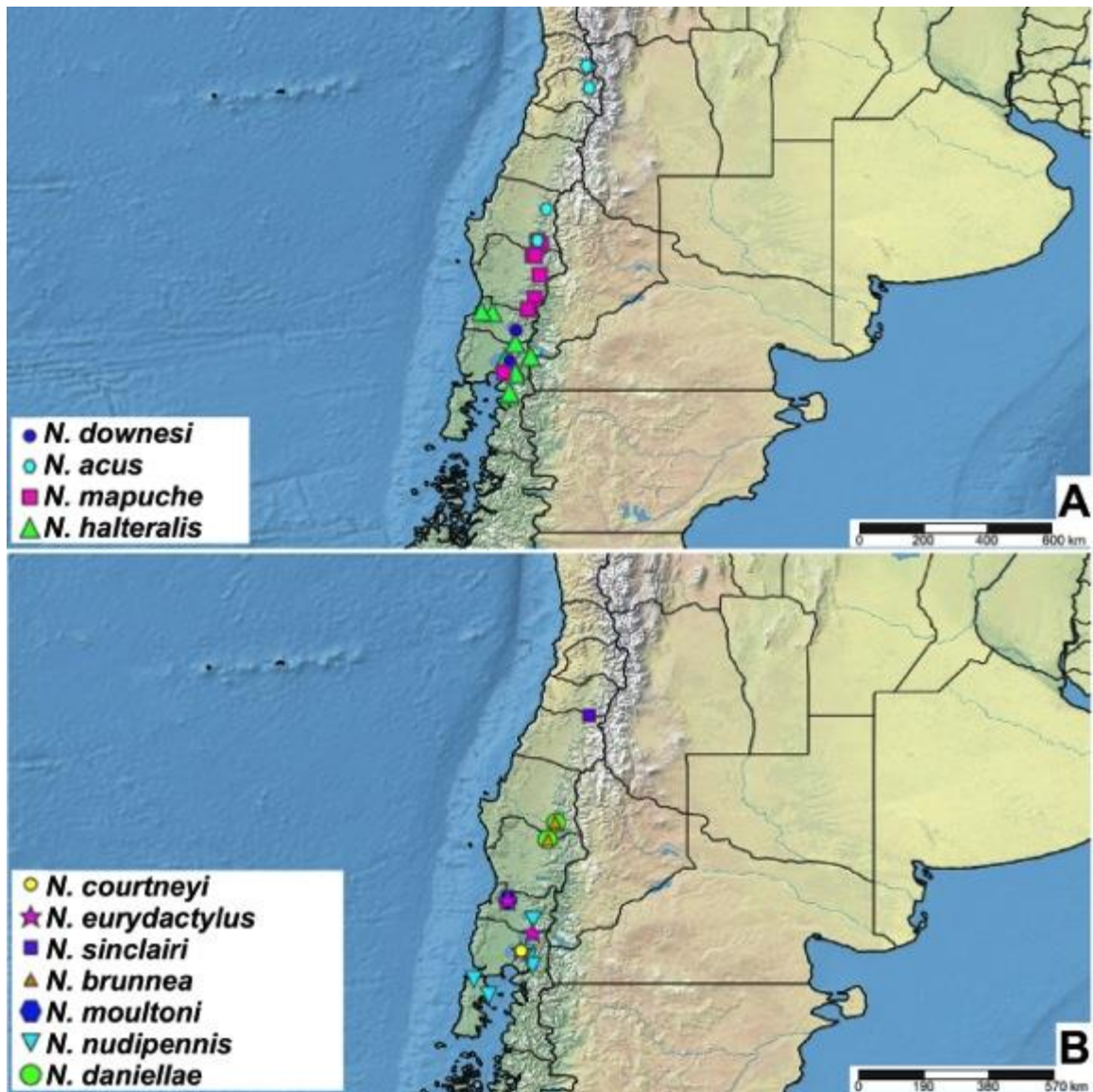


Figure 3.32. Known distribution of Chilean *Niphta*: A, *N. halteralis* group; B, *N. nudipennis* group.

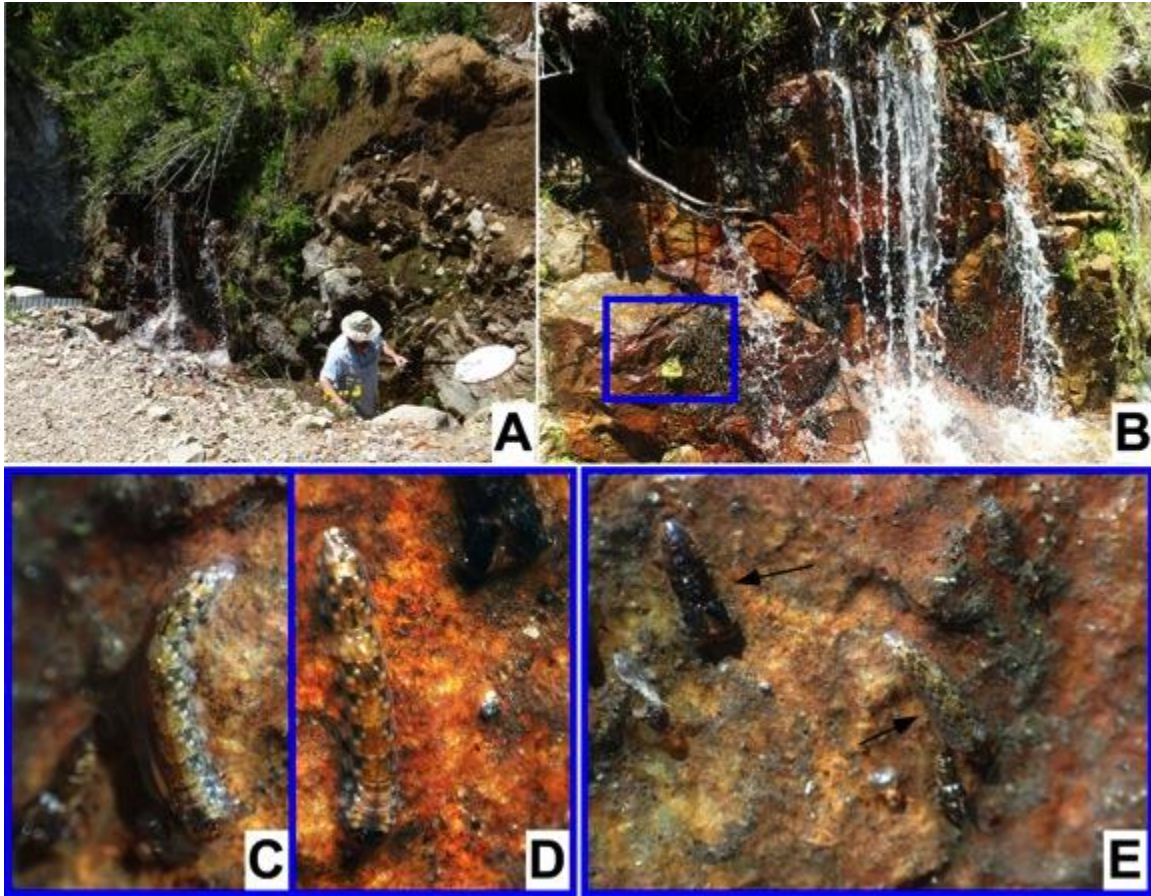


Figure 3.33. Habitat and larvae of *Niphta acus* sp.n. (36°55'02.7"S 71°25'49.6"W): A, Moulton shown next to the falls for scale; B, close up of falls with box indicating where immatures were captured (Note: they were not found in high impact zones); C, lateral view of larva, with adhesive structures visibly in contact with substrate; D, dorsal view of larva illustrating camouflage; E, larva and pupal exuviae on rock face.

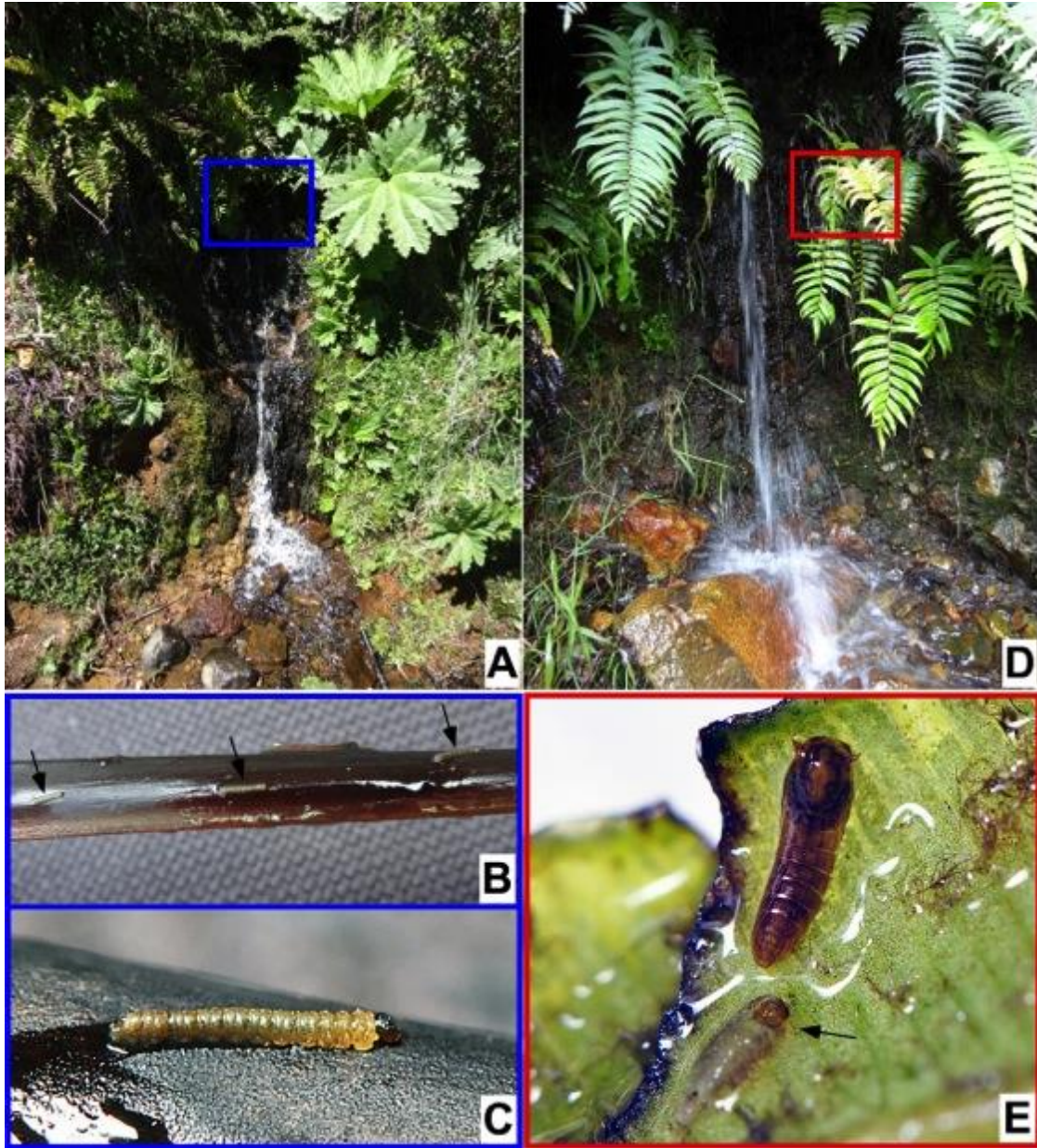


Figure 3.34. Habitat and immatures of members of the *Niphta nudipennis* group: A, type locality of *N. brunnea* **sp.n.** and *N. daniellae* **sp.n.**, box indicating where plant stem in images B & C was taken from (38°14'20.6"S 71°53'46.6"W); B, larvae of *N. brunnea* **sp.n.** on plant stem from splash zone; C, close up of *N. brunnea* **sp.n.** larva, adhesive structure visibly in contact with substrate; D, habitat of *N. nudipennis*, box indicating foliage in splash zone where immatures were found; E, pupa of *N. nudipennis* affixed to leaf with final instar larval exuviae visible.

Fig. 3.35. Examples of thaumaleid habitat in Chile: A, waterfalls where *Niphta downesi* **sp.n.**, *N. eurydactylus* **sp.n.** and *N. nudipennis* were collected by sweeping adjacent foliage (40°19'58.7"S 72°16'54.8"W); B, collection site of *Austrothaumalea spatulata* and *N. sinclairi* **sp.n.** (34°59'48.8"S 70°48'37.0"W); C, type-locality of *N. acus* **sp.n.**, specimens were mainly collected from plant on left (37°46'30.8"S 71°42'03.9"W); D, rock face seep, the type locality of *N. sinclairi* **sp.n.** (34°59'46.7"S 70°49'19.2"W); E, rock face seep where *A. chilensis* and *A. setipennis* were collected (38°13'20.3"S 71°44'41.1"W); F, the northernmost collection site of *A. spatulata* in the Chilean Matorral, inset showing small trickle of water where specimen was collected (34°46'29.2"S 70°40'03.9"W); G, Puente El Salto, *N. halteralis* was collected above the falls (41°31'29.2"S 72°17'14.6"W); H, Chiloé, *N. nudipennis* was collected at a small waterfall next to the larger Cascada Tocoihue, Pivar is shown for scale (42°18'20.3"S 73°26'08.9"W).

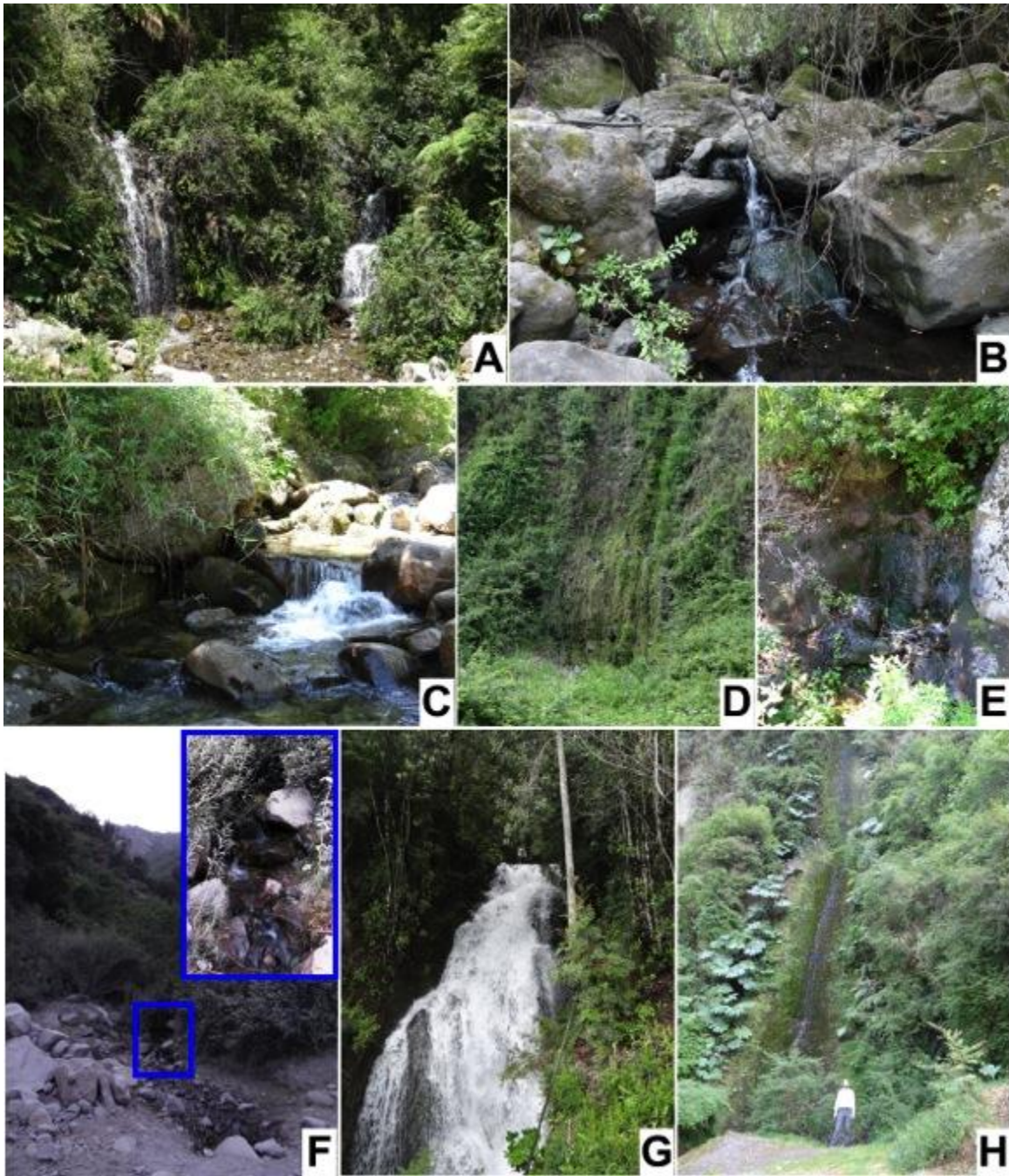


Figure 3.35. See figure caption on previous page.

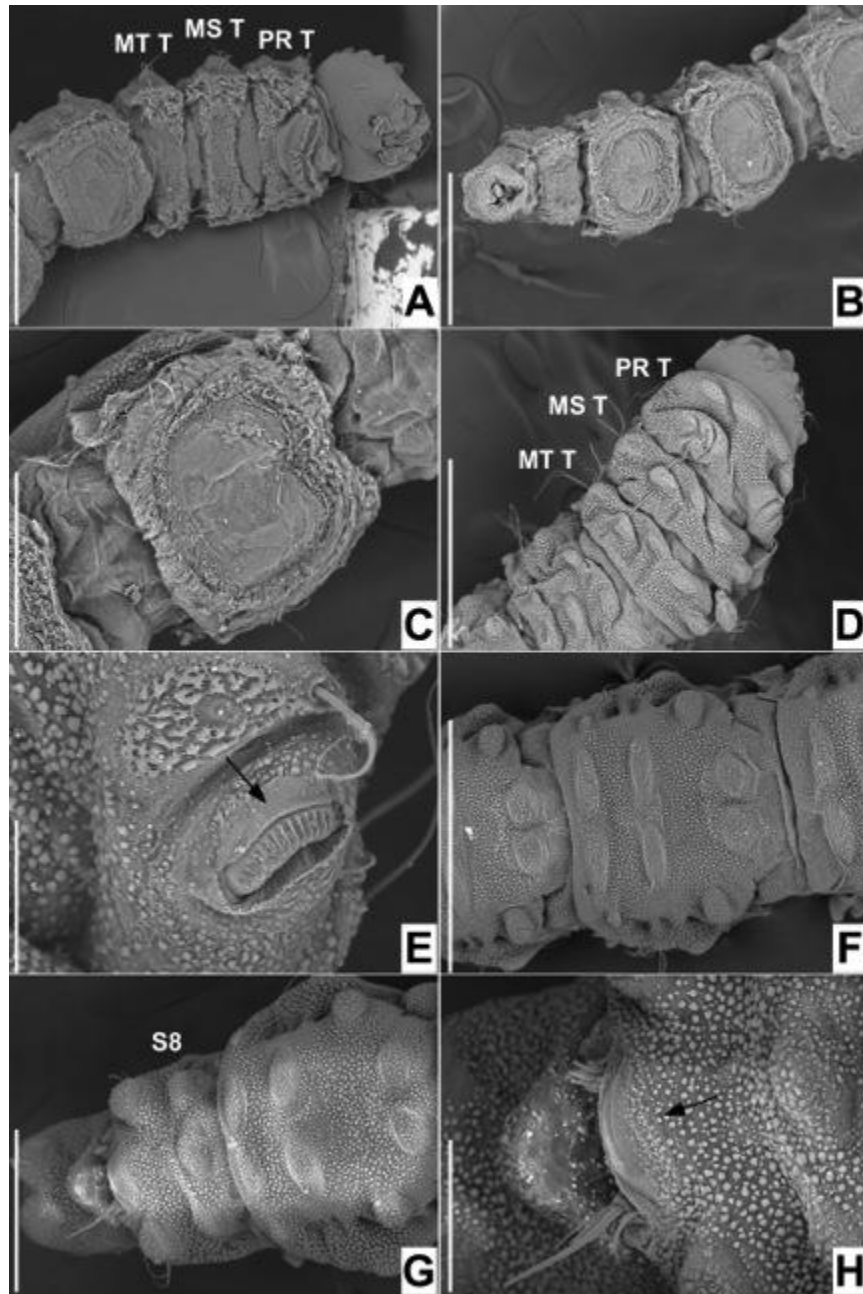


Figure 3.36. Scanning electron micrographs of Australian *Niphta* sp. larva: A, ventral view of head, thorax and abdominal segment 1 (scale bar = 0.5 mm); B, ventral view of abdominal sternites segments 5–9 (scale bar = 0.5 mm); C, ventral view of abdominal segment 3 (scale bar = 0.25 mm); D, dorsal view of head, thorax and abdominal segment 1 (scale bar = 0.5 mm); E, prothoracic spiracle (scale bar = 0.05 mm); F, dorsal view of abdominal segment 6 (scale bar = 0.5 mm); G, dorsal view of abdominal segments 7–9 (scale bar = 0.25 mm); H, close-up of posterior spiracular plate (scale bar = 0.1 mm). Abbreviations: MS T, mesothoracic segment; MT T, metathoracic segment; PR T, prothoracic segment; S, abdominal segment.

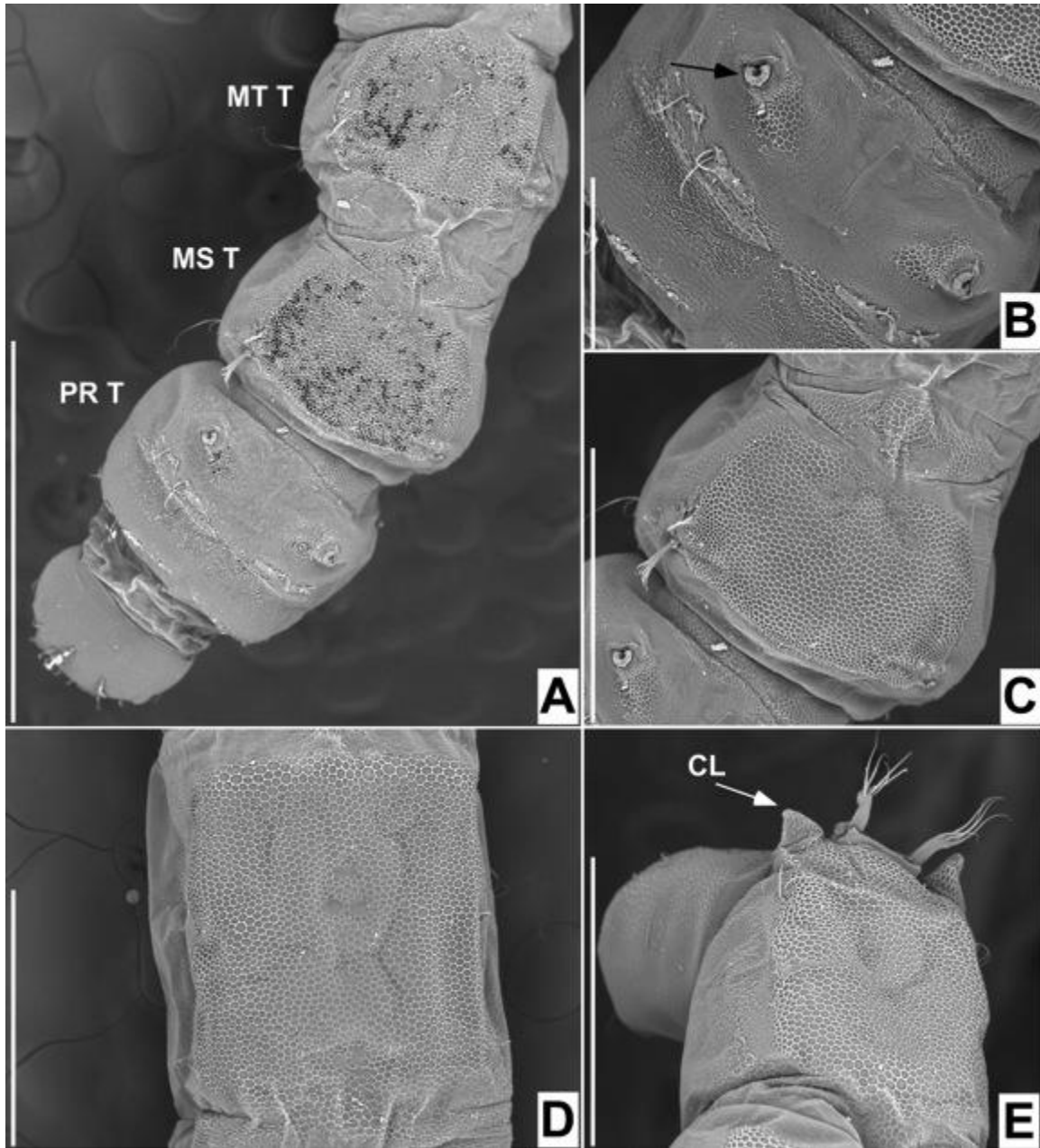


Figure 3.37. Scanning electron micrographs of dorsal view of *Androprosopa americana* larva: A, head and thoracic segments 1–3 (scale bar = 1.0 mm); B, prothorax with spiracles (scale bar = 0.5 mm); C, mesothorax (scale bar = 0.5 mm); D, abdominal segment 4 (scale bar = 0.5 mm); E, abdominal segment 8 (scale bar = 0.5 mm). Abbreviations: CL, conical lobe; MS T, mesothoracic segment; MT T, metathoracic segment; PR T, prothoracic segment.

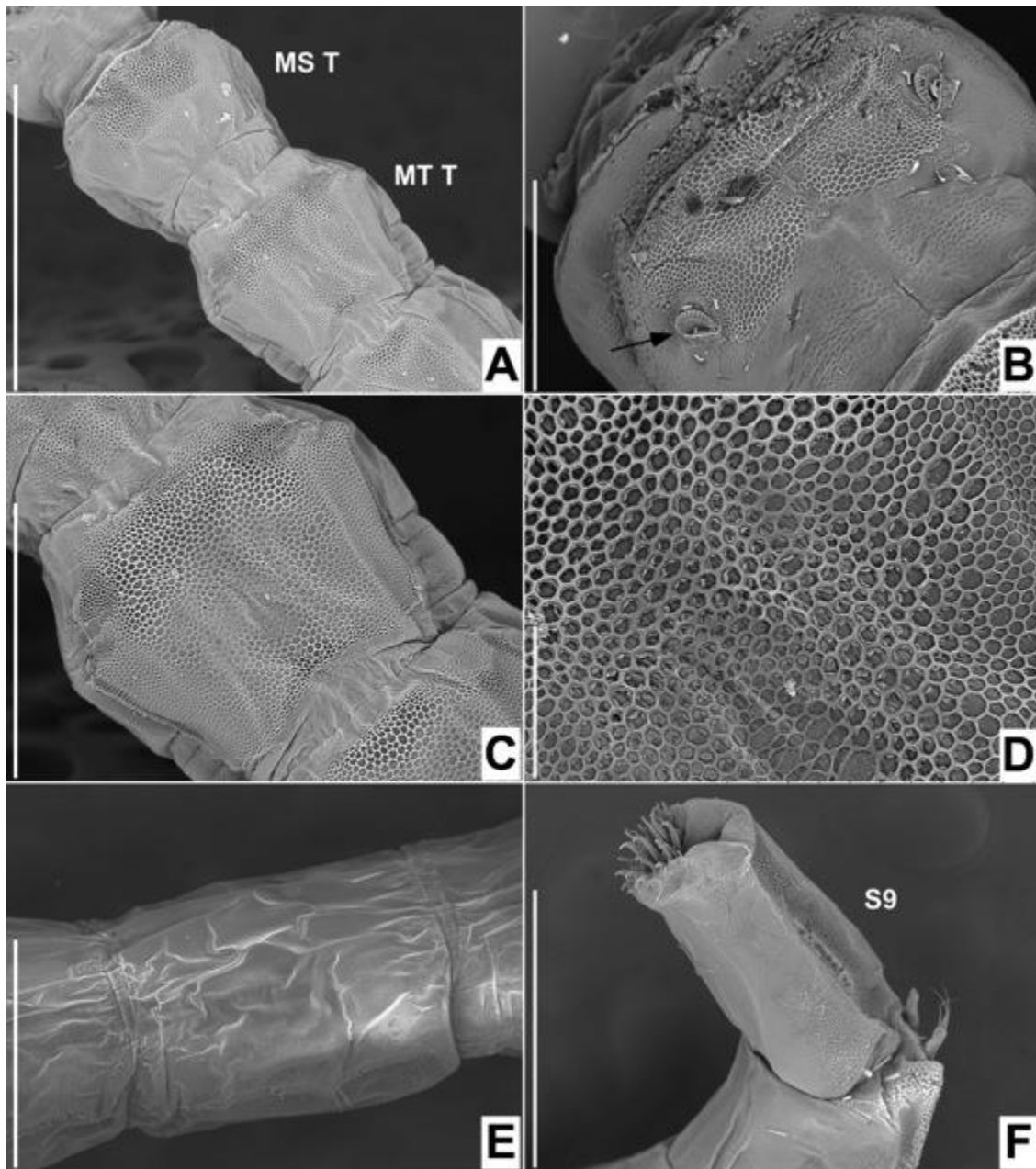


Figure 3.38. Scanning electron micrographs of *Androprosopa becca* larva: A, dorsal view of meso- and metathorax (scale bar = 1.0 mm); B, dorsal view of prothorax with spiracles (scale bar = 0.25 mm); C, dorsal view of metathorax (scale bar = 0.5 mm); D, close-up of dorsal view of metathorax (scale bar = 0.1 mm); E, ventral view of abdominal segment 5 (scale bar = 0.5 mm); F, ventrolateral view of abdominal segment 9 (scale bar = 0.5 mm). Abbreviations: MS T, mesothoracic segment; MT T, metathoracic segment; S, abdominal segment.

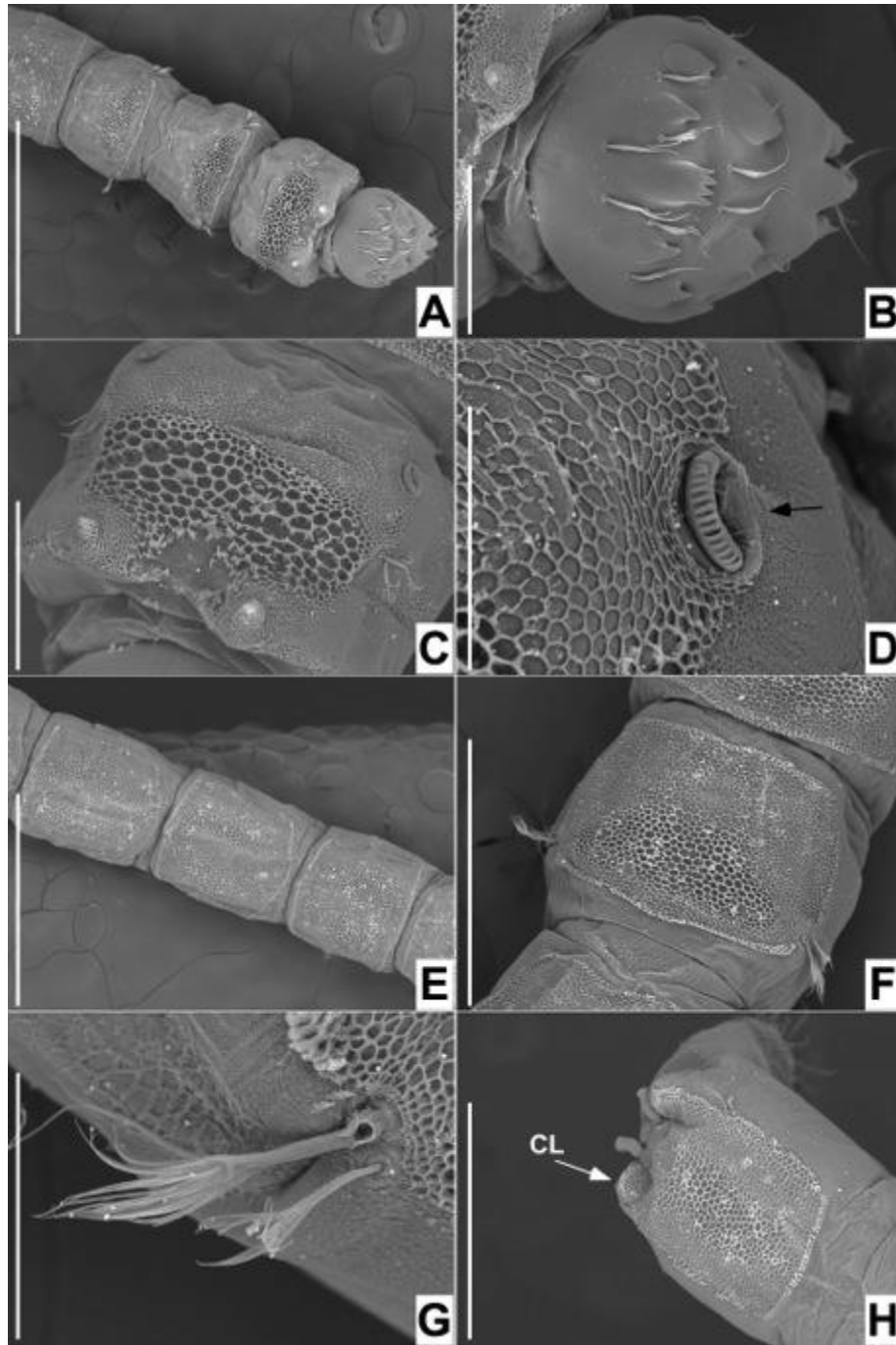


Figure 3.39. Scanning electron micrographs of dorsal view of *Androprosopa striata* larva: A, head and thoracic segments (scale bar = 1.0 mm); B, head (scale bar = 0.25 mm); C, prothorax (scale bar = 0.25 mm); D, prothoracic spiracle (scale bar = 0.1 mm); E, abdominal segments 2–4 (scale bar = 1.0 mm); F, metathorax (scale bar = 0.5 mm); G, close-up of metathoracic setae (scale bar = 0.1 mm); H, dorsolateral view of abdominal segment 8 (scale bar = 0.5 mm). Abbreviations: CL, conical lobe.

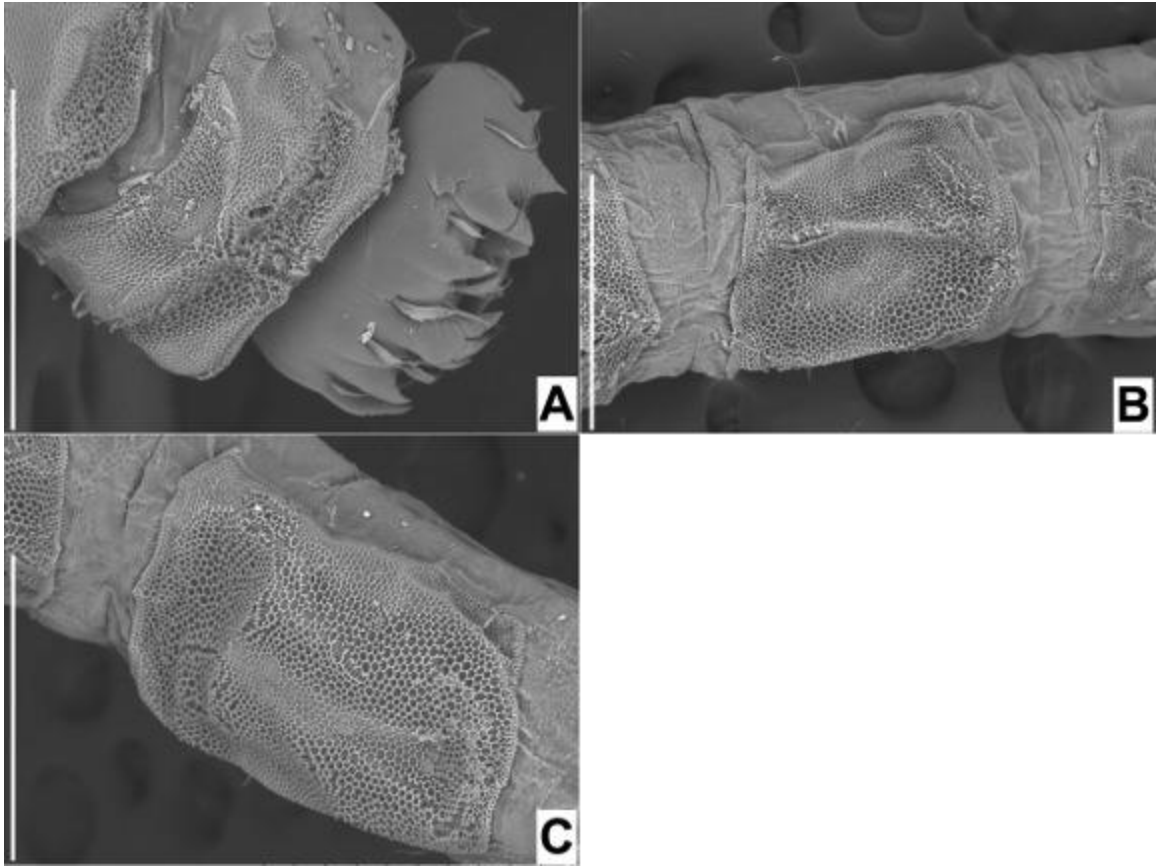


Figure 3.40. Scanning electron micrographs of dorsal view of *Thaumalea verralli* larva: A, head and prothorax (scale bar = 0.5 mm); B, abdominal segment 4 (scale bar = 0.5 mm); C, abdominal segment 4 (scale bar = 0.5 mm).

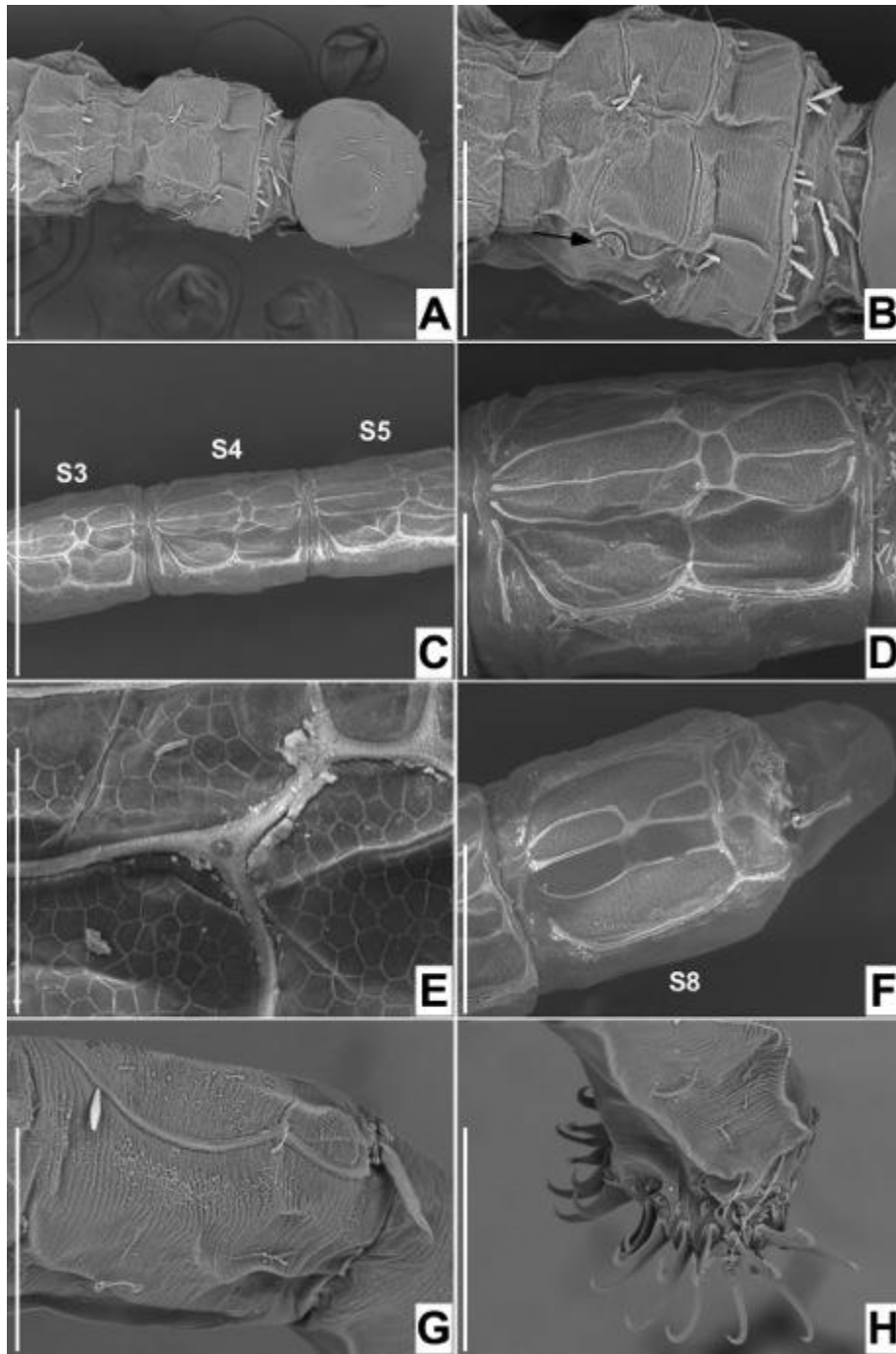


Figure 3.41. Scanning electron micrographs of dorsal view of *Austrothaumalea* sp. larva: A, head, pro- and mesothorax (scale bar = 0.5 mm); B, prothorax (scale bar = 0.25 mm); C, abdominal segments 3–5 (scale bar = 1.0 mm); D, abdominal segment 4 (scale bar = 0.25 mm); E, close up of abdominal segment 4 (scale bar = 0.1 mm); F, abdominal segment 8 (scale bar = 0.25 mm); G, lateral view of abdominal segment 8 (scale bar = 0.25 mm); H, anal proleg (scale bar = 0.25 mm). Abbreviations: S, abdominal segment.

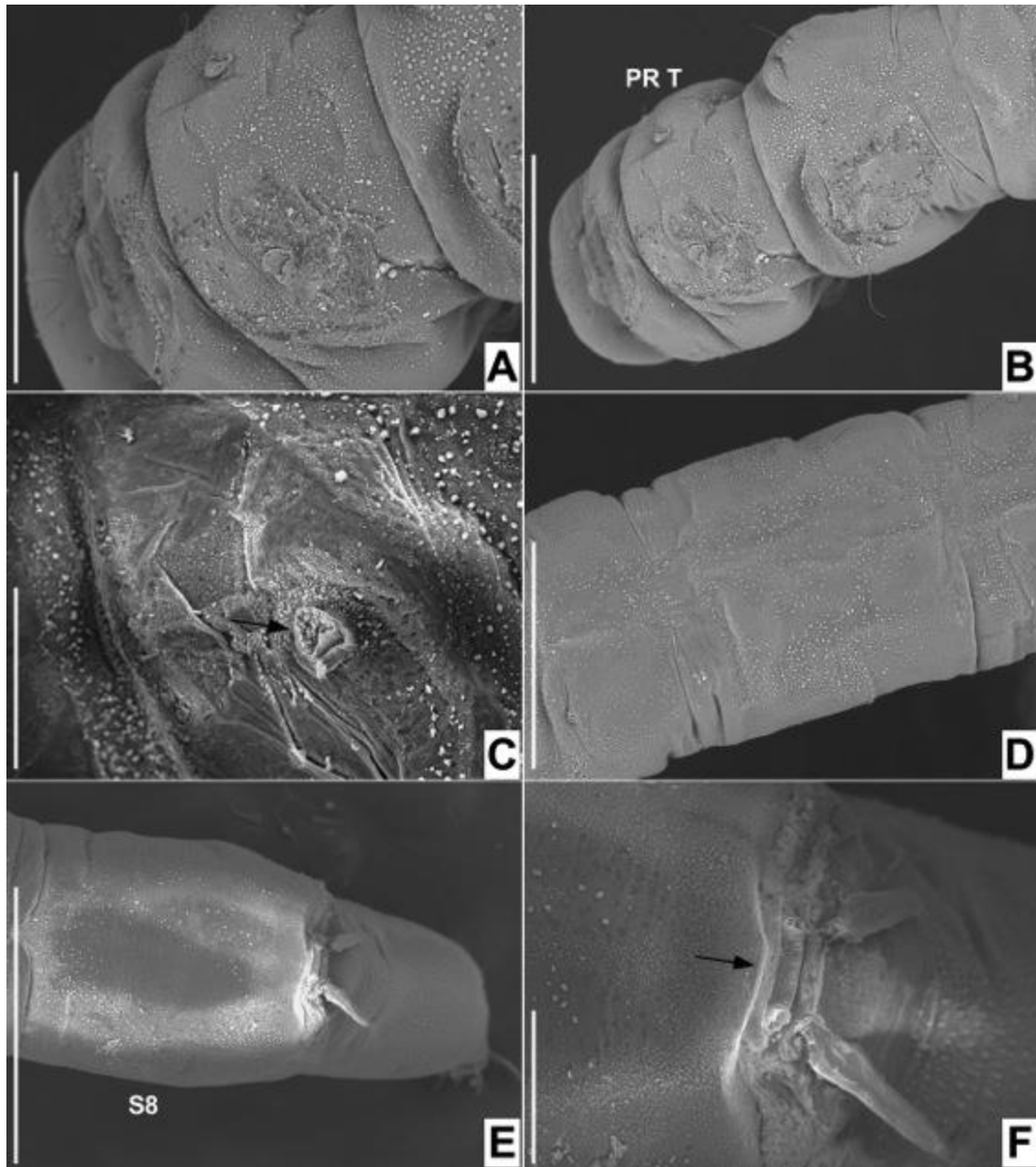


Figure 3.42. Scanning electron micrographs of dorsal view of *Afrothaumalea stuckenbergi* larva: A, head and prothorax (scale bar = 0.25 mm); B, head, pro- and mesothorax (scale bar = 0.5 mm); C, prothoracic spiracle (scale bar = 0.1 mm); D, abdominal segment 4 (scale bar = 0.5 mm); E, abdominal segments 8 & 9 (scale bar = 0.5 mm); F, close-up of posterior spiracular plate (scale bar = 0.1 mm). Abbreviations: PR T, prothoracic segment; S, abdominal segment.

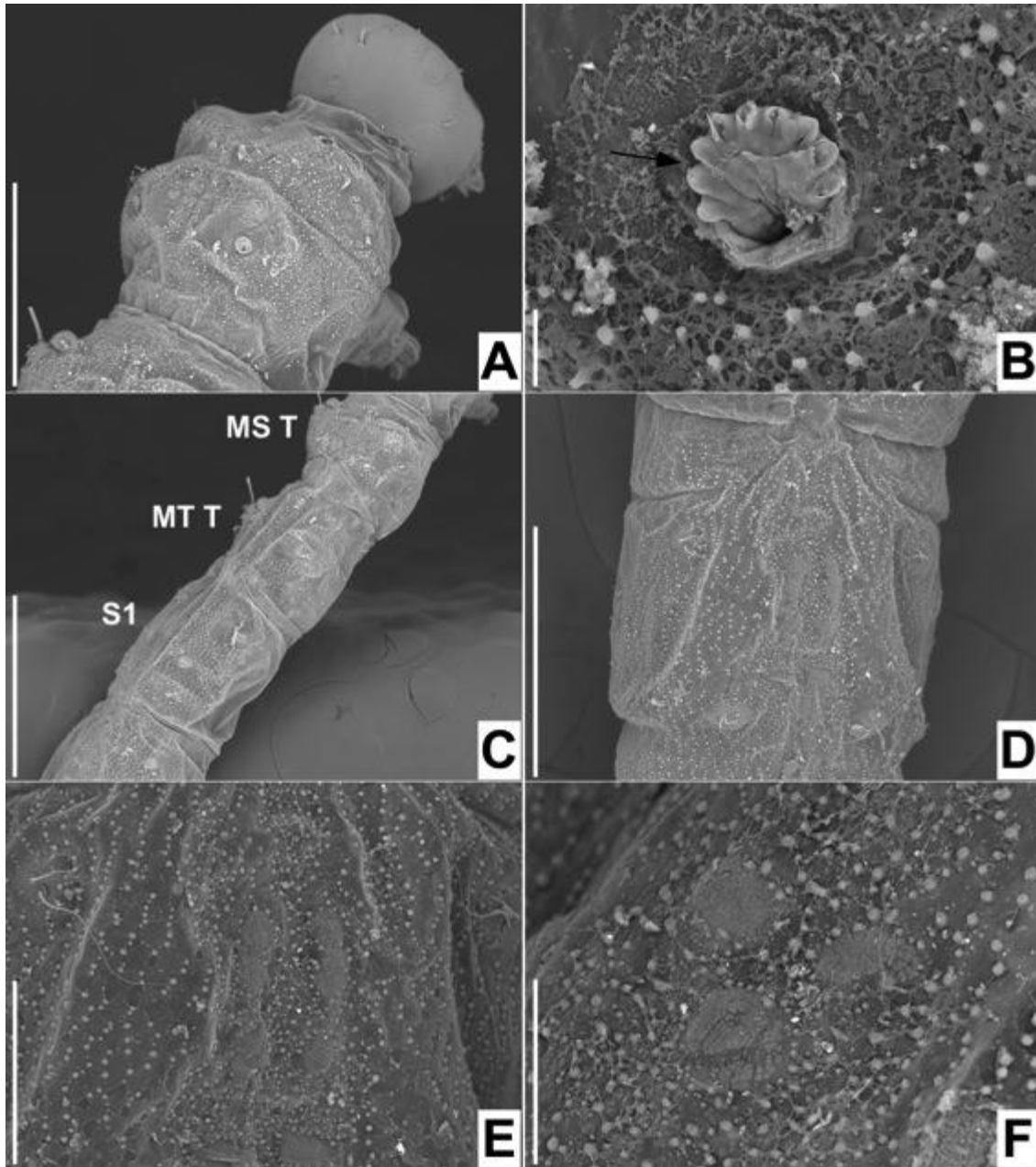


Figure 3.43. Scanning electron micrographs of dorsal view of *Trichothaumalea elakalensis* larva: A, dorsolateral view of head and prothorax (scale bar = 0.25 mm); B, prothoracic spiracle (scale bar = 0.1 mm); C, lateral view meso- and metathorax, abdominal segment 1 (scale bar = 0.5 mm); D, abdominal segment 4 (scale bar = 0.25 mm); E, close up of abdominal segment 4 (scale bar = 0.1 mm); F, close-up of abdominal segment 7 (scale bar = 0.05 mm). Abbreviations: MS T, mesothoracic segment; MT T, metathoracic segment; S, abdominal segment.

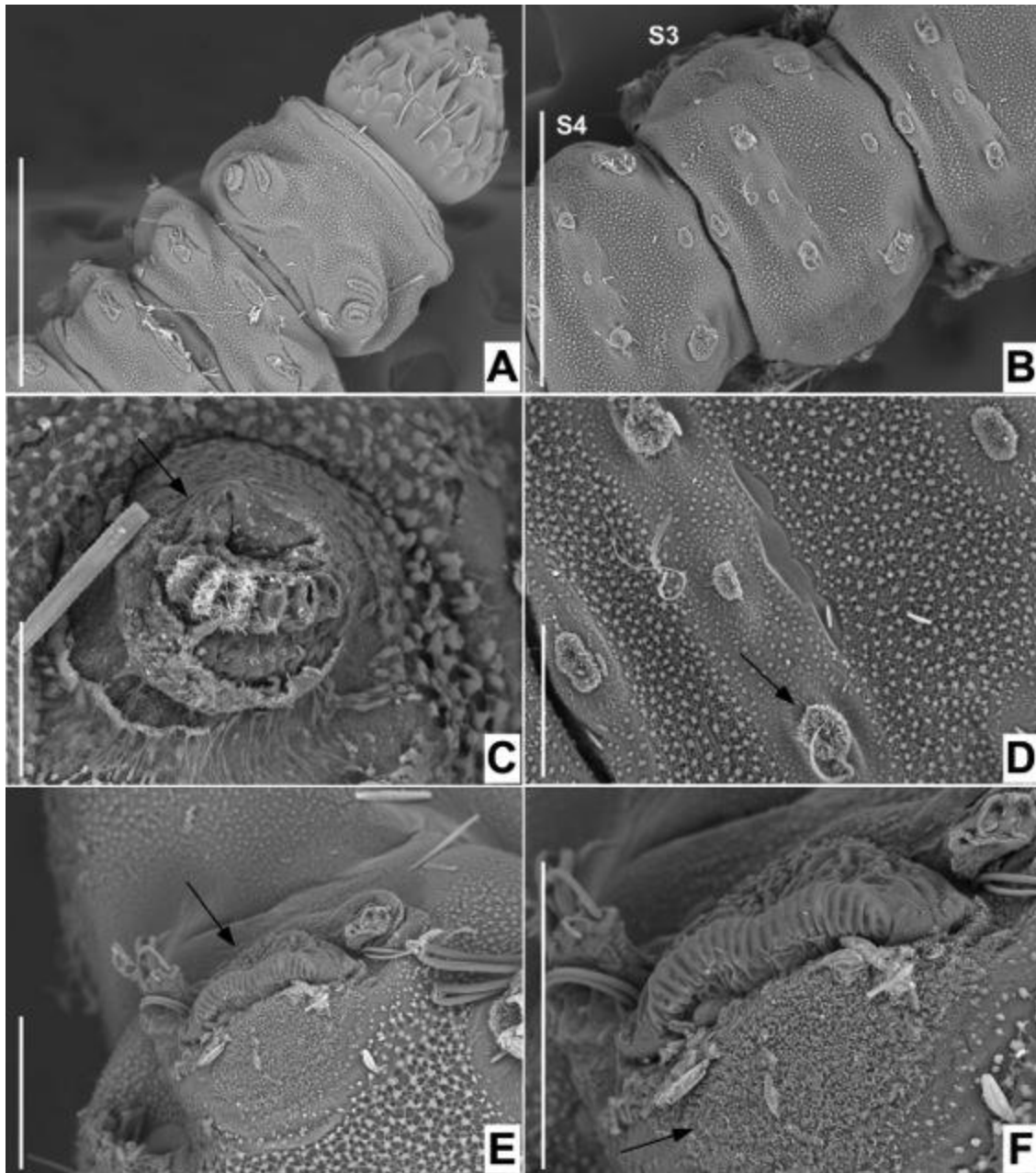


Figure 3.44. Scanning electron micrographs of dorsal view of *Neothaumalea atlantica* larva: A, head, pro- and mesothorax (scale bar = 0.5 mm); B, abdominal segment 3 (scale bar = 0.5 mm); C, prothoracic spiracle (scale bar = 0.25 mm); D, close-up of abdominal segment 2 and protuberances (scale bar = 0.1 mm); E, posterior spiracular plate (scale bar = 0.25 mm); F, close-up of posterior spiracular plate and felt-like cuticle (scale bar = 0.1 mm). Abbreviations: S, abdominal segment.

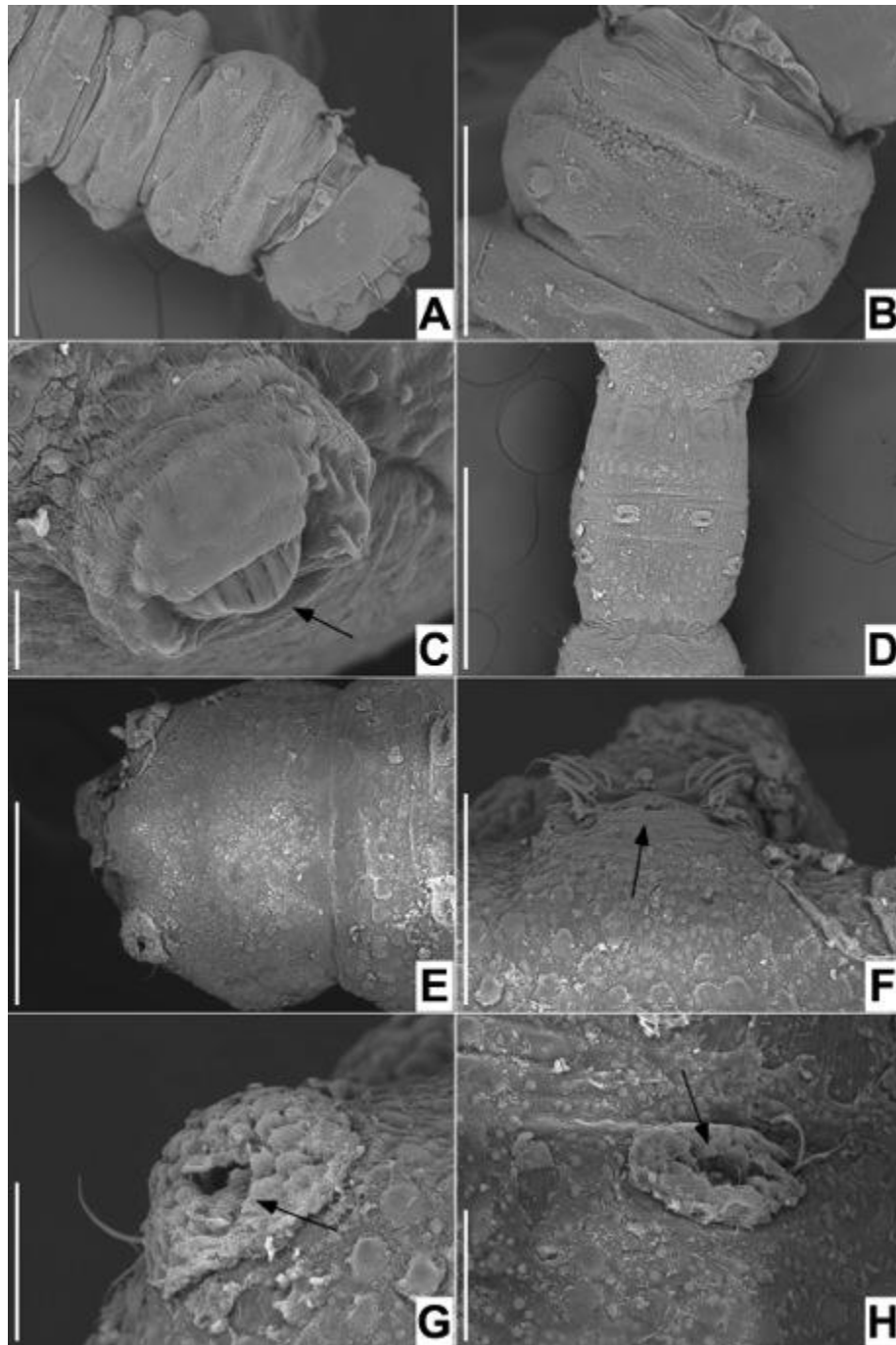


Figure 3.45. Scanning electron micrographs of dorsal view of *Niphta nudipennis* larva: A, head, pro- and mesothorax (scale bar = 0.5 mm); B, prothorax (scale bar = 0.25 mm); C, prothoracic spiracle (scale bar = 0.25 mm); D, abdominal segment 4 (scale bar = 0.5 mm); E, abdominal segment 8 (scale bar = 0.25 mm); F, posterior spiracular plate (scale bar = 0.1 mm); G, close-up of protuberance on abdominal segment 8 (scale bar = 0.05 mm); H, close-up of protuberance on abdominal segment 7 (scale bar = 0.05 mm).

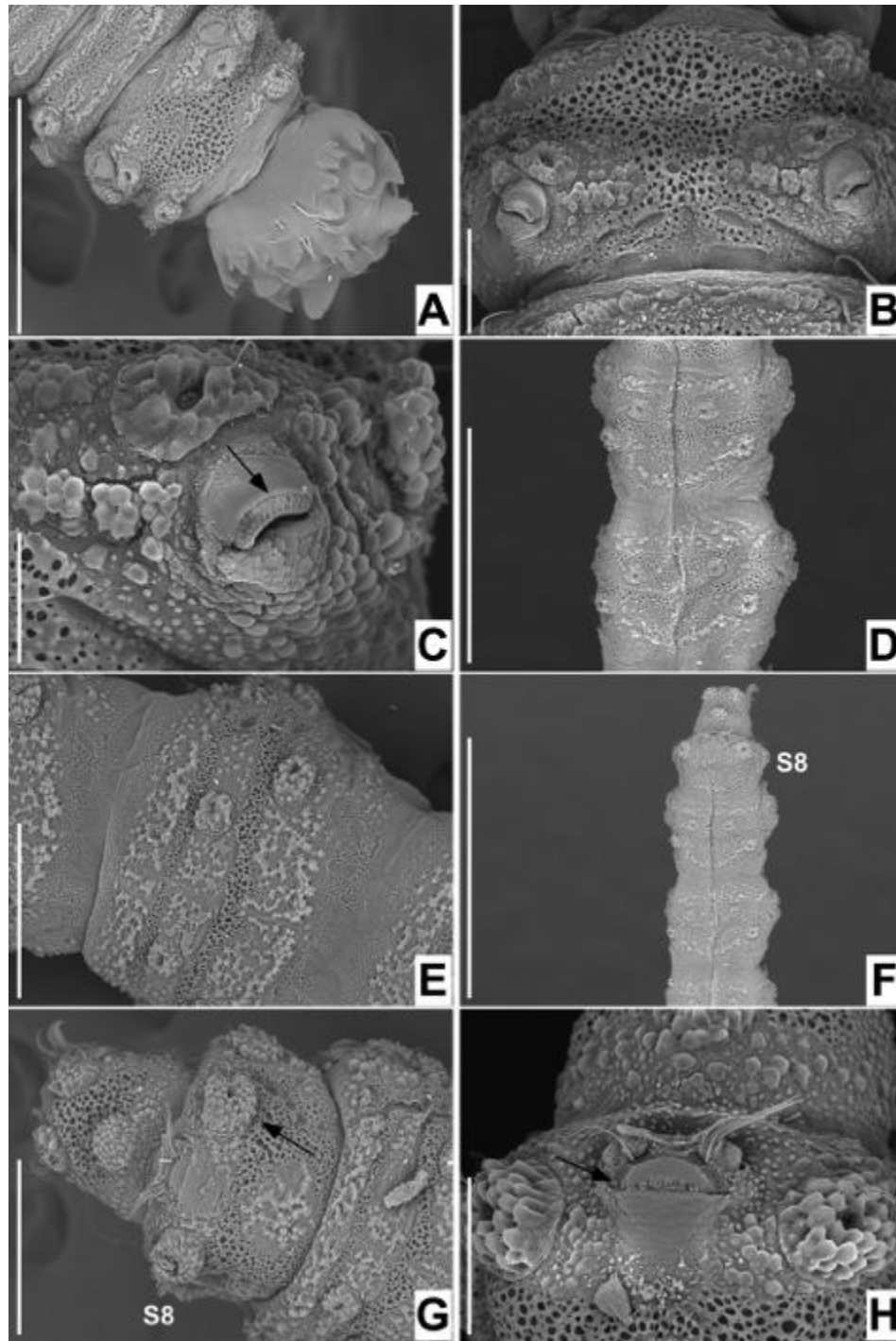


Figure 3.46. Scanning electron micrographs of dorsal view of *Niphta acus* larva: A, head and prothorax (scale bar = 0.5 mm); B, prothorax (scale bar = 0.1 mm); C, prothoracic spiracle (scale bar = 0.1 mm); D, abdominal segments 4 & 5 (scale bar = 0.5 mm); E, abdominal segment 6 (scale bar = 0.25 mm); F, abdominal segments 6–9 (scale bar = 1.0 mm); G, abdominal segments 8 & 9, and protuberances (scale bar = 0.25 mm); H, posterior spiracular plate (scale bar = 0.1 mm). Abbreviations: S, abdominal segment.

CHAPTER 4
FIRST PHYLOGENY DEPICTING GENERIC RELATIONSHIPS IN
THAUMALEIDAE (DIPTERA)

ABSTRACT

The first phylogeny depicting generic relationships within the family Thaumaleidae is presented based upon evidence from three nuclear genes: big zinc finger, elongation complex protein 1 and molybdenum cofactor sulfurase. Sequences were obtained from at least one representative of each genus in order to determine relationships and test the validity of all known genera. Phylogenetic analysis was conducted using Bayesian methods. Two main lineages are recovered: the Northern Hemisphere clade and the Southern Hemisphere clade. The Northern Hemisphere clade includes *Trichothaumalea* Edwards and *Thaumalea* Ruthe, which is expanded to include all species of *Androprosopa* Mik and *Protothaumalea* Vaillant. The Southern Hemisphere clade places *Afrothaumalea* Stuckenberg as sister to the remaining genera: *Austrothaumalea* Tonnoir (expanded to include *Oterere* McLellan) + (*Neothaumalea* Pivar, Moulton & Sinclair + *Niphta* Theischinger). Phylogenetic implications and a hypothesis about the geographic origin of Thaumaleidae are discussed.

INTRODUCTION

The phylogenetic position of the family Thaumaleidae (madicolous midges) is well supported as sister to Simuliidae (black flies) (Pawłowski *et al.* 1996; Moulton 2000; Bertone *et al.* 2008; Wiegmann *et al.* 2011; Borkent 2012; Kutty *et al.* 2018); however, generic relationships within Thaumaleidae have never been rigorously tested. Madicolous midges are commonly referred to as seepage midges due to their highly specialized larval habitat: thin films of vertical flowing water over either rocky (Vaillant 1956; Sinclair and Marshall 1987; Pivar *et al.* 2018a) or vegetative (Pivar, Chapter 3) substrates. Their

restricted habitat and low vagility has led to thaumaleids being under-collected and subsequently understudied. There are approximately 200 described species among 8–9 recognized genera: *Afrothaumalea* Stuckenberg, *Androprosopa* Mik, *Austrothaumalea* Tonnoir, *Neothaumalea* Pivar, Moulton & Sinclair, *Niphta* Theischinger, *Oterere* McLellan, *Thaumalea* Ruthe and *Trichothaumalea* Edwards. Some researchers recognize *Protothaumalea* Vaillant as a ninth valid genus (Wagner 2002), while others consider it to be a synonym of *Androprosopa* (Sinclair 1996).

The vast majority of thaumaleid research has been descriptive in nature. Species-level relationships have been proposed for various genera (McLellan 1988; Wagner 2002; Sinclair 2008b; Pivar *et al.* 2018a), some of which have been tested molecularly (Haubrock *et al.* 2017; Pivar in prep.). While no phylogenies depicting thaumaleid generic relationships have been published, hypotheses have been proposed.

Sinclair (pers. comm.) hypothesized *Thaumalea* + *Androprosopa* is the sister group to all remaining genera. Both of these Holarctic genera are distinguished from all others by the presence of a complete subcostal (Sc) vein. Most described species of *Androprosopa* were initially placed in the genus *Thaumalea* until Sinclair (1996) redefined *Androprosopa* to include all species with obliquely to dorsoventrally directed gonostyli. Edwards (1929), Vaillant (1953), and Sinclair (1996) all noted that both genera are nearly identical and can only be accurately separated on the basis of male genitalia. The validity of *Protothaumalea* is also disputed, as it was synonymized with *Orphnephilina* (= *Androprosopa*) by Vaillant and Vinçon (1988) on the basis of undisclosed intermediate characters possessed by *An. demandana* (Vaillant and Vinçon). Wagner (2002) rejected this synonymy, while Sinclair (1996) accepted it. This

controversy, along with the generic limits of *Androprosopa* and *Thaumalea* must be rectified in order to provide a stable classification system for future research.

Sinclair (pers. comm.) also hypothesized the abbreviated Sc vein as a synapomorphy supporting a lineage comprised of *Trichothaumalea* + *Afrothaumalea* + *Niphta* + *Neothaumalea* + *Oterere* + *Austrothaumalea*. Sinclair noted that the abbreviated Sc vein terminates at the sc-r crossvein in all Southern Hemisphere genera, forming a well-supported monophyletic lineage (Sinclair 2008a). In this hypothesis, *Trichothaumalea* would either be placed with the Northern Hemisphere genera, or on its own, as the Sc vein terminates opposite the branching of Rs (Sinclair and Saigusa 2002). Previously, Sinclair and Stuckenberg (1995) suggested close affinities between *Afrothaumalea*, *Austrothaumalea* and *Niphta*, citing several larval characters. Sinclair (2000) suggested that the absence of apical, spine-like gonostylar setae in *Afrothaumalea*, *Austrothaumalea*, *Niphta* and *Oterere* further supported this hypothesis, but the recent discoveries of *Af. stuckenbergi* Sinclair and *Ne. atlantica* Pivar and Pinho puts this hypothesis into question as they both possess gonostylar spines. *Niphta* and *Afrothaumalea* were also considered to be sister-genera based on reduced macrotrichia on the first wing vein (Sinclair and Stuckenberg 1995; Sinclair 2000), but Sinclair (2015) describes that setae run the entire length of the first vein in *Af. stuckenbergi*, calling into question the value of this character. The validity of the genus *Oterere* is also suspect, as several characters used to diagnose the genus are also found in species of *Austrothaumalea*, perhaps rendering it paraphyletic (Sinclair 2008a,b). The relationship between *Trichothaumalea* and other genera is also in question, as it shares derived characters with *Niphta* (presence of a prominent antealar ridge), *Afrothaumalea* and

Neothaumalea (dorsoventrally flattened pupa), but these are thought to be due to homoplasy (Sinclair & Saigusa 2002; Sinclair 2015; Pivar *et al.* 2018b). Pivar *et al.* (2018b) proposed that *Neothaumalea* was closely allied to *Niphta* based on morphological characters (see Chapter 2).

The absence of a well-supported phylogeny and resultant stable and predictive classification for Thaumaleidae are glaring voids for the family. The objectives of this research are to utilize three nuclear protein-coding genes to test all generic concepts and relationships within Thaumaleidae, in particular, deciphering relationships among the difficult to assess Gondwanan genera. Specifically, the objectives for this paper are as follows:

- Construct the first phylogeny for the entire Thaumaleidae.
- Assess the monophyly and validity of all known genera, in particular *Oterere* and *Protothaumalea*.
- Infer evolutionary relationships between the Gondwanan genera.
- Determine the affinities of *Trichothaumalea*.
- Trace the geographic origin of Thaumaleidae.

This phylogeny will give insight into relationships, evolutionary trends and biogeography within the family. It will also help future studies to determine character polarity, which has been problematic in the past. A rigorously tested and comprehensive phylogeny is imperative for setting the groundwork for future thaumaleid studies.

MATERIALS AND METHODS

Taxon sampling

Samples were collected by the authors or were contributed by colleagues from around the world. Upon collection, specimens were placed immediately into 95% non-denatured ethanol and kept at -20°C until use. Ingroup taxa included, at minimum, one exemplar for each genus (Table 4.1). When possible, multiple species from each genus were included to challenge generic monophyly and shorten branches in reconstructed phylogenies. Attempts to obtain type species for each genus were made when possible.

Outgroups (Table 4.2) were chosen based on relationships established by previous studies regarding the position of Thaumaleidae within the infraorder Culicomorpha (Hennig 1973; Pawlowski *et al.* 1996; Moulton 2000; Bertone *et al.* 2008; Wiegmann *et al.* 2011; Borkent 2012; Kutty *et al.* 2018) and include representatives from the following families: Chironomidae, Culicidae, Dixidae and Simuliidae. In the case of dixids, gene sequences from closely related species were amalgamated to obtain full sequence coverage (Table 4.2). Data extracted from the National Center for Biotechnology Information (NCBI) are indicated with GenBank accession numbers in Table 4.2, otherwise, all sequences were generated in the Moulton lab.

Genomic sampling

Gene regions from three nuclear-coding loci were chosen to carry out this study: big zinc finger (BZF), elongation complex protein 1 (ECP1) and molybdenum cofactor sulfurase (MCS). These genes were chosen because of their proven ability to resolve relationships among both closely related taxa and for constructing deeper level

phylogenies, as well their presence as single copies, high levels of sequence divergence and large size (Senatore *et al.* 2014; Winkler *et al.* 2015; Blaschke *et al.* 2018; Pivar in prep.).

Big zinc finger (BZF)

Senatore *et al.* (2014) demonstrated BZF's capability as a phylogenetic marker when constructing relationships within the *Simulium jenningsi* Malloch species group of black flies. Pivar (2015, in prep) later used, with positive results, the distal two-thirds of BZF to infer relationships between the Nearctic thaumaleid fauna. The entire amplifiable gene region (~3,000 kb) was used for this study. Thaumaleids contain four introns in this region, and upon their removal the average length of the fragment was ca. 2.7 kilobases. The final aligned matrix included 68 terminal taxa: 16 outgroups and 52 ingroup taxa.

Elongation complex protein 1 (ECP1)

The inclusion of ECP1 for this study was based on Senatore *et al.*, 's (2014) success resolving black fly relationships, as well as Moulton's (unpublished) success resolving deep relationships within the Dixidae. Custom primers are available to acquire the entire gene; however, the anterior two-thirds were amplified for this study because it is intron free; the latter third contains three introns in thaumaleids that were problematic to direct sequencing. The average sequence length acquired for 16 outgroup and 52 ingroup taxa, a total of 68 taxa, was 2 kilobases. The highly conserved nature of the gene between conspecifics and the lack of introns make this an ideal gene for DNA fingerprinting thaumaleids, and on numerous occasions successfully identified females,

immatures, and damaged specimens.

Molybdenum cofactor sulfurase (MCS)

MCS has been demonstrated to have a high degree of phylogenetic informativeness for deciphering relationships among tachinid flies (Winkler *et al.* 2015; Blaschke *et al.* 2018) and Nearctic thaumaleids (Pivar in prep.). This study targeted a 2.5 kilobase region of the gene, resulting in an average of 1.8 kilobases after the removal of seven introns that occur in thaumaleids. Problems with direct sequencing through these introns due to allelic length polymorphisms resulted in several taxa having truncated sequences due to the use of 3' primers designed to cleave problematic introns. Terminal taxa included 13 outgroup and 51 ingroup taxa, totaling 64 taxa.

Laboratory methods

Total genomic DNA was extracted from specimens using either Thermo Scientific's GeneJET Genomic DNA Purification Kit #K0722 (ThermoScientific, Waltham, MA, U.S.A.) or Omega Bio-tek's E.Z.N.A.® Insect DNA Kit #D0926-02 (Omega Bio-tek, Inc., Norcross, Georgia, U.S.A.) following the manufacturers' suggested protocols, except for reducing the final elution volume to a total of 100µl. In most cases, only the abdomen was used in order to preserve the integrity of the head and thorax for future mounting and research, such as descriptive studies. This was especially important if only a single specimen was available. Resultant gDNA was stored at -20°C and cleared voucher specimens were stored either in 70% non-denatured ethanol or glycerin vials pinned beneath mounted specimens. Amplifications were performed in

GenePro (Bioer Technology Co., Hangzhou, China) thermal cyclers, using 50µL PCR reactions filled with TaKaRa Ex Taq Hotstart DNA polymerase (Takara Bio, Shiga, Japan) kit components per the manufacturer's suggested protocol, with 1.5µL of template DNA, and 3µl of 20µM custom forward and reverse primers (Table 4.3). Occasionally, reamplification of PCR products was required in order to obtain sufficient yields of DNA for Sanger sequencing using less to nondegenerate taxon or clade-specific internal primers (available upon request). The following PCR regimen was utilized: Initial 90s denaturing step at 94 °C, then 4 cycles of 30s at 94 °C, 30s at 56 °C and 75s at 72 °C, followed by 4 cycles of 30s at 94 °C, 25s at 52 °C and 75s at 72 °C, 9 cycles of 30s at 94 °C, 20s at 48 °C and 75s at 72 °C and finally, 38 cycles of 30s at 94 °C, 20s at 45 °C and 75s at 72 °C. Upon completion, the program ends at 72 °C for 5min., and then finishes with a 15 °C hold to keep the samples cool until removal from the thermal cycler. PCR products were electrophoresed in 1% agarose gels at 115V for 30min, excised, then purified using silica column-based protocols and eluted in 37µL of elution buffer (10mM Tris, pH8.5). Purified PCR products served as a template for sequencing, which was carried out by using a 1:1 dilution of water and the original PCR primers. Purified templates were sequenced in both directions in 20µL reactions using 20-fold diluted (= 0.4µl) BigDye® v3.1 terminators (Applied Biosystems, Carlsbad, California, U.S.A.) including a proprietary 5X sequencing buffer and implementing a three-step touchdown procedure with respect to annealing temperature. Sequencing reactions were cleaned using Centrisep columns (Princeton Separations, Adelphia, NJ, U.S.A.) and dried in a Centrivap Concentrator (LABCONCO, Kansas City, MO, U.S.A.). Dried samples were sent to the University of Tennessee-Knoxville Genomics Core for sequencing. Sequences

from opposing strands were reconciled and verified for accuracy using Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI, U.S.A.). Exon-intron boundaries were determined using the GT-AG rule and subsequently removed, ensuring the presence of a resultant continuous open reading frame of concatenated exons (Rogers and Wall 1980). All introns were excised in Sequencher prior to alignment and phylogenetic analysis. All sequences will be deposited in GenBank at a future date.

DNA alignment and phylogenetic analysis

Final nucleotide data matrices were exported as Nexus-formatted files (Maddison *et al.* 1997) for subsequent alignment and phylogenetic analysis. The amino acid alignment was made in Mesquite 2.72 (Maddison and Maddison 2011) using Opal 2.1.0 (Wheeler and Kececioglu 2007), upon which the DNA nucleotide alignment was created. The final nucleotide alignment was then subjected to Bayesian analysis using MrBayes 3.2.6 (Ronquist *et al.* 2012) as implemented on the online CIPRES Science Gateway (Miller *et al.* 2010). Two runs with four chains each were run for a total of 1×10^7 generations. Markov chains were sampled at every 1000 generations and the first 25% of pre-stationarity trees were discarded as burn-in. Default settings for the priors were used, and base frequencies were estimated from the data. Upon completion of analyses, both output tree files were imported into PAUP* (Swofford 2001) and combined to assemble a 50% majority rule consensus tree. Phylogenetic analyses were conducted on each gene individually, followed by an analysis of all three genes combined, resulting in a total of four trees.

RESULTS

Each gene recovered nearly identical topographies, with the few exceptions occurring mainly among the outgroups. BZF (Fig. 4.1) and ECP1 (Fig. 4.2) performed similarly, although deep nodal support was slightly weaker than that of MCS (Fig. 4.3), which proved to be the most robust gene providing solid support values throughout the tree. MCS was the top-performing gene among several examined for phylogenetic informativeness within Tachinidae (Winkler *et al.* 2015; Blaschke *et al.* 2018). The combined gene tree (Fig. 4.4) is hypothesized to be the best estimate of the evolutionary history of Thaumaleidae based on the more robust node support throughout. Node support for Bayesian inference is represented by posterior probability (pp). Parentheses ‘()’ denote sister groups and square brackets ‘[]’ represent paraphyletic groups.

Outgroups

In the combined gene analysis, twenty-two non-thaumaleid outgroups were included, with the chironomid, *Clunio marinus* Haliday, chosen as the distalmost. Dixids formed the sister clade to Culicidae + Simuliidae + Thaumaleidae with strong support (pp = 0.98). Culicidae + Simuliidae formed a highly supported clade (pp = 0.97) as sister to Thaumaleidae, which was recovered as monophyletic with high support (pp = 0.98). The BZF analysis recovered a similar topography but with much less node support. Dixids were recovered as a monophyletic group (pp = 1.00), but support values within the family are weaker. The arrangement of Culicidae + (Simuliidae + Thaumaleidae) had no significant support, with nodes of 0.76 and 0.42, respectively. The monophyly of Thaumaleidae itself was not supported with BZF alone (pp = 0.66). ECP1 also had issues

resolving outgroup relationships; it recovered a (Culicidae + Dixidae) clade with low support (pp = 0.87) and Simuliidae + Thaumaleidae as sister families with low support (pp = 0.88). It did however support Thaumaleidae as monophyletic (pp = 0.96). MCS had strong support (pp = 0.99) for a clade including (Dixidae + Culicidae + Simuliidae) as sister to the strongly supported, monophyletic Thaumaleidae (pp = 0.98).

Thaumaleidae

Thaumaleidae are represented by two main lineages in each phylogeny. The first lineage consists of genera found in the Northern Hemisphere, and the second with those found in the Southern Hemisphere. The Northern Hemisphere clade and the Southern Hemisphere clade were recovered with strong support by ECP1, MCS and the combined data sets (ECP1 pp = 0.96; MCS pp = 0.98; combined pp = 0.98). BZF also recovered two major clades, but the taxa within each clade differed from the other analyses and statistical support for the groupings was low.

Northern Hemisphere Clade

Topologies within this grouping were nearly congruent between all analyses. ECP1, MCS and combined all recovered *Trichothaumalea* as sister to [*Androprosopa* + *Protothaumalea* + *Thaumalea*] with strong support (ECP1 pp = 0.94; MCS pp = 0.97; combined = 0.97). BZF was the lone analysis to recover the Northern Hemisphere clade without *Trichothaumalea*, but still supported a [*Androprosopa* + *Protothaumalea* + *Thaumalea*] clade with a posterior probability of 1.00. *Trichothaumalea* was always strongly supported as monophyletic (BZF pp = 1.00; ECP1 pp = 0.98; MCS & combined

= 0.99). The [*Androprosopa* + *Protothaumalea* + *Thaumalea*] grouping was strongly supported by all genes (BZF pp = 1.00; ECP1 pp = 0.90; MCS pp = 0.97; combined pp = 0.99). Within this group, western Nearctic species of *Androprosopa* form a clade (BZF pp = 1.00; ECP1 pp = 0.96; MCS pp = 0.95; combined pp = 0.99) that is sister to the [eastern Nearctic/Palearctic *Androprosopa* + *Protothaumalea* + *Thaumalea*] (BZF pp = 0.89; ECP1 pp = 0.90; MCS pp = 0.97; combined pp = 0.98).

Southern Hemisphere Clade

The Southern Hemisphere clade is comprised of *Afrothaumalea* as sister to ([*Austrothaumalea* + *Oterere*] + (*Neothaumalea* + *Niphta*)) (ECP1 pp = 0.90; MCS pp = 0.97; combined pp = 0.98). BZF, as mentioned above, was the only outlier, as it recovered *Trichothaumalea* as sister to the remaining Southern Hemisphere genera, but with no statistical support. BZF did recover *Afrothaumalea* + ([*Austrothaumalea* + *Oterere*] + (*Neothaumalea* + *Niphta*)), but again with no significant support. The paraphyletic [*Austrothaumalea* + *Oterere*] grouping was supported by all genes with strong support (BZF pp = 1.00; ECP1 pp = 0.95; MCS pp = 0.99; combined pp = 0.99). Within this group, taxa from both Australia and Chile formed closer relationships with each other, rather than distinct Australia or Chile clades. Support for *Neothaumalea* + *Niphta* as sisters was significant and was recovered by analyses of all gene (BZF pp = 1.00; ECP1 pp = 0.96; MCS pp = 0.97; combined pp = 0.99). Within *Niphta*, all genes recovered two distinct clades, an Australian clade and a Chilean clade (BZF pp = 1.00; ECP1 pp = 0.94; MCS pp = 0.99; combined pp = 0.99).

DISCUSSION AND CONCLUSIONS

This study represents the first comprehensive phylogenetic analysis of supraspecific relationships among world Thaumaleidae segregates. The targeted loci – BZF, ECP1, and MCS – each yielded trees having largely congruent topologies, with MCS being the lone one providing significant statistical support throughout. When combined, these genes provided robust phylogenetic signal and the resultant phylogeny provides the basis for the following discussion. Some prior hypotheses about generic relationships were validated, while others were not, and several novel hypotheses about thaumaleid relationships were revealed. Here, generic relationships and concepts are discussed, as well intrageneric relationships. Taxonomic implications and a hypothesis about the geographic origin of thaumaleids are also proposed.

Thaumaleidae was recovered with strong support as a monophyletic group. Within the family, two clades are strongly supported: the Northern Hemisphere clade and the Southern Hemisphere clade.

Northern Hemisphere clade

This group consists of four genera, with relationships as follows:
Trichothaumalea + [*Androprosopa* + *Protothaumalea* + *Thaumalea*]. This lineage is supported by the wing venation character Sc reaching or extending beyond the branching of Rs; *Androprosopa*, *Protothaumalea* and *Thaumalea* all share a complete Sc vein (though this could be plesiomorphic), while that of *Trichothaumalea* is incomplete but extends to the branching of Rs (Sinclair 1996; Sinclair and Saigusa, 2002). Additionally, the position of sensory pit 13 on the larval head-capsule is located near the base of seta 5

in all Northern Hemisphere genera (Sinclair 1992; 1996; 2000), and caudal lobes flanking the posterior spiracular plate are present (Saunders 1923; Sinclair 1996; Sinclair and Saigusa 2002).

Trichothaumalea

The monophyly of *Trichothaumalea* was never in doubt, as it is the only genus with macrotrichia on the wing membrane and without a gonocoxal plate (Edwards 1929; Sinclair and Saigusa 2002). Sinclair and Saigusa (2002) also alluded to the short, hook-like parameres and dorsoventrally flattened pupae as autapomorphic within the genus; however, since that publication both *Afrothaumalea* (Sinclair 2015) and *Neothaumalea* (Pivar *et al.* 2018) have been discovered to also possess dorsoventrally flattened pupae. *Neothaumalea* also possesses short, hook-like parameres. Sinclair and Saigusa (2002) hypothesized that *Tr. japonica* Sinclair and Saigusa is sister to the eastern Nearctic species *Tr. elakalensis* Sinclair, while the two western Nearctic species, *Tr. pluvialis* (Garrett) and *Tr. pilosa* (Dyar and Shannon), are sister species. This arrangement was not compatible with the topology observed here, possibly because we were unable to include *Tr. pilosa* (fresh material was unavailable).

Androprosopa*, *Protothaumalea* and *Thaumalea

These three genera are closely related on the basis of the complete Sc, however, generic concepts have been problematic and controversial (see introduction). This study found *Androprosopa* and *Protothaumalea* to be paraphyletic in relation to *Thaumalea*. The paraphyly of *Protothaumalea* is not surprising, given its tumultuous taxonomic

history. Additionally, Haubrock *et al.* (2017) found *Protothaumalea* to be nested within *Thaumalea* in their molecular phylogeny of European Thaumaleidae. That *Androprosopa* is paraphyletic is somewhat unexpected, though previous studies have eluded to its potential for paraphyly. Haubrock *et al.* (2017) recovered *Androprosopa* as a monophyletic sister group to *Thaumalea*, but their taxon sampling was limited to three European species. Pivar *et al.* (in prep.), in a molecular phylogenetic analysis of Nearctic *Androprosopa*, observed *Thaumalea testacea* Ruthe, initially intended to be an outgroup taxon, to be nested within the species of Nearctic *Androprosopa*. The more robust sampling of taxa from disparate geographical areas provided the means to better resolve relationships among species shunted into these two genera. Within the *Thaumalea* clade, the western Nearctic *Androprosopa* forms a monophyletic group, while the eastern Nearctic and Palearctic *Androprosopa* species form another. *Thaumalea* should be expanded to subsume both *Androprosopa* and *Protothaumalea*, as the latter are both junior synonyms. Further investigation is required to determine which morphological characters should be used to define the broadened genus concept. It appears that the gonostylar action that Sinclair (1996) used to separate the genera is not phylogenetically informative. Currently, the most important character useful for defining *Thaumalea* s.l. is the complete Sc vein. The presence of dorsal curved hooks on the caudal segment of the pupae also appears to be a strong synapomorphy, as do the dorsal abdominal setae. Other as yet undiscovered characters in the preimaginal stages would also be worth considering.

Southern Hemisphere clade

A monophyletic Southern Hemisphere clade was hypothesized by Sinclair (2008a) as a well-supported clade on the basis of an incomplete Sc vein that terminates at, or before, the sc-r crossvein, and is well supported in this phylogeny. Additional synapomorphies for this lineage include: the deflexion of the basal section of R into cell br (Sinclair and Saigusa 2002), sensory pit 13 located near the dorsal margin of the antenna in the larval head capsule (Sinclair 1995; 2000; Pivar *et al.* 2018b; Pivar Chapter 3), and caudal lobes flanking the posterior spiracular plate are absent (Sinclair 2000; Pivar Chapters 2 and 3). The Southern Hemisphere clade is represented by five genera with the following relationships: *Afrothaumalea* + ([*Austrothaumalea* + *Oterere*] + (*Neothaumalea* + *Niphta*)).

Afrothaumalea

Afrothaumalea is defined by broad gonostyli, paired finger-like gonocoxal blades, and a triangular-shaped epandrium (Sinclair 2015). The genus consists of three described species, although only *Af. stuckenbergi* was available for this study; it was always placed as the sister to the remaining Southern Hemisphere fauna. This contradicts the hypothesis that *Afrothaumalea* is most closely related to the Australian and South American genus *Niphta*, which has been proposed on the basis of reduced setae on the first vein ($R_1(+R_{2+3})$) (Sinclair and Stuckenberg 1995). Sinclair (2015) questioned the utility of this character after the discovery of *Af. stuckenbergi*, which possesses setae along the entire length of the first vein. As mentioned earlier, the pupa of *Af. stuckenbergi* is

dorsoventrally flattened, similar to that of *Trichothaumalea* (Sinclair and Saigusa 2002; Sinclair 2015), although Sinclair suggested these are likely not homologous.

Austrothaumalea* and *Oterere

Austrothaumalea has numerous diagnostic features (Sinclair 2008b) and its validity as a monophyletic group has never been in doubt. *Oterere*, however, has been hypothesized as rendering *Austrothaumalea* paraphyletic because its defining features are shared with numerous species of *Austrothaumalea*: a well-developed appendage on CuA, macrotrichia on R₄₊₅ and patterned wings (Sinclair 2008a, b). The specimen of *Oterere* used in this study was a female, but it possesses all of the features of *Oterere setipennis* (Edwards), and the authors are certain that it is congeneric, if not conspecific. *Oterere* was recovered as deeply nested within *Austrothaumalea*, supporting Sinclair's (2008b) hypothesis that is synonymous with *Austrothaumalea*.

Several species groups have been proposed for *Austrothaumalea* (McLellan 1988; Sinclair 2008b) and despite the small number of taxa herein, it appears these groups need to be redefined. *Austrothaumalea spatulata* Schmid and *Au. fredericki* Pivar are closely related, as predicted by Pivar (Chapter 3) where he placed them in the *Au. fusca* Theischinger group described by Sinclair (2008b). *Austrothaumalea spiculata* Pivar was also hypothesized as a member of this group (Pivar *et al.* 2016), but it was recovered in a separate clade with members of the *Au. capricornis* Theischinger group: *Au. capricornis* Theischinger *Au. chilensis* Edwards, *Oterere setipennis* (= *Austrothaumalea*) (Edwards), *Au. spinosa* Theischinger and *Au. zentae*. This phylogeny also places *Au. denticulata* in the *Au. capricornis* group, rather than on its own as a member of the *Au. barrydayi*

Theischinger group (Sinclair 2008b). These discrepancies may be a result of undersampling, or the defining characters may not be phylogenetically important. The groups are defined mainly by the shape of the gonocoxal plate, though the parameres and epandrium may also be useful. The species studied herein are morphologically suited to their hypothesized groups, but that they are not recovered that way suggests the gonocoxal plate may not be phylogenetically informative.

Another interesting aspect of the *Austrothaumalea* clade is the relationship between the Chilean and Australian members of the genus; *Au. spiculata* is more closely related to *O. setipennis*, *Au. chilensis* and *Au. sp.2 V69F*, rather than its Australian relatives. This is not unexpected, as the ties to Gondwana have been hypothesized many times previously, and will be discussed further in this paper. These relationships are well supported in the phylogenetic tree and provide evidence of diversification within the genus prior to Gondwanan break-up.

Unfortunately, New Zealand species were unavailable for this study. Although they share many characters with Australian and Chilean species, they possess some marked differences, most notably by the shape of the male parameres and foliate gills of the pupa (McLellan 1983; 1988). The pupa of only a single Australian representative has been described (Sinclair 2000) and it lacked foliate gills. Thus far, foliate gills are not reported from any other known thaumaleid genus, so they represent a strong synapomorphy for the clade that possesses them. Currently, no immatures are known for the South American *Austrothaumalea* species.

Neothaumalea* and *Niphta

The molecular data shows strong support for a *Neothaumalea* + *Niphta* sister group, as does morphology. Pivar *et al.* (2018a) proposed this relationship based upon the presence of a developed antealar ridge, absent in all other Southern Hemisphere genera, and the lack of proepisternal setae adjacent to the anterior spiracle, present in all other genera. *Trichothaumalea* also has a developed antealar ridge, but this is hypothesized to be the result of convergence.

Niphta consists of three species groups, as discussed by Pivar (Chapter 3), all of which are supported by this phylogeny. The Australian *N. bickeli* group is sister to the two Chilean species groups, which is expected based on both adult and larval morphology (Pivar Chapter 3). Unfortunately, the two Australian *Niphta* species in this study were not identified to species and no voucher exists. They were processed for molecular study when it was common practice to sacrifice the entire specimen for DNA recovery. Attempts were made by colleagues in Australia to recollect *Niphta* specimens for this study, but with no success. Recollecting these species and learning more about their immature stages will be informative in tracing the evolution of the immature adhesive structures found in the South American fauna, as well as in at least one Australian species (Pivar Chapter 3). The only described pupa of Australian *Niphta* (Sinclair 2000) lacks adhesive structures and has a more typical thaumaleid appearance. Which Australian species have larvae with adhesive modifications? Are they more closely related to the Chilean species, or has this evolved multiple times within the genus? These answers will only be answered with continued study of the Australian and South American *Niphta* fauna.

Summary of Taxonomic Changes

The reconstructed phylogeny necessitates three taxonomic changes to the current thaumaleid classification to make it phylogenetically valid. The validity of *Protothaumalea* has been disproved and it is proposed here as a synonym of *Thaumalea*. *Androprosopa* was recovered here as a paraphyletic grade including antecedents of *Thaumalea* and thus is also proposed as a synonym of *Thaumalea* (Table 4.4). Finally, *Oterere* is formally synonymized with *Austrothaumalea* (Table 4.5). The Northern and Southern Hemisphere clades are supported molecularly; however, stronger morphological characters are needed before further higher classification schemes are put into place. Updated generic diversity and distributions are presented in Table 4.6.

Geographical origin of Thaumaleidae

The biogeography of Thaumaleidae has not been studied in depth. Most of the discussion regarding the family's biogeography deals with the well-known Gondwanan elements exhibited by the Southern Hemisphere clade (Edwards 1930; Hennig 1966; Theischinger 1986; McLellan 1988; Sinclair 2000; Sinclair 2008a, b; Sinclair 2015; Pivar *et al.* 2016; Pivar *et al.* 2018b; Pivar Chapter 3). Sinclair has discussed the biogeography of island species (2008a), *Trichothaumalea* (Sinclair and Saigusa 2002), and the only transcontinental species of thaumaleid, *Th. verralli* Edwards (1996). Thaumaleid origins have been problematic to evaluate because of the lack of a stable phylogeny hypothesizing relationships. There is also a paucity of fossil thaumaleids; *Mesothaumalea fossilis* Kovalev is the only known fossil. It is a compression fossil recovered from deposits in eastern Russia, which date back to the Late Jurassic to Early Cretaceous

(Kovalev 1989). With the phylogeny proposed herein, it is possible to draw some conclusions about where thaumaleids originated and distributed to their present day distribution.

As stated above, the Southern Hemisphere genera have long been cited as an example of Gondwanan distribution and these relationships are strongly supported by the molecular data. The genera are found on the continents of Australia, South America and the southern tip of Africa, where the African group is sister to the remaining relatives from Gondwana. This follows a very common relationship topography that has numerous examples in Diptera (Hennig 1966; Brundin 1975; Yeates and Irwin 1996; Cranston and Edward 1999; Cranston 2005). Africa was the first to split from Gondwana 100 Mya, followed by New Zealand around 80 Mya, then Australia from eastern Antarctica around 35 Mya, and finally South America from Antarctica around 30 Mya (de Jong 2003; Krosch *et al.* 2011). Given that timeline, *Afrothaumalea* would be the oldest of the Gondwanan genera since it is only found in Africa, suggesting evolution by vicariance. *Austrothaumalea* would likely have evolved next, while the New Zealand + Australia + Antarctica + South America landmass was still intact. This is evidenced by today's distribution of the genus, as well as the species level relationships depicted in the phylogeny. The Australian *Au. spiculata* is more closely related to Chilean representatives, suggesting diversification while the land masses were still connected. *Niphta* may be the youngest genus in the Southern Hemisphere, as suggested by its absence in New Zealand. Unless there are yet undiscovered species in New Zealand, or there have been extinction events, it is likely *Niphta* evolved after the separation of New Zealand from Gondwana, but before the total separation of the supercontinent. Based on

the phylogeny, as well as the presence of the ventrally modified larvae in only some Australian species, *Niphta* may have originated in central (Antarctica) or eastern (Australia) Gondwana and migrated to western Gondwana (South America). The origins of *Neothaumalea* are more obscure since it is known from only a single species. *Neothaumalea atlantica* is known from the Aparados da Serra Geral mountains in the Atlantic Forest of Brazil, which date to the Mesozoic era (Wildner *et al.* 2004). The Atlantic Forest exhibits austral temperate elements (Cranston 2005), and several dipteran families found there have genera that exhibit these elements (Ditomyiidae, Mycetophilidae, Rangomaramidae, etc.) (Amorim and Santos 2018). Although *Neothaumalea* is known from one species, its sister genus *Niphta* also exhibits austral elements; perhaps, *Neothaumalea* follows a similar biogeographic pattern, but extinctions and unknown present day diversity make this difficult to assess. While Gondwanan relationships are fairly well known, Laurasian dispersal is more obscure.

Laurasia was the second supercontinent that resulted after the breakup of Pangea in the late Triassic (215 to 175 Mya). As Cranston (2005) notes, the dispersal of Laurasian taxa is often complex and difficult to interpret, and typically follow a northern trans-Atlantic track (North America + Europe). Cranston (2005) also discussed that the Pleistocene glaciations likely led to random recolonization processes in middle and higher latitudes, further obfuscating phylogenetic patterns. This appears to hold true within the Northern Hemisphere genera. The western Nearctic *Thaumalea* are monophyletic and sister to the remaining members of the genus, which exhibit disjunct relationships. The eastern Nearctic species are most closely related to the European *Th. larvata* Mik, which is more closely related to eastern Asian species, which are closely

related to European species. Some of these relationships are likely a product of the small number of Palearctic species available for this study, but glaciation events probably had major impacts on extinctions within these geographic regions. Sinclair and Saigusa (2002) hypothesized that *Trichothaumalea* exhibits a Trans-Pacific biogeographic distribution, but the analysis herein does not reveal much about species-level relationships. Species level nodes are not well supported and vary between genes. The present day distribution of *Trichothaumalea* in both the Nearctic and east Palearctic regions indicate that it likely took advantage of the Bering land bridge to reach its present-day distribution, much like the caddisfly genus *Manophylax* Wiggins (Trichoptera: Apataniidae) (Chuluunbat *et al.* 2010).

The question remains: where did Thaumaleidae originate? It is clear that the Southern Hemisphere genera are an ancient group with close ties to Gondwana, and while it is more difficult to age the Northern Hemisphere genera, *M. fossilis* is evidence that it has been in the Palearctic Region at least since the early Cretaceous (~125 Mya). Consistent with present day *Thaumalea*, it too has a complete subcostal vein, suggesting the presence of an extinct common ancestor. As hypothesized by the phylogeny, there were two major radiations from some extinct common ancestor that likely originated in the supercontinent Pangea. There is no evidence to suggest exactly where thaumaleids arose, but their presence on both Laurasia and Gondwana suggest a Pangean origin. Upon the break-up of Pangea, the ancient Tethys Ocean would have acted as a barrier preventing the two populations from mixing, and thus they would have continued into the two distinct geographic lineages observed today. If this is the case, Thaumaleidae may be

at least ~160 million years old, similar to the age of Simuliidae (Grimaldi and Engel 2005).

Conclusion

The phylogeny presented herein is the first of its kind for thaumaleids and will act as a foundation upon which to build future studies. Additional taxa will help to further clarify relationships, particularly within *Austrothaumalea* and *Thaumalea*. Future studies should focus on determining morphological character polarity for all life stages within the family. This will provide stronger characters to separate both major clades and further solidify generic characters, especially for *Thaumalea*. Focused collecting efforts will uncover new diversity and unknown life-stages, all of which will contribute to our knowledge of Thaumaleidae.

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APPENDIX

Table 4.1. Ingroup exemplars of Thaumaleidae taxa used in study. Species name, geographical source data, genomic sampling and size of region used (after removal of introns) are included.

Genus	Species	Specimen Locality Information	Locality Coordinates	Gene and # of base pairs (bp)		
				BZF	ECP1	MCS
<i>Afrothumalea</i> Stuckenberg	<i>stuckenbergi</i> Sinclair	RSA: Western Cape: Cederberg Wilderness, Duiwelsgat	-32°24'05" 19°05'16"	2713 bp	2074 bp	2018 bp
<i>Androprosopa</i> Mik	<i>americana</i> (Bezzi)	USA: GA: Towns Co.: Brasstown Bald	34°51'36" -83°48'09"	2848 bp	2106 bp	2045 bp
	<i>coloradensis</i> (Arnaud & Boussy)	USA: CO: San Juan Co.: San Juan N.F.	37°42'29" -107°46'17"	2744 bp	2072 bp	1977 bp
	<i>elnora</i> (Dyar & Shannon)	USA: WA: Spokane Co.: Mt. Spokane SP	47°54'20" -117°06'35"	2715 bp	2089 bp	2055 bp
	<i>ericfisheri</i> (Arnaud & Boussy)	CAN: BC: Golden	51°27'55" -117°21'03"	2786 bp	2042 bp	1877 bp
	<i>gillespieae</i> (Arnaud & Boussy)	CAN: BC: Hwy 23, 13km N Revelstoke	51°04'03" -118°10'31"	2772 bp	2117 bp	1962 bp
	<i>larvata</i> ^a Mik	DEU: Hesse: Rhon Biosphere Reserve	50°28'04" 10°00'45"	2545 bp	2050 bp	2031 bp
	<i>lindsayorum</i> (Arnaud & Boussy)	USA: WA: Mt. Rainier N.P.	46°46'48" -121°45'43"	2580 bp	2067 bp	2013 bp
	sp. (larva)	CHN: Sichuan: Pingwu, Old Creek Reserve	32°30'37.86" 104°43'9.86"	2000 bp	2076 bp	1620 bp
	<i>striata</i> (Okada)	JPN: Kyushu, Kusatsu godoni	N/A	2715 bp	2057 bp	2022 bp
	<i>thornburghae</i> (Vaillant)	USA: GA: Towns Co.: Brasstown Bald	34°51'36" -83°48'09"	2780 bp	2027 bp	1836 bp

Table 4.1. (continued)

Genus	Species	Specimen Locality Information	Locality Coordinates	Gene and # of base pairs (bp)		
				BZF	ECP1	MCS
	<i>vaillantiana</i> Sinclair	USA: NC: Swain Co.: Great Smoky Mountains N.P.	35°33'24" -83°29'39"	2352 bp	1604 bp	1959 bp
	<i>A. waha</i> (Arnaud & Boussy)	USA: WA: Spokane Co.: Mt. Spokane S.P.	47°54'20" -117°06'35"	2707 bp	2067 bp	1955 bp
<i>Austrothaumalea</i> Tonnoir	<i>capricornis</i> Theischinger	AUS: NSW: Mt. Wilson	-33°30'32" 150°22'26"	2590 bp	2024 bp	1977 bp
	<i>chilensis</i> Edwards	CHL: Araucanía, Rte. 71	-38°13'20.3" -71°44'41.1"	2551 bp	2067 bp	1901 bp
	<i>denticulata</i> Theischinger	AUS: NSW: Mt. Wilson	-33°30'32" 150°22'26"	2605 bp	2050 bp	1767 bp
	<i>fredericki</i> Pivar	CHL: Los Lagos, Lago Llanquihue	-41°08'28" -72°35'16"	2712 bp	2121 bp	2050 bp
	sp.1 R71F	CHL: Araucanía, Rte. 71	-38°14'20.6" -71°53'46.6"	2596 bp	2008 bp	2019 bp
	sp.2 V69F	CHL: Los Lagos, Rte. V-69	-41°49'51.5" -72°24'40.0"	2584 bp	2033 bp	1704 bp
	<i>spatulata</i> Schmid	CHL: Maule, Los Queñes	-34°59'48.8" -70°48'37.0"	1979 bp	2042 bp	1995 bp
	<i>spiculata</i> Pivar	AUS: VIC: Grampians NP	-37°06'37.05" 142°24'31.39"	2500 bp	2013 bp	1877 bp
	<i>spinosa</i> Theischinger	AUS: NSW: Blue Mountains NP	-33°37'41" 150°18'41"	2709 bp	2062 bp	2042 bp
	<i>zentae</i> Theischinger	AUS: NSW: Werrikimbe NP	-31°10'55" 152°19'48"	2638 bp	2069 bp	1890 bp

Table 4.1. (continued)

Genus	Species	Specimen Locality Information	Locality Coordinates	Gene and # of base pairs (bp)		
				BZF	ECP1	MCS
<i>Neothaumalea</i> Pivar, Moulton & Sinclair	<i>atlantica</i>^a Pivar & Pinho	BRA: Santa Catarina, Serra do Corvo Branco	-28°03'21" -49°22'01"	2959 bp	2046 bp	2000 bp
<i>Niphta</i> Theischinger	<i>acus</i> Pivar	CHL: Bío Bío, Estero Agua Blanca	-37°46'30.8" -71°42'03.9"	2855 bp	1679 bp	1767 bp
	<i>brunnea</i> Pivar	CHL: Araucanía, Rte. 71	-38°14'20.6" -71°53'46.6"	2730 bp	2050 bp	1650 bp
	<i>courtneyi</i> Pivar	CHL: Los Lagos, Lago Llanquihue	-41°08'28" -72°35'16"	2814 bp	2120 bp	1807 bp
	<i>daniellae</i> Pivar	CHL: Araucanía, Rte. 71	-38°14'20.6" -71°53'46.6"	2791 bp	2077 bp	1745 bp
	<i>downesi</i> Pivar	CHL: Los Ríos, Rte. T-85	-40°19'58.6" -72°16'56.1"	2881 bp	2139 bp	1723 bp
	<i>eurydactylus</i> Pivar	CHL: Los Lagos, Rte. U-99	-41°08'28.2" -72°35'16.8"	2781 bp	2090 bp	1708 bp
	<i>halteralis</i> (Edwards)	CHL: Los Lagos, Camino de Penetracion	-42°03'33.3" -72°27'07.4"	2610 bp	2149 bp	1995 bp
	<i>mapuche</i> Pivar	CHL: Araucanía, Rte. S-365	-38°46'27.0" -71°36'51.0"	2835 bp	2141 bp	2015 bp
	<i>moultoni</i> Pivar	CHL: Los Ríos, Antilhue	-39°49'09.8" -72°56'22.6"	2804 bp	2100 bp	1820 bp
	<i>nudipennis</i> (Edwards)	CHL: Los Lagos, Chiloé	-42°18'20.3" -73°26'08.9"	2795 bp	2100 bp	1805 bp
	<i>sinclairi</i> Pivar	CHL: Maule, Los Queñes	-34°59'46.7" -70°49'19.2"	2721 bp	2116 bp	872 bp
	sp.1 AUS	Australia	N/A	2085 bp	2028 bp	1894 bp
	sp.2 AUS	Australia	N/A	2718 bp	2023 bp	1943 bp

Table 4.1. (continued)

Genus	Species	Specimen Locality Information	Locality Coordinates	Gene and # of base pairs (bp)		
				BZF	ECP1	MCS
<i>Niphta</i> continued	sp.3 T85F	CHL: Los Ríos, Rte. T-85	-40°19'58.6" -72°16'54.8"	2860 bp	2056 bp	1776 bp
<i>Oterere</i> McLellan	<i>setipennis</i>	CHL: Araucanía, Rte. 71	-38°13'20.3" -71°44'41.1"	2495 bp	2061 bp	2060 bp
<i>Protothaumalea</i> Vaillant	<i>tarda</i> ^a Vaillant	ITA: Sicily, Gioiosa Marea	N/A	2680 bp	2027 bp	X
<i>Thaumalea</i> Ruthe	<i>freyi</i> Edwards	AUT: Gesäuse NP	N/A	2725 bp	2065 bp	1769 bp
	<i>miki</i> Edwards	SVK: High Tatra Mountains, Mlynicka valley	N/A	2628 bp	2014 bp	1837 bp
	<i>provincialis</i> Vaillant	ITA: Civiasco	N/A	2464 bp	2071 bp	745 bp
	<i>testacea</i> ^a Ruthe	FRA: Vosges, Bussang	N/A	2714 bp	2132 bp	2033 bp
	<i>truncata</i> Edwards	DEU: Quelle am Morsberg Bei Rasdorf	N/A	2685 bp	2077 bp	1800 bp
	<i>verralli</i> NOR Edwards	NOR	N/A	2696 bp	2043 bp	1929 bp
	<i>verralli</i> GM Edwards	CAN: NL: Gros Morne NP	49°30'28" -58°06'00"	2712 bp	2022 bp	1893 bp
	<i>verralli</i> SJ Edwards	CAN: NL: St. John's Harbour	47°34'05.3" -52°40'56.0"	2738 bp	2057 bp	1759 bp
	<i>verralli</i> FR Edwards	FRA: Vosges, Bussang	N/A	2745 bp	2044 bp	975 bp
<i>Trichothaumalea</i> Edwards	<i>elakalensis</i> Sinclair	USA: WV: Blackwater Falls S.P.	39°06'32" -79°29'56"	2902 bp	1757 bp	2011 bp

Table 4.1. (continued)

Genus	Species	Specimen Locality Information	Locality Coordinates	Gene and # of base pairs (bp)		
				BZF	ECP1	MCS
<i>Trichothaumalea</i> continued	<i>japonica</i> Sinclair & Saigusa	JPN: Yamanashi Pref., Shosenkyo	N/A	2230 bp	1737 bp	1912 bp
	<i>pluvialis</i> ^a (Dyar & Shannon)	CAN: BC: Glacier NP	51°16'20" -117°30'16"	2447 bp	1859 bp	2004 bp

^a Indicates type species

Table 4.2. Exemplars of outgroup taxa studied. Species name, geographical source data, genomic sampling, GenBank accession numbers and size of region used are included.

Family	Species	Specimen Locality Information	Genbank Accession # and # of base pairs (bp)		
			BZF	ECP1	MCS
Chironomidae	<i>Clunio marinus</i> Haliday	N/A	CVRI01000055.1* 2198 bp	CVRI01000072.1* 2048 bp	CVRI01000004.1* 1830 bp
Culicidae	<i>Aedes aegypti</i> (Linnaeus)	N/A	XM_001654141.2 1195 bp	XM_021851986.1 2100 bp	XM_021839169.1 2347 bp
	<i>Anopheles gambiae</i> Giles	N/A	XM_319733.4 1269 bp	XM_318429.4 2223 bp	XM_310528.5 1857 bp
	<i>Culex quinquefasciatus</i> Say	N/A	XM_001861745.1 1239 bp	XM_001848888.1 2082 bp	XM_001870703.1 1905 bp
Dixidae	<i>Dixa blax</i> Dyar & Shannon	USA: CA: Uvas Canyon County Park	2781 bp	2170 bp	X
	<i>Dixa cornuta</i> Johannsen	USA: WA: Colville NF, nr. Metaline Falls	2886 bp	X	X
	<i>Dixa neoaliciae</i> Garrett	USA: AZ: Government Spring	X	1930 bp	X
	<i>Dixa notata</i> Loew	USA: GA: Brasstown Bald	2940 bp ^a	1996 bp	2025 bp ^b
	<i>Dixa rudis</i> Garrett	CAN: BC: Cranbrook	2745 bp	2022 bp	X
	<i>Dixa serrata</i> Garrett	USA: SD: Black Hills NF	2900 bp	2053 bp	X

Table 4.2. (continued)

Family	Species	Specimen Locality Information	Genbank Accession # and # of base pairs (bp)		
			BZF	ECP1	MCS
Dixidae continued	<i>Dixella aliciae</i> (Johannsen)	USA: AZ: Chiricahua Mtns.	X	X	2403 bp
	<i>Dixella</i> pgt.	USA: WA: Gifford Pinchot NF	2544 bp	X	2241 bp
	<i>Nothodixa atrovittata</i> (Edwards)	CHL: Bío Bío, Mulchén	3108 bp	2038 bp	1903 bp ^c
	<i>Nothodixa nitida</i> (Edwards)	CHL: Los Lagos	3102 bp	2065 bp	X
	<i>Nothodixa</i> sp. NZ	NZD	3162 bp	2008 bp	X
Simuliidae	<i>Cnephia ornithophilia</i> Davies, Peterson & Wood	USA: VA: Blackwater River	X	X	2010 bp
	<i>Simulium donovani</i> Vargas	USA: AZ: Pima Co.	X	X	909 bp
	<i>Simulium krebsorum</i> Moulton & Adler	USA: SC: Harman Mill Pond	JX847702.1 2645 bp	2056 bp	938 bp
	<i>Greniera humeralis</i> Currie, Adler & Wood	USA: CA: Lake Co.	X	X	1958 bp
	<i>Parahelodon gibsoni</i> (Twinn)	CAN: ON: Algonquin Prk.	X	2104 bp	900 bp

Table 4.2. (continued)

Family	Species	Specimen Locality Information	Genbank Accession # and # of base pairs (bp)		
			BZF	ECP1	MCS
	<i>Parasimulium stonei</i> Peterson	USA: OR: Aufderheide Rt. 19	2281 bp	1185 bp	X
	<i>Parasimulium crosskeyi</i> Peterson	USA: OR: Multnomah Falls	2463 bp	1618 bp	X

* Sequence pulled from genomic scaffold

^a Actual sequence from *Dixa modesta* Johannsen

^b Actual sequence from *Dixa terna* Loew

^c Actual sequence from *Nothodixa chilensis* (Edwards)

Table 4.3. Primers^a used in this study.

Gene	Primer	F/R	Sequence (5' → 3') ^b	Length
BZF Piece 1	90F	F	GARTGYGARGTNACNGARGARGT	23-mer
	94F (111F) ^c	F	GARGARGTNATHACNGAYGAYTGG	24-mer
	1.5F	F	CCNACNGAYCARGAYGARTA	20-mer
	Thaum 99F	F	GGNGARGARTGYGTNGARGT	20-mer
	Thaum P1R	R	TGYGATCGWTGTGGYATGKCNTT	23-mer
Piece 2	385F (330F) ^c	F	CCNTTYGTNTGYCARCARTGYGG	23-mer
	Thaum P2F	F	TGYGGTGARATGTTYTGGGAYCG	23-mer
	Int2F	F	ACNTGYGGNGAWATGTTYTGGGA	23-mer
	Int2F Rpl	F	GATTCNCCNTTTGARTGYGA	20-mer
	765R	R	TGYTGGAARGCNTTNGCNGAYGG	23-mer
	777R	R	AARCARGARCCNATHCAYACNNG	23-mer
	787R	R	GARAARCCRTAYGTRTGYTCSATNTG	26-mer
	787R Rpl1	R	TGYCCWCGKGCDDTTCAAYCAAMG	23-mer
	Thaum787R Rpl2	R	CAYCAYTCRGCRCMRGAYACNGT	23-mer
Piece 3	Thaum P3F	F	TAYGGYTGYCGYTTYTGYTGG	21-mer
	New Thaum P3F	F	CARATHCAYATHCGYATYCAAYAC	23-mer
	Thaum 1203R (1101R) ^c	R	TTNACNGGNGARCGNAGDATYTCYTC	26-mer
	New Thaum P3R	R	GAYAARTAYATGGAYAARTAYAAYAG	26-mer
ECP1 Piece 1	159F ^c	F	GTNGGNTGGGGNAARAARGARAC	23-mer
	162F ^c	F	GGNAARAARGARACNCARTTYCAYGG	26-mer
	Afro P1R	R	ATCATCTGCATCTACCAC	18-mer
	Austro P1R NEW	R	GGNAGYTGYAGYACYGYTTT	20-mer
	EuroP1R1	R	ACWGCCTTTSTAHTTTYTCYAAAC	22-mer
	NearcP1R MRG	R	TCYKKKTCNATCCAYTTCCARTG	23-mer
	Niph P1R	R	GAGVADTTGRTARAAAATCAC	21-mer
	NiphBrz P1R	R	TCACCCARTGCCARTTRTA	19-mer
Piece 2	Austro P2F	F	GAYGTNACNTCKTTYTAYAT	20-mer

Table 4.3. (continued)

Gene	Primer	F/R	Sequence (5' → 3')^b	Length
ECP1 Piece 2 continued	Dixella P2F	F	GTNGCNGTNATYGAYGG	17-mer
	Euro P2F1	F	TTRTTYGTRTGYTGYCARTA	20-mer
	NearcTh P2F	F	ATHCCNCCNCCNATGTAYGG	20-mer
	Nipht P2F	F	CGCTAYAAAYTGGCAYTGGGT	20-mer
	852R ^c	R	GGNARRTAYTCYTTNGGRTCYTTYTG	26-mer
	853R ^c	R	AANGGNAGRTAYTCYTTNGGRTCYTT	26-mer
MCS Piece 1	55F(Y) ^d	F	TGYTAYYTNGAYCAYGCGYGG	20-mer
	55F(R) ^d	F	TGYTAYYTNGAYCAYGCRGG	20-mer
	Niphta 60F	F	GTNTGGKCNAAAYCCRCAYAC	20-mer
	78F	F	TNWWYTGYAAAYCCNCAYAC	19-mer
	SC+ P1.5F	F	GTNCGNAARGGRAGYGARCA	20-mer
	Austroth P1R	R	CAAGCCSGTWGGRTAYCT	18-mer
	New MCS Thaum Rev	R	GCNCCNGGRTTRCARAARCA	20-mer
	Niph 1.5R	R	ACTATTCCACCTTGMGTGG	19-mer
	Thaum P1R	R	CAYATTTGTGGYGATTCMAAYGA	23-mer
	Thaum P1R5	R	GGNACNCCNTCWAYYAARTC	20-mer
Piece 2	116F	F	GTNGTNTWYCCNGCNCARTGYAA	23-mer
	446F ^E	F	TTYGTNNGNTTYGCGYGARGT	20-mer
	463F ^E	F	YCTNCGNACNCGGNTGYTTYTGAA	24-mer
	464F	F	ACRGGRTGYTTYTGAAAYCC	20-mer
	NiphCH/SC P2F	F	ATTTCACGATKMGCTGAA	19-mer
	Niph spp P2.5R	R	CGNACNGAAGTYTCRTT	17-mer
	838R ^E	R	TGYCARATGATHTGYATHGAYCA	23-mer
	840R ^d	R	CCNGTNSYYTGRTCDATRCADATCAT	26-mer

^a Sequences for additional, less degenerate, clade-specific PCR and sequencing primers used in this study are available upon request.

^b R=A/G; Y=C/T; S=C/G; W=A/T; K=G/T; M=A/C; H=A/C/T; D=A/G/T; V=A/C/G; N=A/T/G/C

^c Primer names published by Senatore *et al.* 2014

^d Primer names published by Winkler *et al.* 2015

^E Primer names published by Blaschke *et al.* 2018, though slightly tailored to suit Thaumaleidae.

Table 4.4. Species included in *Thaumalea* Ruthe

<i>algira</i> Vaillant, 1953b: 127 (<i>Thaumalea</i>) status revised
<i>alpina</i> Vaillant, 1981: 133 (<i>Thaumalea</i>)
<i>alticola</i> Schmid, 1951: 2 (<i>Thaumalea</i>)
<i>americana</i> Bezzi, 1913: 250 (<i>Thaumalea</i>) status revised
<i>angelieri</i> Vaillant & Vinçon, 1986: 65 (<i>Thaumalea</i>)
<i>anolo</i> Schmid, 1970: 501 (<i>Thaumalea</i>) status revised
<i>apache</i> Pivar & Moulton, 2018a: 17 (<i>Androprosopa</i>) comb. n.
<i>aperta</i> Martinovský & Rozkošný, 1976: 190 (<i>Thaumalea</i>)
<i>appendiculata</i> Wagner, 1987: 20 (<i>Thaumalea</i>)
<i>ardechica</i> Vaillant, nomen nudum, 1978b: 460 (<i>Thaumalea</i>)
<i>arnaudi</i> Pivar, 2018a: 21 (<i>Androprosopa</i>) comb.n.
<i>atropatenica</i> Schmid, 1958: 16 (<i>Thaumalea</i>) status revised
<i>austriaca</i> Edwards, 1929: 136 (<i>Thaumalea</i>)
<i>baltasardi</i> Schmid, 1958: 13 (<i>Thaumalea</i>)
<i>baminana</i> Yang, 2003: 587 (<i>Thaumalea</i>)
<i>becca</i> Arnaud & Boussy, 1994: 60 (<i>Thaumalea</i>) status revised
<i>bernardi</i> Vaillant, 1953b: 126 (<i>Thaumalea</i>)
<i>bezzii</i> Edwards, 1929: 134 (<i>Thaumalea</i>)
<i>biacuminata</i> Wagner & Bellstedt, 2015: 485 (<i>Thaumalea</i>)
<i>bispicula</i> Wagner & Rulik, 2008: 322 (<i>Androprosopa</i>) comb. n.
<i>bosnica</i> Schmid, 1958: 11 (<i>Thaumalea</i>)
<i>botosaneanui</i> Vaillant, 1969: 690 (<i>Thaumalea</i>)
<i>brevidens</i> Edwards, 1929: 131 (<i>Thaumalea</i>)
<i>brincki</i> Vaillant, 1963: 28 (<i>Thaumalea</i>)
<i>brothersi</i> Arnaud & Boussy, 1994: 63 (<i>Thaumalea</i>) status revised
<i>buckae</i> Arnaud & Boussy, 1994: 66 (<i>Thaumalea</i>) status revised
<i>carinthica</i> Wagner, 1995: 67 (<i>Thaumalea</i>)
<i>carpathica</i> Vaillant, nomen nudum, 1978b: 460 (<i>Thaumalea</i>)
<i>carthusiana</i> Vaillant, 1978a: 699 (<i>Orphnephilina</i>) comb. n.
<i>carthusiensis</i> Vaillant, nomen nudum, 1978b: 460 (<i>Thaumalea</i>)
<i>caudata</i> Bezzi, 1913: 257 (<i>Thaumalea</i>)
<i>cebennica</i> Vaillant, 1978a: 702 (<i>Thaumalea</i>)
<i>chandlerorum</i> Arnaud & Boussy, 1994: 77 (<i>Thaumalea</i>) status revised
<i>chiosica</i> Wagner, 1981: 148 (<i>Thaumalea</i>)
<i>coloradensis</i> Arnaud & Boussy, 1994: 79 (<i>Thaumalea</i>) status revised
<i>confracta</i> Arnaud & Boussy, 1994: 82 (<i>Thaumalea</i>) status revised
<i>corsica</i> Edwards, 1928: 174 (<i>Thaumalea</i>)
<i>curtior</i> Schmid, 1958: 6 (<i>Thaumalea</i>)
<i>decussiferens</i> Vaillant, 1967: 396 (<i>Thaumalea</i>)
<i>demandana</i> Vaillant & Vinçon, 1988: 282 (<i>Orphnephilina</i>) comb. n.
<i>digitata</i> Edwards, 1929: 138 (<i>Thaumalea</i>)
<i>dinarica</i> Schmid, 1958: 10 (<i>Thaumalea</i>)
<i>dirghakantaka</i> Schmid, 1970: 510 (<i>Thaumalea</i>) status revised

Table 4.4 (continued)

<i>divaricata</i> Bezzi, 1913: 256 (<i>Thaumalea</i>)
<i>edwardsi</i> Tjeder, 1949: 106 (<i>Thaumalea</i>)
<i>elnora</i> Dyar & Shannon, 1924: 434 (<i>Thaumalea</i>) status revised
<i>ericfisheri</i> Arnaud & Boussy, 1994: 87 (<i>Thaumalea</i>) status revised
<i>falciformis</i> Arnaud & Boussy, 1994: 91 (<i>Thaumalea</i>) status revised
<i>freyi</i> Edwards, 1929: 130 (<i>Thaumalea</i>)
<i>furva</i> Edwards, 1929: 130 (<i>Thaumalea</i>)
<i>fusca</i> Garrett, 1925: 10 (<i>Orphnephila</i>) comb. n.
<i>galibierensis</i> Vaillant, 1953b: 125 (<i>Thaumalea</i>)
<i>gerecki</i> Wagner, 1988a: 10 (<i>Thaumalea</i>)
<i>gillespieae</i> Arnaud & Boussy, 1994: 97 (<i>Thaumalea</i>) status revised
<i>gredosensis</i> Schmid, 1951: 4 (<i>Thaumalea</i>)
<i>heterodoxa</i> Schmid, 1958: 19 (<i>Thaumalea</i>) status revised
<i>himalayana</i> Schmid, 1958: 18 (<i>Thaumalea</i>) status revised
<i>horvati</i> Wagner, 1992: 212 (<i>Thaumalea</i>)
<i>hyrcanica</i> Schmid, 1958: 16 (<i>Thaumalea</i>) status revised
<i>idahoensis</i> Arnaud & Boussy, 1994: 101 (<i>Thaumalea</i>) status revised
<i>ikariae</i> Wagner, 1981: 148 (<i>Thaumalea</i>)
<i>inflata</i> Bezzi, 1913: 251 (<i>Thaumalea</i>)
<i>intermedia</i> Schmid, 1958: 11 (<i>Thaumalea</i>) status revised
<i>japonica</i> Okada, 1938: 390 (<i>Thaumalea</i>) status revised
<i>karakoramica</i> Schmid, 1958: 20 (<i>Thaumalea</i>) status revised
<i>kykladica</i> Wagner, 1981: 144 (<i>Thaumalea</i>)
<i>larvata</i> Mik, 1888: 243 (<i>Orphnephila</i>) comb. n.
<i>lesbica</i> Wagner, 1981: 148 (<i>Thaumalea</i>)
<i>libanica</i> Edwards, 1932: 33 (<i>Thaumalea</i>)
<i>lindsayorum</i> Arnaud & Boussy, 1994: 105 (<i>Thaumalea</i>) status revised
<i>longicauda</i> Vaillant, 1970: 168 (<i>Psychothaumalea</i>) comb. n.
<i>macedonica</i> Schmid, 1958: 10 (<i>Thaumalea</i>)
<i>major</i> Bezzi, 1913: 254 (<i>Thaumalea</i>)
<i>malickyi</i> Theischinger, 1979: 51 (<i>Thaumalea</i>)
<i>martinovskiyi</i> Joost, 1979: 89 (<i>Thaumalea</i>)
<i>melanderi</i> Arnaud & Boussy, 1994: 108 (<i>Thaumalea</i>) status revised
<i>micomicon</i> a Schmid, 1951: 5 (<i>Thaumalea</i>)
<i>miki</i> Edwards, 1929: 131 (<i>Thaumalea</i>)
<i>minuta</i> Wagner, 1981: 146 (<i>Thaumalea</i>)
<i>mixta</i> Edwards, 1929: 129 (<i>Thaumalea</i>)
<i>monikae</i> Wagner & Bellstedt, 2015: 485 (<i>Thaumalea</i>)
<i>nanarupa</i> Schmid, 1970: 503 (<i>Thaumalea</i>) status revised
<i>nevadensis</i> Vaillant & Vinçon, nomen nudum, 1988: 281 (<i>Thaumalea</i>)
<i>nigra</i> Loew, 1871: 30 (<i>Orphnephila</i>) comb. n.
<i>nigrinitida</i> Papp, 2006: 122 (<i>Thaumalea</i>)
<i>pachystyla</i> Vaillant, 1978a: 701 (<i>Thaumalea</i>)

Table 4.4. (continued)

<i>palouse</i> Arnaud & Boussy, 1994: 110 (<i>Thaumalea</i>) status revised
<i>popovi</i> Joost, 1978: 118 (<i>Thaumalea</i>)
<i>provincialis</i> Vaillant, 1978a: 702 (<i>Thaumalea</i>)
<i>pulla</i> Edwards, 1929: 130 (<i>Thaumalea</i>)
<i>pyrenaica</i> Edwards, 1929: 135 (<i>Thaumalea</i>)
<i>rainierensis</i> Pivar & Sinclair, 2018a: 48 (<i>Androprosopa</i>) comb. n.
<i>rangifer</i> Martinovský, 1999: 133 (<i>Androprosopa</i>) comb. n.
<i>remota</i> Martinovský & Rozkošný, 1976: 197 (<i>Thaumalea</i>)
<i>restonica</i> Vaillant, 1970: 170 (<i>Thaumalea</i>)
<i>rivosecchii</i> Wagner, 1997a: 183 (<i>Thaumalea</i>)
<i>rumanica</i> Edwards, 1929: 136 (<i>Thaumalea</i>)
<i>sandaliae</i> Wagner, 1987: 18 (<i>Thaumalea</i>)
<i>santaclaraensis</i> Brothers, 1972: 121 (<i>Thaumalea</i>) status revised
<i>schmidi</i> Martinovský & Rozkošný, 1976: 198 (<i>Thaumalea</i>)
<i>schmidiana</i> Arnaud & Boussy, 1994: 116 (<i>Thaumalea</i>) status revised
<i>seguyi</i> Vaillant, 1969: 697 (<i>Thaumalea</i>)
<i>serbica</i> Wagner, 1980: 15 (<i>Thaumalea</i>)
<i>serrata</i> Edwards, 1929: 139 (<i>Thaumalea</i>)
<i>similis</i> Wagner, 1988b: 204 (<i>Thaumalea</i>)
<i>sonorensis</i> Arnaud & Boussy, 1994: 119 (<i>Thaumalea</i>) status revised
<i>spinata</i> Vaillant, 1955: 94 (<i>Thaumalea</i>)
<i>stelviana</i> Wagner & Rulik, 2008: 324 (<i>Thaumalea</i>)
<i>striata</i> Okada, 1938: 392 (<i>Thaumalea</i>) status revised
<i>subafricana</i> Becker, 1908: 70 (<i>Orphnephila</i>)
<i>suborientalis</i> Schmid, 1958: 18 (<i>Thaumalea</i>) status revised
<i>suzonae</i> Schmid, 1958: 14 (<i>Thaumalea</i>)
<i>tarda</i> Loew, 1847: 369 (<i>Thaumalea</i>) status revised
<i>tatrica</i> Vaillant, 1969: 699 (<i>Thaumalea</i>)
<i>tchakrarupa</i> Schmid, 1970: 507 (<i>Thaumalea</i>) status revised
<i>tchaturskona</i> Schmid, 1970: 503 (<i>Thaumalea</i>) status revised
<i>testacea</i> Ruthe, 1831: 1211 (<i>Thaumalea</i>)
<i>thhammeri</i> Zilahi-Sebess, 1956: 433 (<i>Thaumalea</i>)
<i>thomasi</i> Vaillant, 1968: 83 (<i>Thaumalea</i>)
<i>thornburghae</i> Vaillant, 1959: 35 (<i>Thaumalea</i>) status revised
<i>tiriaschi</i> Schmid, 1970: 507 (<i>Thaumalea</i>) status revised
<i>tiriasdanta</i> Schmid, 1970: 509 (<i>Thaumalea</i>) status revised
<i>tridanta</i> Schmid, 1970: 504 (<i>Thaumalea</i>) status revised
<i>tridhatu</i> Schmid, 1970: 504 (<i>Thaumalea</i>) status revised
<i>toscanica</i> Vaillant, 1981: 135 (<i>Thaumalea</i>)
<i>truncata</i> Edwards, 1929: 137 (<i>Thaumalea</i>)
<i>urdhvakantaka</i> Schmid, 1970: 508 (<i>Thaumalea</i>) status revised
<i>vaillanti</i> Martinovský & Rozkošný, 1976: 203 (<i>Thaumalea</i>)
<i>vaillantiana</i> Sinclair, 1996: (<i>Androprosopa</i>) comb. n.

Table 4.4. (continued)

<i>vakrakantaka</i> Schmid, 1970: 509 (<i>Thaumalea</i>) status revised
<i>valdesia</i> Schmid, 1951: 5 (<i>Thaumalea</i>)
<i>vartula</i> Schmid, 1970: 507 (<i>Thaumalea</i>) status revised
<i>veletensis</i> Vaillant, 1978a: 704 (<i>Thaumalea</i>)
<i>verralli</i> Edwards, 1929: 132 (<i>Thaumalea</i>)
<i>waha</i> Arnaud & Boussy, 1994: 121 (<i>Thaumalea</i>) status revised
<i>zelmae</i> Arnaud & Boussy, 1994: 124 (<i>Thaumalea</i>) status revised
<i>zempoala</i> Sinclair & Huerta, 2010: 443 (<i>Androprosopa</i>) comb. n.
<i>zernyi</i> Edwards, 1929: 129 (<i>Thaumalea</i>)
<i>zhejiangana</i> Yang, 1998: 249 (<i>Thaumalea</i>)
<i>zwicki</i> Wagner, 1980: 13 (<i>Thaumalea</i>)

Table 4.5. Species included in *Austrothaumalea* Tonnoir

<i>apicalis</i> Edwards, 1930: 113 (<i>Austrothaumalea</i>)
<i>appendiculata</i> Tonnoir, 1927: 112 (<i>Austrothaumalea</i>)
<i>australis</i> Theischinger, 1986: 296 (<i>Austrothaumalea</i>)
<i>barrydayi</i> Theischinger, 1986: 297 (<i>Austrothaumalea</i>)
<i>bickeli</i> Theischinger, 1988: 212 (<i>Austrothaumalea</i>)
<i>bicornis</i> Sinclair, 2008b: 4 (<i>Austrothaumalea</i>)
<i>bifida</i> Sinclair, 2008b: 4 (<i>Austrothaumalea</i>)
<i>caledonica</i> Sinclair, 2008a: 292 (<i>Austrothaumalea</i>)
<i>capricornis</i> Theischinger, 1986: 298 (<i>Austrothaumalea</i>)
<i>cervula</i> Theischinger, 1986: 298 (<i>Austrothaumalea</i>)
<i>chilensis</i> Edwards, 1930: 111 (<i>Austrothaumalea</i>)
<i>commoni</i> Theischinger, 1986: 300 (<i>Austrothaumalea</i>)
<i>concava</i> Sinclair, 2008b: 6 (<i>Austrothaumalea</i>)
<i>crosbyi</i> McLellan, 1988: 566 (<i>Austrothaumalea</i>)
<i>denticulata</i> Theischinger, 1986: 301 (<i>Austrothaumalea</i>)
<i>fredericki</i> Pivar, 2018: Chapter 3 (<i>Austrothaumalea</i>) sp.n.
<i>fusca</i> Theischinger, 1986: 301 (<i>Austrothaumalea</i>)
<i>gibbsi</i> McLellan, 1988: 571 (<i>Austrothaumalea</i>)
<i>macalpinei</i> Theischinger, 1986: 301 (<i>Austrothaumalea</i>)
<i>macfarlanei</i> McLellan, 1988: 566 (<i>Austrothaumalea</i>)
<i>maxwelli</i> McLellan, 1988: 571 (<i>Austrothaumalea</i>)
<i>minnamurrae</i> Theischinger, 1986: 304 (<i>Austrothaumalea</i>)
<i>neozealandica</i> Tonnoir, 1927: 110 (<i>Austrothaumalea</i>)
<i>ngaire</i> McLellan, 1988: 567 (<i>Austrothaumalea</i>)
<i>oliveri</i> McLellan, 1988: 573 (<i>Austrothaumalea</i>) status revised
<i>pala</i> McLellan, 1988: 567 (<i>Austrothaumalea</i>)
<i>queenslandensis</i> Sinclair, 2008b: 8 (<i>Austrothaumalea</i>)
<i>ramosa</i> Sinclair, 2008b: 8 (<i>Austrothaumalea</i>)
<i>setipennis</i> Edwards, 1930: 113 (<i>Austrothaumalea</i>) status revised
<i>similis</i> Theischinger, 1986: 305 (<i>Austrothaumalea</i>)
<i>simplex</i> Theischinger, 1986: 306 (<i>Austrothaumalea</i>)
<i>sinuosa</i> Theischinger, 1986: 307 (<i>Austrothaumalea</i>)
<i>spatulata</i> Schmid, 1970: 501 (<i>Austrothaumalea</i>)
<i>spiculata</i> Pivar, 2016: 595 (<i>Austrothaumalea</i>)
<i>spinosa</i> Theischinger, 1986: 307 (<i>Austrothaumalea</i>)
<i>tasmanica</i> Theischinger, 1986: 307 (<i>Austrothaumalea</i>)
<i>theischingeri</i> Sinclair, 2008b: 8 (<i>Austrothaumalea</i>)
<i>tonnoiri</i> Theischinger, 1986: 309 (<i>Austrothaumalea</i>)
<i>uloola</i> Sinclair, 2008b: 9 (<i>Austrothaumalea</i>)
<i>uptoni</i> Theischinger, 1986: 310 (<i>Austrothaumalea</i>)
<i>victoriae</i> Theischinger, 1986: 311 (<i>Austrothaumalea</i>)
<i>walkerae</i> McLellan, 1988: 567 (<i>Austrothaumalea</i>)
<i>zentae</i> Theischinger, 1986: 313 (<i>Austrothaumalea</i>)

Table 4.5. (continued)

<i>zwicki</i> McLellan, 1988: 569 (<i>Austrothaumalea</i>)
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Table 4.6. List of Thaumaleidae genera, with their distribution and number of species after classification changes.

Genus	Distribution	Described species
<i>Afrothaumalea</i> Stuckenberg	Afrotropical (South Africa)	3
<i>Austrothaumalea</i> Tonnoir	Neotropical (Argentina, Chile); Australasia (Australia (mainland, Tasmania), New Caledonia, New Zealand).	44
<i>Neothaumalea</i> Pivar, Moulton and Sinclair	Neotropical (Brazil)	1
<i>Niphta</i> Theischinger	Neotropical (Chile); Australasia (Australia).	14
<i>Thaumalea</i> Ruthe	Indomalayan (Thailand); Nearctic (Canada, Mexico, U.S.A.); Palearctic (Albania, Algeria, Andorra, Austria, Bosnia & Herzegovina, Bulgaria, China, Croatia, Czech Republic, Denmark, Finland, France (Corsica, mainland), Germany, Great Britain, Greece, Hungary, Iceland, India, Iran, Ireland, Italy (mainland, Sicily), Japan, Lebanon, Macedonia, Nepal, Norway, Pakistan, Poland, Portugal (Madeira), Romania, Russia, Serbia, Slovenia, Slovakia, Spain (Canary Islands, mainland, Mallorca), Sweden, Switzerland).	140
<i>Trichothaumalea</i> Edwards	Nearctic (Canada, U.S.A); Palearctic (Japan).	4
		Total: 206

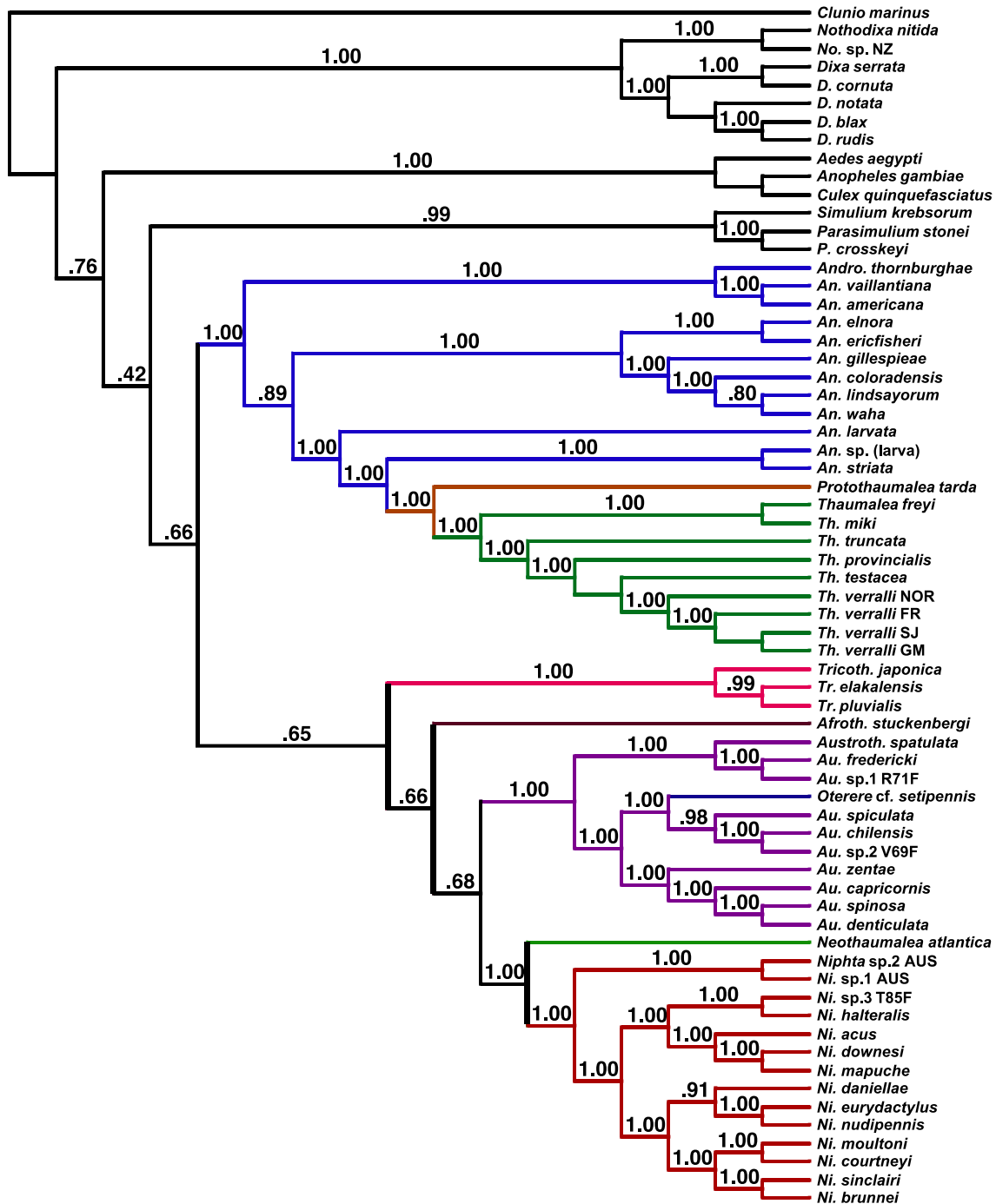


Figure 4.1. Hypothesized phylogenetic relationships of Thaumaleidae genera based on Bayesian analysis of big zinc finger (BZF) data set. Values above branches represent posterior probabilities.

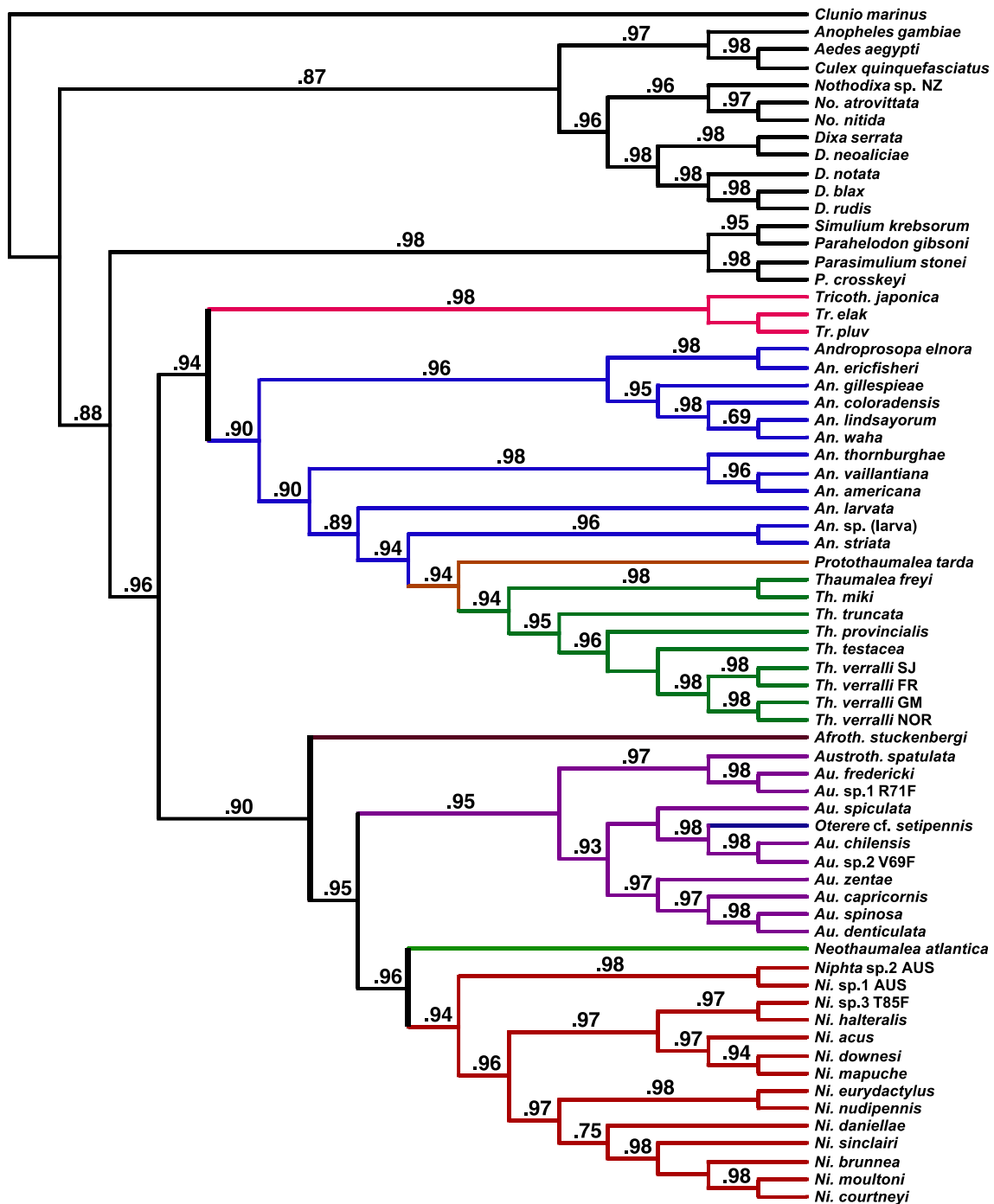


Figure 4.2. Hypothesized phylogenetic relationships of Thaumaleidae genera based on Bayesian analysis of elongator complex protein 1 (ECP1). Values above branches represent posterior probabilities.

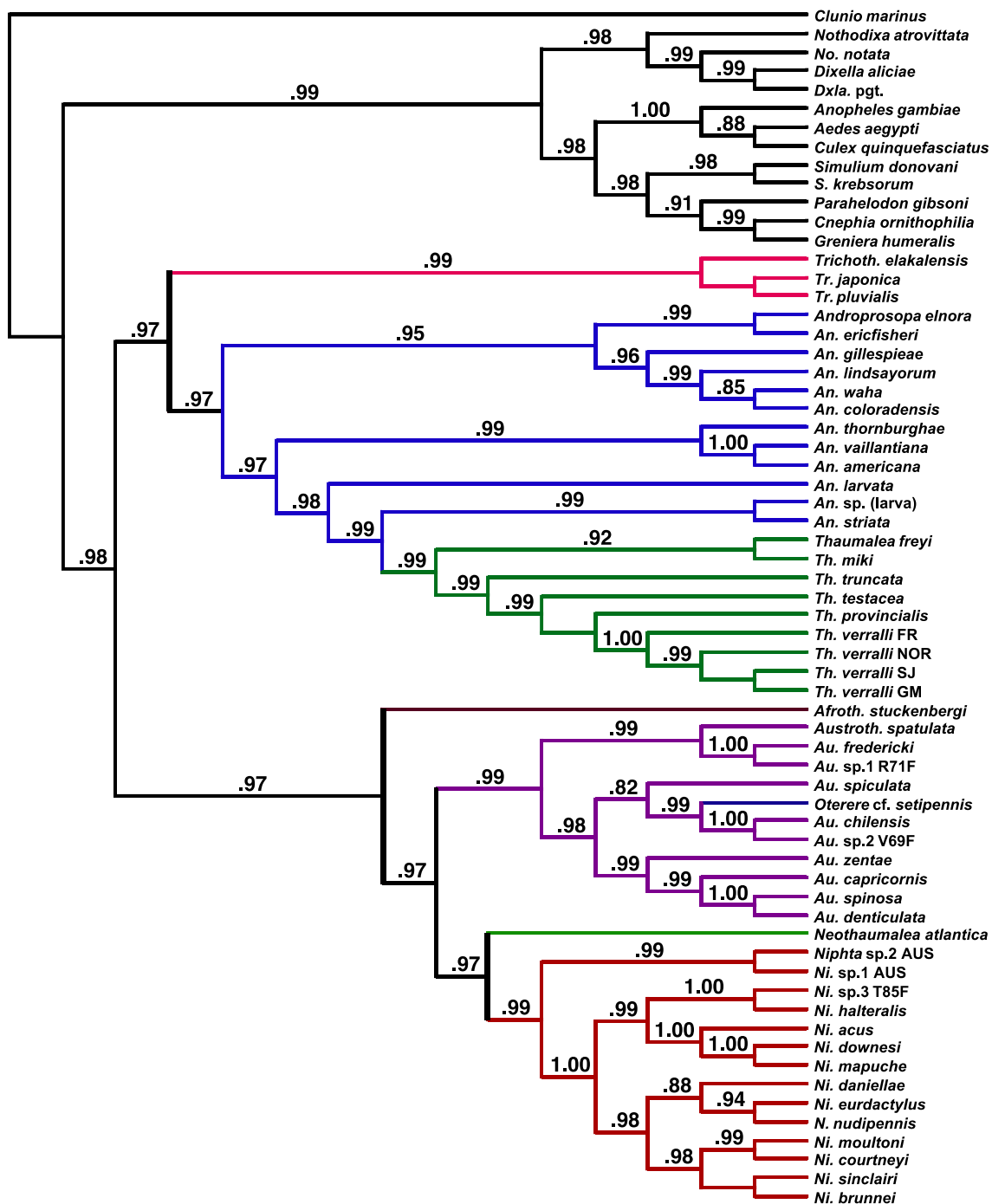


Figure 4.3. Hypothesized phylogenetic relationships of Thaumaleidae genera based on Bayesian analysis of molybdenum cofactor sulfurase (MCS). Values above branches represent posterior probabilities.

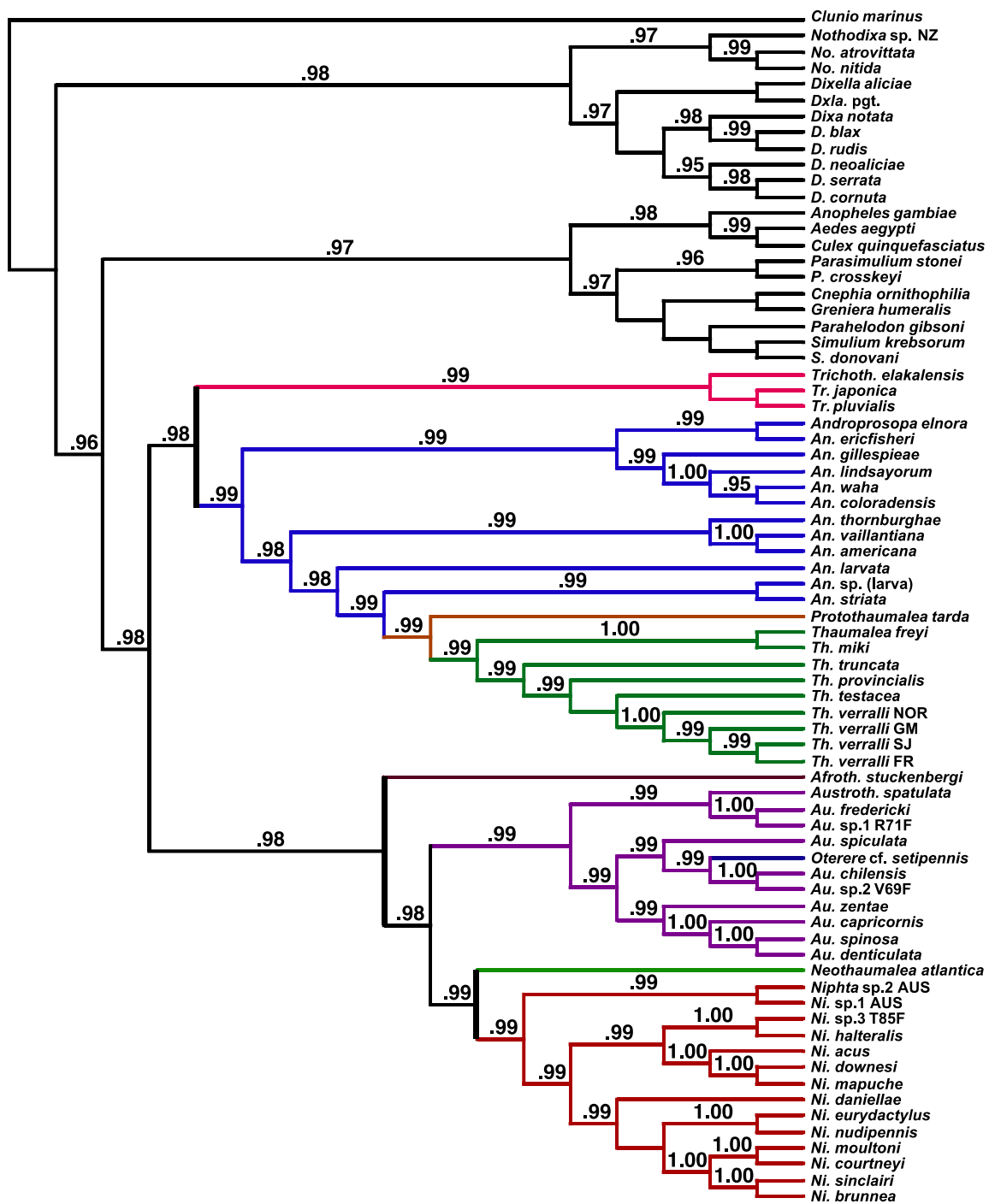


Figure 4.4. Hypothesized phylogenetic relationships of Thaumaleidae genera based on Bayesian analysis of combined molecular data set (BZF, ECP1 & MCS). Values above branches represent posterior probabilities.

CHAPTER 5
GENERAL CONCLUSIONS

Thaumaleidae is an understudied family of Diptera and after this research contains 206 species. Although the family is widespread and found on nearly all continents, there remains much to be discovered. The research in this dissertation yields a wealth of new information, which can be built upon by future research.

Neothaumalea Pivar, Moulton and Sinclair, is a newly described genus discovered from Brazil (Chapter 2). This is an important discovery because it gives new insight into phylogenetic affinities between closely related genera and also solidifies synapomorphies within the Southern Hemisphere genera. It is also the first thaumaleid collected east of the Andean mountains, leaving the door open for future research in the region.

Chapter 3 examines the poorly known Andean thaumaleid fauna, with a focus on southern Chile. This research led to many new discoveries, including 10 new species (1 *Austrothaumalea* and 9 *Niphta*), unique genitalic characters in both sexes, a new larval morphotype and the discovery of immatures living on vegetation rather than rocky substrates. These discoveries are particularly important for the genus *Niphta* Theischinger, as it was previously known only from five species. The increased number of species and new morphological characters have allowed for a thorough study of the genus.

Chapter 4 hypothesizes the first phylogeny depicting thaumaleid generic relationships and tests the validity of all known genera. This is perhaps the most important chapter of this dissertation, as it provides a predictive framework on which to build future studies of the family. The molecular phylogeny suggests that three genera are invalid (*Androprosopa* Mik, *Oterere* McLellan and *Protothaumalea* Vaillant), that two major lineages exist (Northern and Southern Hemisphere clades) and it also hypothesizes

intergeneric relationships. Future research can build upon this phylogeny by using it as a means to determine morphological character polarity to further strengthen generic concepts, as well as to explore intrageneric relationships.

A common theme throughout each of these chapters is discovery. Despite all the research that has been done on this small family, there are still plenty of opportunities for new discoveries. In particular, the understudied Afrotropical and Neotropical regions both need focused collection efforts to determine the true extent of thaumaleid diversity and will undoubtedly yield many new and exciting finds. Many questions still exist with regards to thaumaleids, including: what are the northern limits of South American Thaumaleidae? Do they overlap with Nearctic genera? How is the New Zealand fauna related to Australian and South American fauna? What do the immature stages of Neotropical *Austrothaumalea* Tonnoir look like? Which Australian species of *Niphta* have ventral adhesive structures? How many thaumaleid species exist in Southeast Asia? These questions will only be answered through continued collecting and research of Thaumaleidae.

VITA

Robert J. Pivar was born in Milton, Ontario, Canada and grew up in Burlington, ON. He has always had an interest in nature and could often be found outside trying to spot wildlife. Robert became interested in insects at a young age, and when it came time to decide which path he would take for his education, he decided to attend The University of Guelph (Guelph, Ontario). It is here that he became passionate about insects, and flies in particular, under the mentorship of Dr. Stephen A. Marshall. After graduating with his B.Sc. in Environmental Biology in 2010, Robert continued to be involved in entomology. He worked in Dr. Marshall's lab conducting an arthropod survey on Manitoulin Island, worked as a nursery pest scout and taught Horticultural Entomology at Niagara College. In 2013, Robert decided to continue his education by pursuing his Master's degree at the University of Tennessee with Dr. J.K. Moulton. Upon receiving his Master's degree in 2015, Dr. Moulton gave Robert the opportunity to pursue his Ph.D. degree, so he stayed to continue his research on Thaumaleidae. Robert will move back home to Ontario with his wife, Danielle, after completion of his Ph.D. studies and he hopes to find a stimulating career in entomology.