

AN ENIGMATIC CHIRONOMIDAE (DIPTERA): FIRST LARVAL DESCRIPTION FOR *NANDEVA* WIEDENBRUG, REISS AND FITTKAU AND EVIDENCE FOR PHYLOGENETIC POSITION IN TANYTARSINI

Peter S. Cranston

Evolution & Ecology, Research School of Biology, Australian National University, Canberra, A.C.T. 2601, Australia. E-mail: pscranston@gmail.com

Abstract

The previously unknown larva of the genus *Nandeva* Wiedenbrug, Reiss and Fittkau (Chironomidae: Chironominae) is suggested to be a candidate from tropical northern Australia belonging to the tribe Tanytarsini. The basis for the assumption is DNA analysis showing maximum values of Bayesian posterior probability and Bootstrap support for an exclusive sister relationship of the un-reared larva with a male of *Nandeva* from South America. The monophyly of *Nandeva* has high support as a member of the subtribe Tanytarsina, although its precise relationships within this subtribe lack support. Larval morphology is described and illustrated, and conforms to the subtribe Tanytarsina in tribe Tanytarsini. The historical tempo is discussed in relation to fossils and dating of pertinent nodes and the term ‘pseudo-gondwanan’ is used to describe an extant restricted austral range, but with fossils showing a wider past distribution in the north.

Introduction

Since the ‘Holarctic Chironomidae’ project (Wiederholm 1983, 1986, 1989), it has been desirable to incorporate morphology of immature stages in descriptive studies, faunal guides and classifications. Continuing unknown immature stages often are associated with atypical and under-sampled life histories such as terrestrial habitats or immersed wood (e.g. Cranston 2003, 2006) and/or from infrequently surveyed and remote places, such as New Zealand’s subantarctic islands (e.g. Sublette and Wirth 1980). Amongst such genera with the larva unknown yet clearly aquatic is *Nandeva* Wiedenbrug, Reiss and Fittkau (1988) that is documented from diverse lotic pupal exuviae but until now with no associated larva.

Nandeva, based on the genotype *Nandeva gaucha* Wiedenbrug, Reiss and Fittkau, was described from the male imago and pupa from Rio Grande do Sul (southern Brazil), plus two species based solely on distinctive pupal exuviae from the Amazon basin (Brazil) and southern Chile, respectively

(Wiedenbrug et al. 1998). Further undescribed species suggest an extensive distribution in southern America, with an adult known from as far north as Panama.

Near simultaneously, this ‘neotropical’ genus was found in tropical northern Australian streams, with the sole species described as *Nandeva fittkau* by Cranston (1999). Although all adults were pharate, associated pupae had the diagnostically posterior hook rows on tergites II-V, and lacked a thoracic horn, frontal setae, pedes spurii A and B, spur / comb on posterior segment VIII, and anal lobe fringe. Pupal exuviae were intercepted in drift from 6 streams in north-eastern Queensland between latitudes 16° to 18°S. Despite intensive conventional searching using kick and drift nets and breaking immersed wood, no candidate for the larval *Nandeva* was found.

Neotropical *Nandeva* diversity increased subsequently with two species described from São Paulo State, Brazil, together with a new pupal / adult association by Sæther and Roque (2004) who also keyed males of the then 5 described species. Additional species diversity was revealed by Andersen *et al.* (2011) in describing adults of a second species from Chile, another from Rio de Janeiro state in Brazil, with range extending to Mexico and Venezuela. Epler (2017) added Costa Rica to the meso-American distribution of the widely distributed species *Nandeva latiloba* Sæther and Roque. However, despite reports from aquatic ecological and biomonitoring researchers, no larval association was found (see e.g. Sæther and Roque 2004).

Because the larva remained unknown, its morphology could not be incorporated into phylogenetic studies. This was problematic as the adult male and pupa are morphologically very divergent (highly apomorphic) compared to prospective relatives. Regarding phylogeny, originally Wiedenbrug et al. (1988) stated only that the adult male belonged to the tribe Chironomini and, although keying to *Pagastiella*, *Nandeva* differed substantially in details of phylogenetic significance. An early phylogenetic

ic estimate was based on morphology in a matrix of 118 characters from all life-history stages for 60 genera of Chironominae, with larval features for *Nandeva* treated as 'missing data' (Cranston 1999). Analysed under strict parsimony, *Nandeva* fell within the tribe Chironomini, associated with Gondwanan-distributed genera *Nilodosis* Kieffer, *Imparipecten* Freeman, *Fissimentum* Cranston and Nolte and 'K1' (subsequently *Xylochironomus* Cranston). Some trivially less-parsimonious trees placed *Nandeva* as sister to Tanytarsini, or even embedded within the tribe. This latter placement found support from Sæther and Roque (2004), who reinterpreted wing vein RM as a continuation of direction of R_{4+5} , diagnostic of tribe Tanytarsini. With this character state score emended in a revised matrix, analysed under a range of weighting schemes, Sæther and Roque (2004) proposed several positions for *Nandeva*, including as sister to, or within Tanytarsini, but with various different internal relationships. The position of *Nandeva* was argued as near or within tribe Tanytarsini based on male characters from the only known fossil species, *N. pudens*, from Fushun amber (early Eocene, NE China) by Gilka et al. (2016). Some morphology seemed driven by shared reductions likely to have been convergent, as suggested also by Cranston (1999). No proposed relationships had significant statistical support.

Detection of an Australian larva collected for molecular phylogenetic studies and linked by DNA sequences with an adult of *Nandeva* from Brazil, now allows association, and description of the larva. This provides better understanding of the phylogenetic relationships and the tempo of diversification.

Material and methods

Diverse and specifically targeted taxa of phylogenetic significance were sought for molecular phylogenetic studies across the Chironomidae (Cranston et al. 2011). Immature stages were obtained for ease of collection and relevance in aquatic biomonitoring programs. From a tropical submontane stream, larvae were recovered from semi-immersed leaf litter packs by coarse-sieving (1 cm mesh) to remove larger material, and then using a 125 μ mesh sieve to retain larvae. Samples were inspected live in the field under dissector microscope and larvae 'of interest' were placed individually in rearing vials. The larva subsequently recognised as possibly belonging to *Nandeva*, died prior to metamorphosis and 100% isopropanol was added to the vial.

The Brazilian adult was collected into ethanol in a

light trap located at Fazzara Creek, the type-locality for both *Nandeva latiloba* Sæther and Roque and *N. strixinorum* Sæther and Roque (2004).

Treatment of material for molecular study and protocols to obtain and align sequences and phylogenetic analyses were as reported in Cranston et al. (2011) and Krosch and Cranston (2012). Subsequent analyses with increased taxon sampling from tribe Tanytarsini follow Krosch et al. (2019). For the larva of *Nandeva*, DNA sequences (GenBank accessions in parentheses) were obtained for a mitochondrial protein-coding gene (*COI*, HQ440940), a ribosomal gene (*18S*, HQ440625), and two sections of the nuclear protein-coding gene (*CAD*, HQ440478, HQ440312). For the adult, only *COI* was obtained (HQ440941).

Specimens are vouchered (MV) as microscope slides in the Australian National Insect Collection, CSIRO, Canberra, Australia (ANIC).

Results

Taxonomy

Nandeva Wiedenbrug, Reiss and Fittkau 1998

Type-species: *Nandeva gaucha* Wiedenbrug, Reiss and Fittkau 1998, by original designation.

Larval material examined: 1L, AUSTRALIA, QUEENSLAND, Mt. Lewis, Mary Ck, 16°35.2'S 145°17.5'E, 4.ix.2005, P.S. Cranston, MV FNQ9-3 (ANIC).

Adult ♂, BRASIL, São Paulo, São Carlos, Córrego do Fazzari, 21°58'S 47°53'W, 10.viii.2007, F.O. Roque, MV BRZNAND (ANIC).

Description (Fig. 1).

Small, colour in life unknown. Body length 3 mm., head length 250 μ m, postmentum 90 μ m. Dorsal head comprising frontoclypeal apotome with straight anterior margin, separate labrum and labral sclerites 3-5 distinct. Antenna (Fig. 1a) with pedestal undeveloped, 5-segmented, with swollen basal segment, segments decreasing successively in length from 2nd to 5th: length (in μ m) 58, 20, 13, 11, 6; Antennal ratio 1.1. Lauterborn organs prominent, equal to length of 3rd antennal segment, both apical / subapical on 2nd segment, one slightly retracted compared to the other. Style long. Ring organ near middle of segment 1, seta present. Blade extends well beyond flagellum, accessory blade very short. Labrum (Fig. 1b): SI plumose with fused bases; SII plumose, arising from small pedestals, SIII short, slender; SIV short, normally developed. Seta premandibularis simple, stout. Labral lamellae pectinate with c. 25 teeth. Pecten

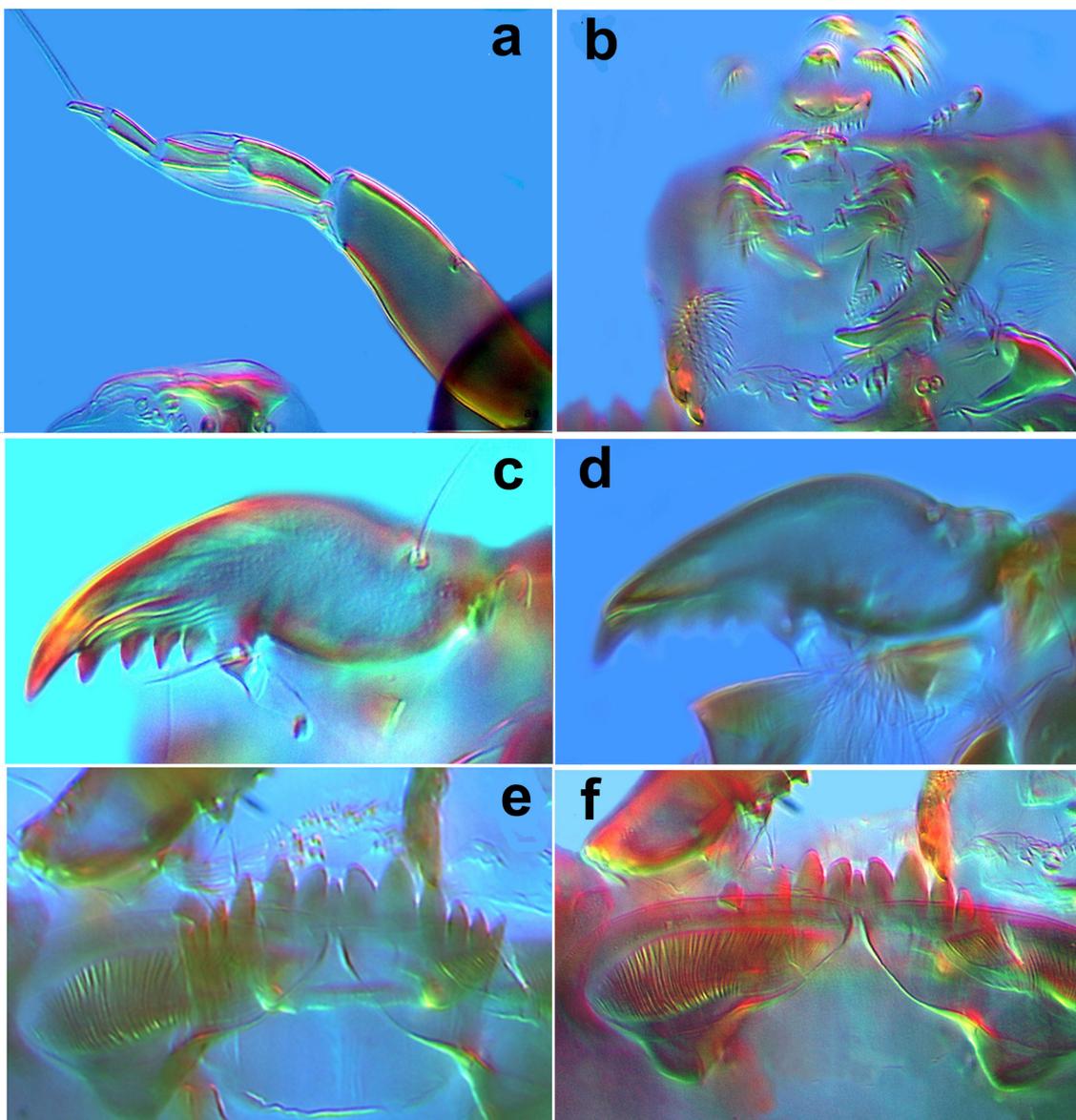


Figure 1. *Nandeva fitzkau* Cranston, putative larva. a. Antenna, b. Labrum, c. Mandible ventral, c. Mandible ventral, e. Mentum, with teeth enhanced, f. Mentum with ventromental plates enhanced.

epipharyngis tripartite, each tooth pectinate with 5-6 teeth distally. Premandible 40 μm long, with 3 teeth, with beard. Mandible (Figs 1c, d) 70 μm long, with stout dorsal tooth; apical tooth 2x length of one of 3-4 inner teeth; all teeth pale brown. Pecten mandibularis absent. Seta subdentalis inserted dorsally, narrow, reaching middle inner tooth. Seta interna multi-branched, inserted on dorsal surface. Mentum (Figs. 1e, f) 62 μm wide, with all teeth pale brown. Median (ventro-) mentum with three teeth, the central one recessed, smaller; with 5 pairs of lateral teeth, declining in size; pointed outermost tooth lies somewhat displaced laterally relative to the inner. Ventromental plates (Fig. 1f) 60 μm wide, distinctively subovoid, in contact

medially, with smooth anterior margin, striae concentrated in medio-lateral area and absent from median sector. Setae submenti apparently simple. Body lacks lateral or ventral tubules. Segment 11 dorsomedially without appendages or hump. Procerus simple, unpigmented, arising directly from membrane, with few short anal setae.

Taxonomic and Ecological Comments

Although there are no formal diagnoses for the tribe Tanytarsini, these can be derived from Sæther (1977) including also for subtribes Tanytarsina and Zavreliina, and from keys such as Epler *et al.* (2013) for Holarctic larvae. The larva proposed here as belonging to *Nandeva* conforms to

tribe Tanytarsini in the subfamily Chironominae, largely based on features of the labro-epipharynx. Notably the labral SI and SII setae are plumose, the bases of S1 setae are fused and SII are on pedestals. The antenna is 5 segmented with antennal seta, with sessile, opposite Lauterborn organs not on pedestals. The ventromental plates are in near median contact, diagnostic of subtribe Tanytarsina. Within this subtribe, the recessed median mental tooth and the mandible with dorsal tooth and seta subdentalis appearing to be inserted dorsally (which is seen otherwise only in tribe 'Pseudochironomini') appear to be diagnostic.

The Australian larva is associated with South American *Nandeva* at 89% similarity of sequence from molecular evidence (Cranston et al. 2011, Krosch et al. 2019). The larval collection site is a 2nd order creek at 1000 m. above sea level in tropical north Queensland. All other Australian records of *Nandeva* are from the characteristic pupal exuviae from streams in Far North Queensland including from Mary Creek that is the larval locality (Cranston 1999). No such larvae have been found in any other stream from which this pupal type derives, despite intensive sampling for over two decades. Circulating photographs of the Australian larvae amongst South American colleagues has not elicited recognition and this larval type remains unknown there.

Phylogeny

Seeking data for an extensively sampled molecular phylogenetic reconstruction, Cranston et al. (2011) obtained representatives across all available major branches of the Chironomidae. Survey strategy often targeted larvae with distinctive morphologies (see Cranston et al. 2011, including Supplementary Material) and most were un-reared. Amongst these was a solitary larva from immersed leaf litter from Mary Creek at 1000 m. elevation above sea level on Mount Lewis on the Atherton Tableland of tropical northern Queensland, which is the type-locality of *Nandeva fittkawi*. Amongst material collected by colleagues was an adult *Nandeva* from the Fazzari Stream in Sao Carlos, Brazil was made available to us by Fabio Roque. As shown in Cranston et al. 2011: fig. 2), the sequence data from the isolated Australian larva and the Brazilian adult midge form a tight cluster, allowing the inference that they were each other's closest relatives, likely to be congeners. Phylogenetic analyses (loc. cit.) implied that *Nandeva* was sister to *Riethia*, represented by four specimens, belonging to only 2 species (Cranston 2019). Although the subtending node lacked support, at the next level

the sister group was proposed as tribe Tanytarsini (Cranston et al. 2010: node C5, with 100% support). Although polythetic, morphological taxonomic concepts of tribe and subtribes thus find strong support from molecular analyses (Cranston et al. 2011; Krosch et al. 2019). Further elaborations of expanded morphological matrices and analyses under various forms of parsimony have shown *Nandeva* as belonging within the well-circumscribed Tanytarsini (Andersen et al. 2011) - in an arrangement found in some slightly suboptimal molecular results. Now a recent analysis combines new and existing molecular data, from more intensive sampling within Tanytarsini and greatly expanded by addition of *Pseudochironomus* and many more species of *Riethia* (Krosch et al. 2019). The analysis (Fig. 2, modified from Krosch et al. 2019, fig. 1) now clearly locates *Nandeva* within Tanytarsini, with 100% Bayesian Posterior Probability (PP) and .93 Bootstrap support (BS)), and within subtribe *Tanytarsina* (99% PP, .85 BS). *Nandeva* attaches as sister to the clade comprising (*Sublettea* (*Paratanytarsus* + *Micropsectra*), but with weak support of only 90% (PP). Contrary to previous predictions, addition of larval characters to the morphological matrix and re-analysed using parsimony, does not strengthen support for any of the previously inconsistent proposed relationships (Cranston pers. obs.).

Tempo of diversification

The major difference between analyses of the tempo of diversification in Cranston et al. (2010, 2011) and Krosch et al. (2019) is the use of new and different fossils to calibrate the BEAST analyses. Well-dated, newly studied, amber fossils include those assigned to Tanytarsini, including *Tanytarsus* (Gilka 2011; Gilka et al. 2013; Zakrzewska and Gilka 2014, 2015b; Zakrzewska et al. 2016; Zakrzewska et al. 2018) and to *Nandeva* (Gilka et al. 2016). Temporal analysis (Fig. 2, red # below line is median age in Ma (million years before present) shows two highly-supported splits, between subtribes Tanytarsina and Zavreliina of 74 Ma (with range 61.1—90.8 Ma) and the later split between *Cladotanytarsus* + *Tanytarsus* and remaining members of the subtribe at 56 Ma (51.3—63.8 Ma). Although the node connecting *Nandeva* within Tanytarsini is unsupported, a date (and range around this node) of 42 Ma (33.2—49.6 Ma) can be postulated. This is expected, as we had applied the Eocene calibration fossil *Nandeva pudens* (Gilka et al. 2016) to the stem at 49.5 Ma, a youngest age attributed to Fushun amber, with the value of 42 Ma applying to the crown age.

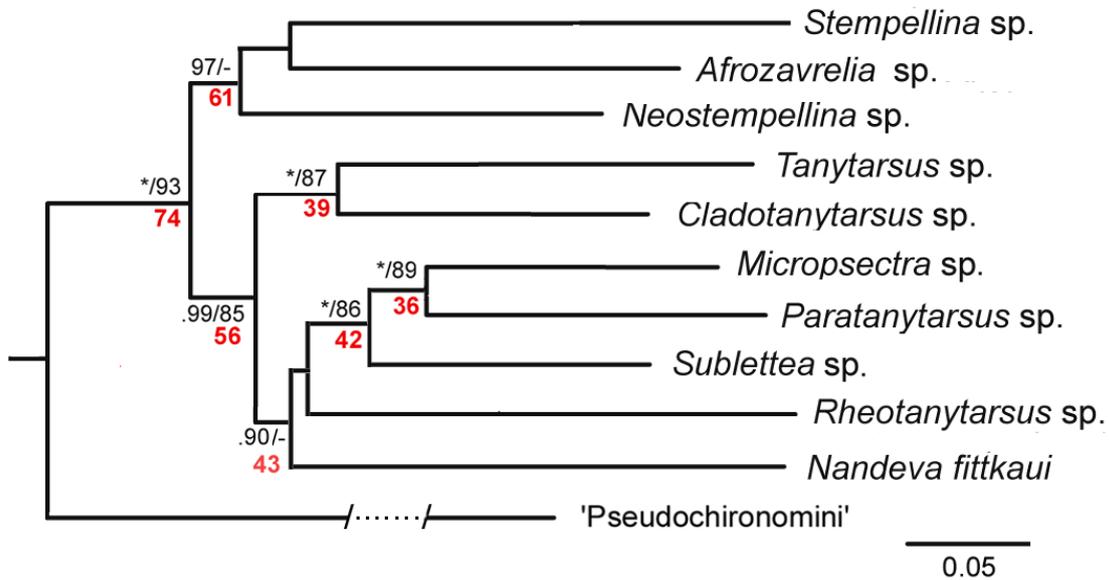


Figure 2. Bayesian dated tree for Tanytarsini, ‘Pseudochironomini’, terminals reduced from Krosch et al. (submitted 2019). Above node, black # Support statistics. * - maximum Bayesian posterior probability (1.00), - = maximum bootstrap (100), .xx/yy Bayesian posterior probability / bootstrap value. Below node, red # is mean date in Ma (million years before present). indicates attenuated distance.

Thus stem Tanytarsini originated in the late Cretaceous and commenced radiation as the extant tribe (crown group) in the end Cretaceous with the progenitors of the two subtribes. Subsequent radiation into extant genera of Tanytarsini took place through the Palaeogene, although fossil evidence is lacking, with most median dates of splits in the Palaeocene and Eocene. Sampled extant genera including *Nandeva* are postulated as existing crown groups by the late Eocene, which is in keeping with the fossil evidence.

Discussion

Studies of the Chironomidae have elucidated southern hemisphere (‘austral’) biogeography, commencing with Brundin’s seminal works (1963, 1965, 1966). Although Brundin used ‘manual’ Hennigian phylogenetics and somewhat subjective temporal reconstructions, studies substantially confirm Brundin’s vicariance-based reconstructions, notably Cranston *et al.* (2011) across the whole family and for subfamilies Podonominae (Cranston et al. 2010), Orthoclaadiinae (Krosch et al. 2011) and Tanytarsini (Krosch et al. 2017). Well-dated reconstructions recover a tempo of midge evolution that tracks Gondwanan fragmentation, even involving southern Africa that allows inference of a vicariance date of > 100 Ma (Cranston et al. 2011). Younger patterns include New Zealand (e.g. Krosch and Cranston 2013),

but most vicariant taxa are separated from South America - Australian connection via Antarctica (van der Ende et al. 2017).

Calculations of the tempo of diversification relative to earth history have been greatly enhanced since Brundin’s time. A cadre of insect palaeontologists are describing well-preserved and carefully dated fossils, particularly from amber-preserved specimens dating back up to 100 Ma. With respect to this study, Giłka, Zakrzewska and colleagues continue to provide valuable data on Eocene amber inclusions. In addition to dated and taxonomically informative ‘calibration points’, are improvements in phylogenetic and temporal diversification analyses, based on molecular data using both Maximum Likelihood and Bayesian methods of analysis.

In a dated phylogeny (Cranston et al. 2011) both sampled *Nandeva* were monophyletic, with the stem node (although as sister to *Riethia*) dated at 47 Ma (30–67 Ma) (unpublished supporting information). Note that this analysis did not use *Nandeva* in calibration. The South American *Nandeva* had to be excluded from the analysis of the Krosch et al. (submitted 2019) analysis of the tempo of diversification because its molecular representation was only by COI. However, with or without the S. American *Nandeva*, and independent of close calibration points in Tanytarsini,

including for *Nandeva*, the median date for stem *Nandeva* converges on early-mid Eocene, which is the date of the fossil *N. pudens*.

The locations and diversity of extant *Nandeva* imply concordance with timing for south American vicariance from Australia via Antarctica which was temperate and connected until the opening of the Drake Passage at c. 30 Ma, and the final break from Australia via the Antarctica landbridge, along the South Tasman Rise at c. 45 Ma (van der Ende et al. 2017). However, that this represents a “southern disjunct” or “amphibiotic” (Cranston 2005) distribution may be tempered by *Nandeva* occurring in more tropical streams as well as the usual cool temperate distributions of such clades. Although Amorim and Santos (2017) demonstrate that Brazil’s Southern Atlantic Forest contains amphibiotic elements, and *Nandeva* are found there, it is clear that they occur also in truly tropical streams.

Of greater significance is the undeniable presence of *Nandeva* in early Eocene Fushun (Palaeartic China) and the fossil genus *Eonandeva* (possibly stem *Nandeva*) from slightly younger Baltic amber (Zakrzewska and Gilka 2015b). Such ancient presence of stem groups of ‘gondwanan’ crown taxa, are discussed by Gilka et al. (2016) for Chironomidae. Other examples include termites of the family Mastotermitidae, the sister group to the remaining extant termites, is known from the Miocene to Eocene of Europe, Mexico, and the Dominican Republic and perhaps Cretaceous fossils in the Palearctic is reduced now to *Mastotermes darwiniensis*, a pest in northern Australia. The bulldog ants (subfamily Myrmeciinae), now restricted to Australia, indubitably include fossil taxa from Argentina and the Baltic. The biting midge *Austroconops* (Ceratopogonidae, Leptoconopinae) abundant in Lebanese and other Cretaceous ambers, is represented now by two species in Western Australia. Differential extinction in the northern hemisphere with survival in the south, seems to be frequent, giving rise to a ‘pseudo-gondwanan’ distribution (Cranston and Gullan 2005). *Nandeva* appears to qualify for this term.

One more issue remains to be addressed here, that the Australian *N. fittkai* differs from the studied neotropical species in enough features that could encourage allocation to a different genus to the neotropical species (Andersen et al. 2011). Some structures are difficult to confirm due to Australian adult material all being in a pharate state. As documented by Andersen et al. (2011) differences include the absence of lateral anteprenotals, the bare squama, spatulate anal point and superior

volsella with more basal setae and strong microtrichia. In the female genitalia the ventrolateral lobe is strongly microtrichiose and in the same plane as the dorsomesal lobe, the spermathecal ducts are nearly straight, a floor seems absent, and the setae of tergite IX are divided into two groups. The pupal tergites VI–VII lack the antero-medial patches of stronger spinules seen in the Neotropics. We still lack non-teneral adults for confirmation of the adult differences, yet can be sure that even if a new rank is ‘needed’ for *N. fittkai*, it surely will be sister to the neotropical clade, and inferences here concerning evolution and biogeography will be unaffected.

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