Evidence from molecules and morphology expands *Podonomopsis* Brundin (Diptera: Chironomidae: Podonominae) to include 'genus Chile'.

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Abstract. The informal taxon 'genus Chile' of Brundin, based solely on pupal exuviae of a podonomine Chironomidae, has remained inadequately known for half-a-century. New collections reveal life associations, and provide molecular data to hypothesise a precise phylogenetic placement in the austral Podonominae. A densely sampled molecular phylogeny based on two nuclear and one mitochondrial DNA marker shows 'genus Chile' to be the sister group to *Podonomopsis* Brundin 1966. Within *Podonomopsis* a clade of south American species is sister to all Australian species. We discuss how to rank such a sister group taxon and treat 'genus Chile'. as a new subgenus *Araucanopsis* **subg. n**. with the new species, *Podonomopsis* (*Araucanopsis*) *avelasse* **sp. n**. from Chile and Argentina as genotype of the monotypic subgenus. We describe *P. (A) avelasse* in all stages and provide an expanded diagnosis and description of *Podonomopsis* to include *Araucanopsis*. A dated biogeographic hypothesis (chronogram) infers the most recent common ancestor (tmcra) of expanded *Podonomopsis* at 95Ma (68-122Ma 95% HPD), 'core' *Podonomopsis* at 83Ma (58-108) and Australian *Podonomopsis* at 65Ma (44-87). All dates are prior to South America – Australia geological separation through Antarctica, supporting previous conclusions that the taxon distribution is 'Gondwanan' in origin. *Podonomopsis*, even as expanded here, remains unknown from New Zealand or elsewhere on extant Zealandia.

Additional keywords: phylogenetics, taxonomy, rank, biogeography, Podonominae, Insecta, Gondwana.

Introduction

The midge subfamily Podonominae Thienemann & Edwards in Thienemann (1937) (Diptera: Chironomidae) is highly significant in the discipline of phylogenetic biogeography (Hennig 1966; Brundin 1965, 1966, 1972; Cranston *et al*. 2010). Initially the subfamily included only few western Palaearctic boreal (northern cold temperate) midges, which formed a minor component of bog and stream invertebrate assemblages. Immature stages were included in the justification for distinction and the rank given (Thienemann 1937). The group remained essentially a curiosity until Brundin (1966) monographed the Podonominae (and the entirely austral Aphroteniinae and Heptagyiae [Diamesinae]) revealing high diversity in the southern hemisphere. Phylogenetic and biogeographic analyses of these transantarctic midges encouraged Brundin (1966) to argue that clade distributions supported Gondwanan vicariance as a major mechanism generating the observed patterns (Brundin 1966, 1972). The proposals were not universally accepted, partly because some critical life history stages were unknown, elucidation of some Gondwanan areas was inadequate and some proposed relationships were ambivalent or without explicit support. However, subsequent studies show Brundin's thesis holds up against more recently discovered immature stages (Cranston *et al*. 1987; Cranston and Edward 1998; Boothroyd and Cranston 1999), new distributional data (e.g. Cranston *et al*. 1987), critical amber fossils (Brundin 1976; Krzeminski *et al*. 1994; Krzeminski and Jarzembowski 1999), interpretation of female genitalic morphology (Sæther 1977) and addition of diversity (e.g. Siri and Brodin 2014). Most tellingly, molecular analyses based on sampling across the taxonomic and geographic range of the subfamily (Cranston *et al*. 2010) confirm monophyly (excepting one monotypic genus) with relationships coinciding substantially with those proposed on morphology (Brundin 1966; Cranston and Edward 1998; Cranston *et al*. 2010). Analyses also confirmed Mesozoic diversification of higher taxa with timing appropriate for Gondwanan vicariance, including early African separation. Some shallower nodes (i.e. species within genera) postulated both younger vicariance and recent dispersal, including some southern to northern hemisphere movement in the New World (Cranston *et al*. 2010).

The peculiar pupa described and discussed by Brundin (1966) remained enigmatic until the molecular study of Cranston *et al*. (2010). This 'genus Chile' showed some affinities with *Podonomopsis* Brundin 1966, erected for taxa from Australia and South America. Here the complete life history stages are associated and described, and the

morphological and molecular basis is provided for its phylogenetic placement in the context of an austral radiation.

Materials and Methods

Sampling, specimen handling, photography and microscopy; abbreviations

Collection involved interception in flowing water of drifting larvae, pupae and drowned adults using 300µm mesh drift nets. As used by Brundin, this 'Thienemann technique' (Cranston 2000) has proved especially valuable for lotic podonomines. We sought different life stages for sequence-based associations, and certain putative taxa were sampled repeatedly from geographically separated populations.

All samples were field sorted, targeting podonomines, under a binocular microscope, preserved into 95-100% isopropanol and refrigerated as soon as practicable. All specimens destined for DNA extraction and sequencing were extracted from whole bodies (see below) and carcasses vouchered as microscope slides using Euparal mountant, or occasionally Hoyers (larvae) and subsequently remounted in Euparal. Slides, including of molecular vouchers are labelled appropriately and preserved in the collections of the Australian National Insect Collection, CSIRO, Canberra, Australia (ANIC). Paratype larval slides are placed in Museo de la Plata, La Plata, Argentina (MLPA) and Museo Nacional de Historia Natural, Santiago, Chile (MNHNC).

Line drawings (made by P.S.C.) used either a camera lucida attached to Olympus™ BX41 (to 400x magnification), or were based on stacked photographs using an Automontage™ system attached to Leica™ DMR microscope with oil immersion optics. (x1000). Ink on drafting film drawings were scanned at 600 dpi monochrome and manipulated subsequently using Abode Photoshop™. Line drawings were scanned similarly from Brundin (1966) with permission. Colour images were produced using Automontage™ on a Leica™ DMR microscope with Nomarski Interference optics, and also manipulated with Abode Photoshop™. Colour photographs complement judicious line drawings – neither are fully satisfactory alone.

We use standard abbreviations for morphological structures as follows: Adult: A.R - antennal ratio (apical flagellomore divided by all preceding flagellomeres summed); ac – acrostichal setae, apn – antepronotal setae; dc – dorsocentral setae, pa – prealar setae; sct – scutellar setae, ta – tarsomere. Pupa (P): apn – antepronotals (d. – dorsal, l. lateral); dc, dorsocentrals; pc – precorneals; Pe – pupal exuviae. Larva (L): A.R. antennal ratio (basal segment divided by apical segments 2-5 summed).

DNA extraction, PCR amplification, and sequencing

In the laboratory, total genomic DNA was extracted using the ISOLATE II Genomic DNA Kit (Bioline, Alexandria, Australia) following the protocol provided by the manufacturer. Modifications to the protocol to enhance extraction from individual pupal exuviae and from older samples followed Krosch and Cranston (2012). One region each of the nuclear rRNA gene *28S*, the nuclear protein-coding gene *CAD*(*CAD3*) and the mitochondrial protein-coding gene *COI* were amplified. Primers, reaction protocols and cycle programs used for amplification of *28S*, *CAD3* and *COI* follow Krosch *et al*. (2011). Amplification products were purified using an ISOLATE PCR and Gel Kit® (Bioline) according to manufacturer's instructions. Direct sequencing of PCR products was performed using the ABI Big Dye® Terminator v.3.1 chemistry and was carried out in an ABI 3500 Capillary Electrophoresis Genetic Analyser at the Molecular Genetics Research Facility (QUT, Brisbane). All new sequences have been lodged with GenBank (Accession Numbers KT633411-KT633502, Table 1).

Data Analysis

COI and *CAD* sequences were compiled and edited by eye using BioEdit v.3.0.9 (Hall, 1999), whereas *28S* sequences were aligned initially by eye and refined using MUSCLE 3.7 (Edgar 2004). Potential heterozygous sites in nuclear sequence data were recognised by double peaks in sequence chromatograms and were coded as ambiguous bases according to IUPAC codes. Eighteen alignment gaps were recognised among *28S* sequences and all were excised following analysis using GBlocks Version 0.91b (Castresana 2000) with default settings.

Sequences were concatenated and individual partitions assigned for each locus; models of evolution were applied to each partition individually. We included representatives of outgroup taxa from outside the Podonominae (specifically, *Aphroteniella* Brundin 1966, *Buchonomyia* Fittkau 1955, *Harrisonina* Freeman 1956 and *Telmatogeton* Schiner 1866) and from other non-target genera within the subfamily; including, *Afrochlus* Freeman 1964, *Archaeochlus* Brundin 1966, *Austrochlus* Cranston 2002, *Boreochlus* Edwards 1938, *Lasiodiamesa* Kieffer 1924, *Paraboreochlus* Thienemann 1939, *Podonomus* Philippi 1865, *Podochlus* Brundin 1966, *Parochlus* Enderlein 1912, and *Trichotanypus* Kieffer 1906. Outgroup sequences derive from

Cranston *et al*. (2010) supplemented by additional new collections of podonomine diversity sequenced for this study (Table 1).

Phylogenies were inferred for single locus datasets and for the concatenated partitioned dataset. Protein-coding genes (COI and CAD3) were partitioned into first, second and third codon base positions for all phylogenetic analyses. Bayesian phylogenetic inference was performed in MrBayes Version 3.2.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) under the GTR model of sequence evolution, incorporating a gamma distribution of nucleotide frequencies. Two simultaneous runs of four chains (two cold, two hot) were performed for 5 million generations for single locus datasets and 20 million generations for the concatenated partitioned dataset. Convergence was maximised by ensuring the standard deviation of split frequencies fell below 0.01 and potential scale reduction factors approached 1.0 for all parameters. Maximum likelihood (1000 bootstraps) reconstruction was performed using RAXML Version 8.0.24 (Stamatakis 2006) under the GTRGAMMA model of sequence evolution. All analyses were conducted on the CIPRES Science Gateway High Performance Computing platform (http://www.phylo.org; Miller *et al*. 2010).

The concatenated partitioned molecular dataset was trimmed to include one representative of each species to allow estimation of times to most recent common ancestor (tmrca) in BEAST Version 1.8.1 (Drummond *et al*. 2012). The rootheight of the tree was calibrated following Cranston *et al*. (2010), using an exponential prior distribution with a zero offset of 201Myr and mean of 15, which corresponded to the oldest unambiguous fossil chironomid *Aenne triassica* Krzemiński and Krzemińska 1999 (209.6 ± 1Myr, Krzemiński & Jarzembowski 1999) and allowed the 97.5% Highest Posterior Density (HPD) to encompass an appropriate degree of uncertainty. An additional internal calibration was applied as per Cranston *et al*. (2010) to the node connecting all sampled Podonominae specimens (Node A), which corresponded to the oldest known fossils of extant crown Podonominae that were sampled in the tree (*Lasiodiamesa* and *Paraboreochlus*; 54.8-33.7Myr, Seredszus & Wichard 2007). This calibration used an exponential prior distribution with a zero offset and a mean of 40Myr. We explored the effect of codon position by partitioning the protein coding genes in three ways: a) unpartitioned, with independent HKY+G models applied to each locus; b) first and second codon positions as a single partition separate to third base positions, with independent GTR+G models applied to each position (Shapiro *et al.* 2006); and c) all codon positions separate, applying independent HKY+G models to each partition (Yang

1996). We unlinked substation rates, rate heterogeneity models and base frequencies across codon positions. A HKY+G was applied to the non-protein coding 28S locus in every analyses. We implemented the 'Speciation: Yule Process' tree prior and allowed substitution rates to vary across branches in accordance with a uncorrelated relaxed lognormal molecular clock prior (Drummond *et al.* 2006) . Two runs of 100 million generations were performed for each dataset, sampled every 1000 generations, from which ten million generations were removed as burnin from each run prior to combining log files in LogCombiner Version 1.8.1 (Drummond *et al*. 2012), producing a total run of 180 million generations. Convergence of runs was assessed by viewing log files in Tracer Version 1.5 (Rambaut & Drummond, 2009). A chronogram was produced from the stationary distribution by first removing 10% of trees from each run as burnin prior to combining tree files from each run in LogCombiner, and annotating in TreeAnnotator Version 1.8.1 (Drummond *et al*. 2012).

Results

Phylogeny

We collected, vouchered and identified austral podonomines, especially *Podonomopsis* and relatives from the tribe Parochlini, predominantly in the immature stages, from Australia and South America. We sought material without success in New Zealand over several years. New molecular data were obtained from 39 specimens, all of which provided sequence for *28S*, 38 for *CO1* and 16 for *CAD*. Exclusion of hyper-variable regions resulted in a final multilocus dataset of 1754 characters. Lengths by locus were: *COI*: 723 bp, *28S*: 407 bp, *CAD*: 624 bp. Details of all specimens, codes, collection locations, dates, collectors, life stage and GenBank accession numbers are listed in Table 1. The concatenated partitioned dataset produced topologies well supported across the tree for both the total and reduced datasets.

The subfamily Podonominae is confirmed as monophyletic (Fig. 1, node A, PP 1.0, BS 68) as in all previous analyses, both morphological and molecular. The 'tribe' Boreochlini was paraphyletic with *Boreochlus* + *Paraboreochlus* alone sister to tribe Podonomini (node B, PP 1.0, BS 100) as in Cranston *et al*. (2010). However *Lasiodiamesa* is postulated (without support) to be sister to *Trichotanypus* in the 'remnant Boreochlini', which otherwise is as in Cranston *et al*. (2010). No additional specimens of grade 'Boreochlini' were included in this study, in contrast to many Podonomini added. Reduction of near duplicate sequences, and/or addition of Australian

exemplars to *Podochlus*, *Parochlus* and *Podonomus* affected neither the monophyly (node C, PP 1.0, BS 82) of this clade, nor relationships of all included generic-rank taxa, (including synonym of *Zelandochlus* Brundin 1966 within *Parochlus*) with respect to Cranston *et al*. (2010).

Addition of many specimens from South America and Australia to *Podonomopsis* did not perturb monophly (node F, PP 0.90, BS 64) although support is weaker than in a previous analysis that lacked South American species (Cranston *et al.* 2010: node P11, PP 1.0 BS 0.98). The two included South American species are monophyletic (node J, PP 1.0 BS 1.0). In contrast to the near identity of the two *P. muticus* specimens from different locations in Argentina, *P. illiesi* from Peru shows internal structuring with differentiation between the Cotohuasi (COT) and Santa (SAN) basins. Australian *Podonomopsis* are monophyletic (node G, PP 0.99, BS 72) with two strongly supported component sister clades (node H, PP 1.0 and node I, BS 99 &100). Specimens of 'genus Chile' from two localities in Chile (Table 1) cluster as identical sequences, and are sister to *Podonomopsis* (node E, PP 1.0 BS 100) in the similarly strongly supported relationship found by Cranston *et al*. (2010) with fewer specimens and lacking S. American taxa.

Evolutionary and biogeographic tempo

Implementation of different codon-partitioning strategies had varying impacts on our analyses: the Yang (1996) model (all positions separate) did not fit the data well, with poor convergence of the runs associated with third position base frequencies, whereas the Shapiro *et al.* (2006) model ([1+2], 3) and the unpartitioned analyses were very similar (data not shown). Here we report estimated dates from unpartitioned analyses. Our data (Fig. 2, Table 2) supports podonomine diversification beginning around 180Ma (95% HPD: 138-217Ma)

Clade ages for *Austrochlus*, *Archaeochlus* and *Podonomus* were roughly contemporaneous, ranging from 37-45Ma (combined range of 12-79Ma). In contrast, *Parochlus* is estimated to have begun divergence earlier, around 56Ma (36-78Ma), as were both the previous concept of *Podonomopsis* (83Ma; 58-108Ma), and that expanded by inclusion of 'genus Chile' (95Ma; 68-122Ma). Within *Podonomopsis*, divergence of mainland Australian and Tasmanian lineages within the two described species was estimated to be of Eocene age (*P. discoceros*: 41Ma [24-59Ma]; *P. evansi*: 38Ma [16-61Ma]). Populations of *P. illiesi* that inhabit different river drainages in Peru diverged around 11Ma (5-18Ma).

Morphology

In the Podonominae congruence between molecular data and identity at genus and species group level is high, probably due to the use of morphology from all semaphoronts (larvae, pupae and adults) in contrast to adults alone, as noted by Cranston *et al.* (2010). In this study, initial morphological identifications of any life stage (including the quite homogeneous larva) concurred with the phylogenetic placements suggested in Fig. 1. Specifically, examination of the previously unknown larva and pharate adult male associated with pupae identical to 'genus Chile' extends Brundin's (1966) recognition of pupal similarity to *Podonomopsis* to other stages. Review of the added diversity of morphology of specimens allocated to *Podonomopsis* in addition to Brundin's (1966) descriptions (with revised terminology where appropriate) suggests 'genus Chile' can be incorporated under a modestly expanded diagnosis of *Podonomopsis* (see further discussion below).

Discussion

Our analysis of a selectively expanded dataset of Podonominae suggests that, with the exception of 'rogue taxon' *Lasiodiamesa,* the phylogenetic hypothesis for the subfamily by Cranston *et al*. (2010) finds substantial support. Additional specimens of *Podonomus* from Australia suggest the described diversity (two species, one mainland and one Tasmanian) is an underestimate. Three Australian mainland submontane specimens are highly differentiated in their DNA and form a clade embedded in the widely sampled South American 'P. decarthrus group' of Brundin (1966).

Within Australian *Podonomopsis* more internal structure is revealed than would be expected for only two described species (*P. discoceros* Brundin 1966 and *P. evansi* Brundin 1966). For example, Tasmanian specimens ('TAS') each appear distinctive from their respective 'conspecifics'. Only in the 'core' *P. discoceros* and *P. evansi* are all stages aligned into genetically coherent clusters. Untangling an expanded species diversity in Australia and Tasmania requires more extensive material across all life stages. Outliers of *P. discoceros* (TAS1 and TAS13.6) are respectively a single larva and pupa; outliers for P. *evansi* (ACTCon1 and AUPE) are solitary pupa and larva respectively.

The phylogenetic position that our results suggest for Brundin's 'genus Chile' (Fig. 1) leads to possible different taxonomic proposals: a new genus-level taxon, expansion of diagnosis of genus *Podonomopsis* or inclusion of an intermediate, subgeneric rank taxon

within the existing hierarchy. Even with a long tradition of phylogenetic analysis within the Chironomidae, little consensus exists for a consistent arbiter for any supraspecific category (rank). With or without a phylogenetic hypothesis, too frequent use of autapomorphy of a single life stage, usually adult male, has 'justified' new higher taxa. Delineation of rank by age, proposed early by Hennig (1966) and Brundin (1966) but little applied, at least appears now more feasible with increasing estimates of the tempo of diversification (Avise & Johns, 1999, Holt & Jønsson, 2014). In Chironomidae several calibrated phylogenies (Cranston *et al*. 2010; Cranston et al, 2012, Krosch & Cranston, 2015) allow investigation of a temporal banding approach in which time-calibrated phylogenies can be cut at upper and lower bounds around a particular rank to delineate more consistent hierarchical taxonomic ranks (Holt & Jønsson, 2014). Clade ages for (non-monotypic) genera of podonomines in our studies average 56-60Ma, spanning oldest to youngest of 95-37Ma (late Cretaceous to Eocene). Some dates here differ significantly from previous calculations (Cranston *et al.* 2010; Cranston *et al.* 2012) including deeper dates for mrca nodes for *Podonomopsis* and *Podonomopsis* + 'genus Chile'. Never-the-less, with a temporal banding rationale 'genus Chile' could be considered as equivalent in rank by age with other podonomine genera. However, we do not advocate this rank for several reasons. (1) A monotypic higher rank contain less information content than recognition of a sister group relationship to *Podonomopsis*, which shares much ecology and morphology of all stages. (2) We acknowledge that *Rheochlus* Brundin 1966 must be considered in this context. Established on adults alone from Australia and South America, the genus is elusive (although recently revised by Siri and Brodin, 2104)) and remains absent from our studies. Based on adult morphology, Siri and Brodin (2014) suggested it was prospective sister group to *Podonomopsis* (as did Brundin 1966) but with ambiguous support. Since 'genus Chile' lies in this position in our study it implies proximity to the molecularly-unknown *Rheochlus*, and thus encourages our conservative taxonomic approach to nomenclature. (3) We further acknowledge that two rather similar species of *Podonomopsis* from South America are unsampled, namely *torrentium* Brundin 1966 and *brevipalpis* Brundin 1966. Their absence should not contradict the two Australian species forming a clade, despite their pupal morphological divergence. This contrasts with Brundin's argument that *P. evansi* was close to the Andean pair of *P. illiesi* and *P. andinus.* Seemingly the highly heterogeneous forms of the thoracic horn in this (and other) genera of Podonominae are compromised as indicators of relationships. (4) The idea that temporal banding provides

a less arbitrary approach is of limited value since it can provide only relative equivalence in ranks within a group and cannot provide wider relativity without progressive collateral disruptive rank-changing outside the target group. In a phylogeny-based temporal banding approach the late Mesozoic-early Palaeogene ages associated here with generic rank in Podonominae are equivalent to ordinal rank or deeper in birds and mammals (Avise & Johns, 1999; Holt & Jønsson, 2014). A universal taxonomic yardstick appears unlikely.

As with other dated chronologies for several different groups of Chironomidae, our results lend further support to Brundin's (1966) contention that the fragmentation of Gondwana promoted the observed current-day patterns of diversification. Major inter-continental dates are prior to South America – Australia geological separation through Antarctica. Aside from the Podonominae (Cranston et al, 2010) such appropriately timed divergences have been proposed within the Orthocladiinae (Krosch *et al*., 2012) and for the genus *Stictocladius* (Krosch & Cranston, 2013). Absences from New Zealand could be due to its isolation prior to ancestral radiation, loss (extinction) due to Oligocene marine transgression (Krosch & Cranston, 2013) or as yet inadequate sampling for molecular studies. At the finer scale, divergences within South America appear likely to be due to Andean orogeny, and within Australia, Tasmanian isolation from the mainland may have been important although clearly not the most recent event, as surmised for *Cricotopus* (Krosch et al., 2015).

Taxonomy

Podonomopsis Brundin

(Figs. 3-9)

Type-species: *Podonomopsis muticus* (Edwards, 1926) by original designation.

Generic redescription

Adult male

Head (Fig. 3*A*): Occiput setose with 2 strong post-orbitals. Clypeus squat. Eye microtrichiose extending slightly beyond facets. Palp variably developed from reduced to long, lacking any ('5th') basal segment. Antenna (apex, Figs 3*B-D*) with pedicel with 1 seta; 14 flagellomeres with short apical flagellomere and penultimate ranging from subequal in length to apical to 3-4 times its length; A.R. c. 0.1. ***Thorax***: Brown with little

evidence of vittae; apn, ac, dc, pa, sct with relatively high numbers of long setae; postnotum seemingly bare. ***Legs***: Coxae and trochanters large and setose, femora and tibiae relatively short; all tibial spurs (Fig. 3*E*) weakly developed to seemingly absent; ta4 subequal to ta5, claws all long, simple. ***Wing***: Densely setose in all cells (except *P. brevipalpis* gp., bare), with free-end of costa reaching apex; width of anterior (radial) cells R_1 and R_5 may be specifically variable. Squama setose, alula bare. ***Hypopygium*** (Figs. 3*F-K*): Tergite IX small, with many long setae; internally with narrow, gently arched sternapodeme, short phallapodeme and hyaline aedeagal lobe. Gonocoxite slender, cylindrical and conventionally developed with long setae. Gonostylus comprising setose / microtrichiose basal part, distally divided in variable lengthed, bare dorsal lobe terminating in lamella or spine, and ventrally a tapering gonostylar extension, sometimes with hyaline lateral flange, terminating in hyaline lamella or spine. 'P seta' long, 'x' and 'y' setae retracted, small or seemingly absent.

Pupa

Cephalothorax*:* with 1-2 pairs of dark stout frontal setae always located on dorsolateral corner of frontal apotome and 2nd pair, when present, more antero-median (Fig. 4*A*). Thoracic setae very fine: 1-2 d.apn, 1-2 l.apn, pc not detectable, 4 dc located far posterior. Thoracic horn varies, always flat, ovoid to parallel-sided (Fig. 4*B-H*) with ovoid to elongate rectangular / parallel-sided porous plate, some apically with disto-lateral projections; stalk spinose, well-developed or near lacking. Narrow tubular horn chamber connects internal trachea to variably developed lumen of plastron plate, with distinct opening at border between stem and granular horn sac. ***Abdomen***: flat or flexed ventrally (arched), segments essentially quadrate, with minimal microsculpture or microtrichiae, with very reduced chaetotaxy including very fine L setae. Distally postero-lateral margin of segment VIII is raised, posteriorly partially isolated from adjacent segment as a 'flap' bearing 5-7 setae, close-set with near contiguous bases (Fig. 4*I-L*),. These setae long, strong and sinuous ('wavy') in *Podonomopsis* s.s.; shorter, much weaker and hooked only apically in *Auraucanopsis* subg. n. (Fig. 8*C, D*). Segment IX bilobed bearing either two wavy setae subapically and 2-3 more distal setae more apically (*Podonomopsis* s.s.) or with only 1 short seta (*Auraucanopsis* subg. n.). Genital sheaths of male extend beyond segment IX, sometimes quite narrow.

Larva.

Up to 6 mm long, with thoracic segments swollen and fused (Fig. 9*C*); brown head capsule (Fig. 5*A*) nearly parallel-sided, slightly longer than broad, frontoclypeal apotome

present, mentum, mandible and occipital margin darker; antenna arises from pale area. Eye spot single.

Head: Antenna (Figs. 5*B*, 9*A*) about 50% of head capsule length; 5-segmented, 1st segment long and quite narrow, segments 2 and 3 annulate, with segments 4 and 5 subequal, short; blade not extending to apex of segment 2, accessory blade absent. A.R. ca. 1.4-1.6. Style very short, hyaline; without Lauterborn organs. Two or 3 Ring organs in basal 1/3, strong seta inserted in middle 1/3. Labrum (Fig. 5*C, F*) with SI, SII and SIII elongate and enlarged, sickle-shaped in lateral view, all arising from distinct pedestals; SIVa rod-shaped, arising from pedestal longer than rod, SIVb rod-shaped. One simple chaeta, seta premandibularis and well-developed, slightly curved labral rod. Pecten epipharyngis of three basally-fused long scales. Premandible absent. Epipharynx with 3-4 pectinate, 2 simple elongate chaetulae laterales, and 2 robust, simple chaetulae basales; without basal sclerite. Mandible (Figs. 5*D*, 9*B*). With 5-6 teeth (distal mola may be dark, tooth-like); median tooth larger than subequal remainder. Seta subdentalis apically pointed, arising from anterior surface of mola, seta interna plumose, located very apically on dorsal mandible. Mentum (Fig. 5*E*, 9*G*). Relatively narrow, flat medially with median tooth narrow and (if unworn) medially either with slight notch or nipple; small first laterals and first 2 larger laterals forming level section, flanked by outer 5-6 small lateral teeth diminishing in even slope. Ventromental plate not recognisable. Setae submenti simple, displaced posterior to base of mentum. Prementо-hypopharyngeal complex (Fig. 5*F*, 9*D*) with 3 pairs of elongate dark lobes of uncertain homology extending far anterior to mentum.

Body. Anterior parapods fused basally, with separate crowns of many serrate claws but without interconnecting fine claws. Spiracles absent. Procercus (Figs. 5*G, H*, 9*F*) hyaline, almost indistinguishable from anal tubules, sometimes with small postero-median pigment patch, apically with tuft of short stout setae. Posterior parapods stout, with numerous pale, simple, long claws arranged in an apical crown (Figs. 5*I*, 9*E*).

New material of *Podonomopsis* examined.

Podonomopsis illiesi Brundin. PERU: 3P♂, Cotahuasi, Cotahuasi R., Ocona Basin, 13.viii.2011, 15°04'S 72°41'W, N. Prat. MV – COTP1-3 (P♂).

Podonomopsis muticus (Edwards). ARGENTINA: P♂, P♀, S. El Bolson, Arroyo Pedrogosa, 42°08'12"S 71°23.21"W, 10.ii.2010, 286m., P.S. Cranston. MV – ARG10.x.5 (P♂), ARG10.x.2 (P♀).

Podonomopis evansi Brundin and *P. discoceros* Brundin: numerous specimens from eastern Australia to Tasmania: new molecular material as detailed in Table 1.

Subgenus ***Araucanopsis*** Cranston & Krosch subgen. nov.
(Figs 6-9)

'genus Chile' Brundin 1966: 288, figs 392-3.

Type-species: *Podonomopsis (Araucanopsis) avelasse* Cranston & Krosch sp. nov., by original designation.
urn:lsid:zoobank.org:act:88329D9B-D916-4781-BED3-4636BE783BB5

Diagnosis
Subgenus *Araucanopsis* with its single species *avelasse* is differentiated from congeneric males in *Podonomopsis* by the elongate dorsal gonostylar lobe and complete lack of tibial spurs or comb, in the pupa by the strong development of anterior tergal tubercles and reduced structures of the distal segments (reduced 'wavy' setae) but in the larva perhaps only by slightly stronger apical procercal spines and perhaps in details of the unworn median mentum. The posterior displacement of the setae submenti is shared with *Podonomopsis*. All features of all known stages lie within the expanded generic diagnosis for *Podonomopsis* (above).

Etymology.
Named for the South American region of Araucana, coterminous with the historical range of the Mapuche people. The region encompasses many austral chironomid midge distributions. Linked to 'opsis', Greek, indicating likeness or resemblance, taken from *Podonomopsis*.

Species description

Podonomopsis (Araucanopsis) avelasse Cranston & Krosch sp. nov.
(Figs. 6-9)
urn:lsid:zoobank.org:act:B242009B-0CEF-41CA-8C78-17B9D3B57025

Material examined

Holotype. P♂, Chile: IX Araucania Region: n.e. L. Villarica, Estero La Casilla, 39˚14'S 71˚56'W, 4.x.2007, 240 m., P.S. Cranston, MV (Molecular Voucher) CH07-4.1 (Australian National Insect Collection, Canberra, Australia: ANIC).

Paratypes. L, as holotype (ANIC); **CHILE**: L(P)(MV CH07-1.1), 15L, IX Araucania Region, Parque Nacionale Villarrica, Rio Palquin, 39˚26'13"S 71˚47'25"W, 14.ii.2006, 805 m., P.S. Cranston (ANIC, 1 to MLPA, 1 to MNHNC); Pe, X Los Lagos Region, Parque Nacionale Puyehue, Rio Chaleufu, 450m., 40°44'11"S 72°18'26'W, drift, 20.ii.2006, P.S. Cranston (ANIC).

ARGENTINA: 4Pe, Neuquen, Puerto Blest, Arroyo Blest, 40°14'S 71°22'W, 2.i.1997, P.S. Cranston (ANIC).

Diagnosis

Follows subgenus *Araucanopsis* subg. n. above.

Description

Adult male (n=1, pharate, part dissected from exuviae; female unknown)

Head: Occiput densely setose anteriorly with approximately 40 setae each side, with 2 strong post-orbitals. Clypeus squat, with 8 setae. Eye densely microtrichiose extending slightly beyond facets; Palp (Fig. 6*A*) moderately developed with no evidence of any '5th' basal segment, palpomere lengths 37, 45, 50, 40, dense with setae up to 80µm. Antenna (apex, Fig. 6*B*). Pedicel with 1 long seta. With 14 flagellomeres of lengths (base to apex, in µm) 42, 26-30, 30-32, 30-32, 30-32, 30-32, 30-32, 30-32, 30-32, 30-32, 32, 26-30, 32-35 A.R. 0.09. ***Thorax:*** Brown without evidence of vittae, with 8 apn, 36 ac, 28-36 dense dc in uni-biserial rows in paler cuticle, pa 17 extending far anterior, sct 17; postnotum seemingly bare. ***Legs***: Coxae and trochanters large and setose, femora and tibiae shorter and stouter than usual, tibial spurs apparently absent on all legs, ta4 subequal to ta5, claws all long, simple. Ratios not measurable in teneral specimen. ***Wing:*** Length 5.5 mm, densely setose, with costa reaching apex. Anterior (radial) cells appear narrow. Squama with 13-14 setae, alula bare. ***Hypopygium*** (Fig. 6*C*): Tergite IX with anterior and lateral bands of many long setae, lacking such setae in posterior half; with 88µm long, narrow, gently arched sternapodeme and short (20µm) phallapodeme connected antero-medially with +/- rectangular, 25µm x 12µm (length to width) hyaline aedeagal lobe. Gonocoxite 130µm long, cylindrical and conventionally developed, without any lobes, with extremely long setae especially laterally (most extend in genitalia

pupal sheath well beyond apex of gonostylus. Gonostylus comprises setose and microtrichiose 35µm long, basal part, broadly contiguous with apex of gonocoxite, abruptly becoming divided into (*a*) dorsally an elongate (40µm long), bare lobe, curving dorsad and terminating in 15µm long, 'tear'-shaped lamella and (*b*) ventrally, a tapering, bare, 60µm long gonostylar extension, with slight evidence of hyaline flange towards apex, terminating in a narrow 10µm long, hyaline, tapering spine. 'P seta' long (c. 100µm), extending well beyond apex of gonostylus, originating from strong tubercle on gonostylus beside base of dorsal lobe; 'x' and 'y' setae seemingly absent.

Female unknown.

Pupa (n=5)

Pale brown to golden yellow. Total length 2.0-2.4 mm.

Cephalothorax: Length 250-275µm. 1 pair of dark brown, slightly sinuous, stout frontal setae 5µm wide, 25-42µm long, located on dorsolateral corner of frontal apotome (Fig. 8*A*). Thorax in profile posterodorsally swollen [may be Brundin's (1966) 'ventral (sic) air cavity'].

All thoracic setae fine and difficult to locate: 1 d.apn, perhaps 1 l.apn, pc not detectable, 4 dc located far posterior (clustered dorsal to posterior part of wing sheath and posterior swollen region of thorax). Thoracic horn flat, ovoid to subtriangular (Figs. 7*D*, 8*B*) with large oval porous plate and short, distally broadened, scaly stem, filled with horn chamber that links to lumen of porous plate in distinct opening at border between stem and plate. ***Abdomen*** (Fig. 7*A*), flexed ventrally (arched) in lateral view (Fig. 7*B*), anteriorly with transverse sclerotised ridges on tergites II-VII, segments essentially quadrate. Anterior tergites (I-IV) with paired large conical protuberances, conspicuously larger on II and III, located mid-tergite close to posterior margin. Segments VII and VIII tapering to small segment IX. Weak microsculpture of microspinules and polygons more distinct on tergites I-VII. 5D setae on II-VII, D_5 seta incorporated into tuberculose area on II-VII, fewer V setae, all fine. Lateral setae small anteriorly on margin, posteriorly on submargin, none taeniate: on segment VIII 5 close-set, terminally hooked weakly 'wavy' setae isolated on posterior margin of segment; segment IX with only 1 small seta (Figs. 7*C*, 8*C, D*). Genital sheaths of male narrow and elongate, terminating in ventrally-directed hook: contain elongate hypopygial setae (in pharate specimens) and extend well beyond apex of gonostylus.

4th instar Larva. (n=10)

Head capsule: dark brown with paler circular areas at base of antenna and dorsolateral on posterior genae. Dorsal length of head 325-340µm, length of postmentum 205-210µm. Antenna (Fig. 9*A*)) arises from slightly elevated area, total length 167-175µm, 5 segmented, tightly annulate on segment 2 (except base) and all of 3; lengths (in microns (µm), base to apex): 98-100, 38-42, 17-18, 2-3, 3-4, A.R.1.46-1.60. Blade 32-35µm. Minute style (or perhaps Lauterborn organ) on apex of segment 2. Basal antennal segment with 3 ring organs and strong seta 50µm long, located at c 40% length of segment. Mentum (Fig. *9G)* narrow, 52-55µm wide, with single median tooth flanked by small 1st laterals then 2 pairs of slightly larger teeth at same level: 7 central mental teeth are more or less transverse, and then flanked by 5 teeth each side declining in size and appressed to one another. Paired maxillary setae lie adjacent to base of mentum; stout 60-65µm long setae submenti are quite retracted toward occipital margin. Mandible (Fig. 9*B*) 75-88µm long, 'hump-backed' with 6 teeth including enlarged median tooth.

Body: Colour in life not recorded, greenish tinge remnant after mounting uncleared larva into Hoyers (Fig. 9*C*). Body length 3.4-4.0 mm (total including head 3.8-4.4 mm). Posterior parapod length 320-350µm, with apical 'brush' of slender, simple, golden-brown claws of lengths c. 90-105µm, arranged in a single dense horseshoe shape (Fig.9*E*). Procercus (Fig. 9*F*) 150-185µm, hyaline excepting basal, oval, dark patch; apically with tight cluster of 5-6 dark brown, 18-22µm long spines (anal setae). One pair of elongate-ovoid 250-300µm long anal tubules (Fig. 9*E*).

Distribution

Brundin's pupal exuviae came from a montane stream and a river near Peulla in province Llanquihue in the Chilean Lake District. Additional pupae and the molecularly-associated larvae come from similar flowing waters from Araucania and Los Lagos districts, Chile and pupal exuviae from a cold stream in Argentina, close to the border with Chile at Puerto Blest.

Etymology.

The species name 'avelasse' recognises Lars Brundin, pioneer of austral biogeography and chironomid systematics by combining 'ave' – Latin for hail or farewell - and 'lasse', in Sweden the diminutive or nickname of Lars, and the name by which Brundin was known to colleagues. To be treated as noun in apposition.

Acknowledgments

Professor Narcis Prat, Universitat de Barcelona, kindly made available specimens of Neotropical *Podonomopsis* suitable for molecular study – we are grateful for this collaboration. Martin Testorf, Naturkistoriska riksmuseet, Stockholm, Sweden kindly gave permission to reproduce figures from Brundin's (1966) seminal study. Many authorities provided permits to seek Podonominae, including in New Zealand where no *Podonomopsis* have yet been found. Our molecular studies and related travel concerning austral podonomines were supported by an endowment to the University of California at Davis (2000-2010) by the late Evert Schlinger. We acknowledge a subsequent contribution from the National Science Foundation grant MIDGEPEET: A Collaborative Effort to Increase Taxonomic Expertise in Understudied Families of Nematocerous Diptera award 0933218 to PI John (Kevin) Moulton.

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Figure legends

Fig. 1. Majority rule consensus maximum likelihood topology for the partitioned dataset. Relevant nodal support values are shown, which correspond to Bayesian posterior probabilities and ML bootstrap support, respectively; '-' denotes nodes unresolved by ML and '*' denotes posterior probabilities of 1.00 or bootstrap support of 100. Lettered nodes are those for which tmrca was estimated and correspond with Table 1, black stars indicate nodes to which prior calibrations were applied. Tip label codes correspond to molecular voucher codes listed in Table 1.

Fig. 2. BEAST chronogram from a dataset of single representatives per species/clade which corresponds with Table 2. Lettered nodes and black stars are as per Figure 1. Timescale is in millions of years before present.

Fig. 3. Adult male of *Podonomopsis* Brundin *(A)* Anterior of head of *P. discoceros* Brundin; *(B)* distal antenna of *P. discoceros*; *(C)* distal antenna of *P. evansi* Brundin; *(D)* distal antenna of *P. torrentium* Brundin; *(E)* apex of hind tibia of *P. torrentium*; *(F- K)* Gonostylus of (F) *P. illiesi* Brundin; *(G) P. torrentium*; *(H) P. evansi* (aberrant double flattened seta); *(I) P. andinus* Brundin; *(J) P.brevipalpis* Brundin; *(K) P. discoceros*. All from Brundin (1966).

Fig. 4. Pupa of *Podonomopsis* Brundin. *(A)* Ventral head of *P. discoceros* Brundin; Thoracic horns of *(B) P. muticus* (Edwards); (*C*) *P. illiesi* Brundin; *(D) P. evansi* Brundin; *(E) P. andinus* Brundin; *(F) P. torrentium* Brundin; *(G) P. brevipalpis* Brundin; (*H) P. discoceros* Brundin.

Abdominal morphology (*I) P. andinus* Brundin; (*J*) *P. discoceros* Brundin. (*K) P. evansi*; (*L*) *P. illiesi* (LSVIII attenuated*).* B,C, E-G, I,L after Brundin (1966), A,D,H,J,K after Cranston (1966).

Fig. 5. Larva of *Podonomopsis* Brundin. *(A)* Dorsal head of *P. illiesi* Brundin; *(B)* Antenna of *P. discoceros* Brundin; *(C)* Labrum (lateral) of *P. illiesi*; *(D)* Mandible of *P. evansi* Brundin; *(E)* Mentum of *P. evansi*; *(F)* mentum, ventral labrum and maxilla of *P. illiesi*; *(G)* Procercus of *P. evansi*; *(H)* Procercus of *P. evansi* (containing pharate pupal setae); (*I*) dorsal posterior segments of *P. illiesi.* A, C, F, H, I after Brundin (1966), B, D, E, G after Cranston (1966).

Fig. 6. Adult male of *Podonomopsis* (*Araucanopsis*) *avelasse* sp. n. *(A)* Palp; (*B*) Antennal apex; (*C*) Hypopygium.

Fig. 7. Pupa of *Podonomopsis* (*Araucanopsis*) *avelasse* sp. n. *(A)* Pupal abdomen, dorsal, L. – Tergites I-IV, R. tergites V-anal lobe; *(B)* Terminal segments, lateral; *(C)* Terminal segments, dorsal; *(D)* Thoracic horn.

Fig. 8. Pupa of *Podonomopsis* (*Araucanopsis*) *avelasse* sp. n. *(A)* Cephalic area; (B) Thoracic horn; *(C)* Posterior abdomen; (*D*) Tergites VIII, IX and genital sheath of ♂ (L side only). B, C, after Brundin, 1966.

Fig. 9. Larvae of *Podonomopsis* (*Araucanopsis*) *avelasse* sp. n. *(A).* Antenna (L. apex, R. complete); *(B)* Mandible; *(C)* Anterior body 4th instar larva; *(D)* Hypopharynx; *(E)* Posterior parapod; *(F)* Procercus; *(G)* Mentum.

Tables (2 separate files)

Table 1. List of taxa, codes, life-stage, locations, GenBank accessions

Table 2. BEAST dates for selected nodes (see Fig. 2).