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Phylogeny of the Ecnomidae (Insecta: Trichoptera)

Kjell A. Johanson[a,*] and Marianne Espeland[a,b]

[a]*Department of Entomology, Swedish Museum of Natural History, PO Box 50007, SE-10405 Stockholm, Sweden;* [b]*Zoological Institute, University of Stockholm, SE-10691 Stockholm, Sweden*

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Abstract

Ecnomidae are a family of seven previously accepted extant genera having a typical Gondwanan distribution, except one genus (*Ecnomus*) being widely distributed also in the Oriental and Palearctic regions. We analysed a molecular data set of 3379 characters representing the sum of four different protein-coding genes (COI, CAD, EF-1a and POL-II). Six equally most parsimonious trees were generated from the combined data set, distributed into two distinct islands. In all maximum parsimony (MP) trees the Ecnomidae is monophyletic when the genus *Zelandoptila* (Psychomyiidae) is included. The sister group to Ecnomidae including *Zelandoptila* is *Pseudoneureclipsis*, previously classified in the other families. This sister-group relationship contradicts earlier findings that the Polycentropodidae are the sister group to Ecnomidae. A Bayesian analysis resulted in a monophyletic Ecnomidae when accepting inclusion of *Pseudoneureclipsis*, which contradicts the results from the MP analysis by leaving *Zelandoptila* as the sister group to Ecnomidae including *Pseudoneureclipsis*. In the majority rule tree from this analysis Polycentropodidae form the sister group to Ecnomidae. We were not able to obtain a monophyletic *Ecnomus* due to the inclusion of *Psychomyillodes*. We conclude that the genus *Zelandoptila* or *Pseudoneureclipsis* probably belongs to the Ecnomidae, and that *Psychomyiellodes* and *Ecnomus* are synonyms. Three additional, as yet undescribed monotypic genera from Australia and New Caledonia remain to be erected in Ecnomidae.

Taxonomy

The family Ecnomidae comprises about 375 described extant species in seven genera. In addition, Navás (1934) described the monotypic genus *Chilocentropus* based on an adult of unknown gender and that is now considered lost. The genus, originally described within the Polycentropodidae and transferred to the Ecnomidae by Flint et al. (1999), is presently considered *nomen dubium* (Holzenthal et al., 2007b). The family was first mentioned in the literature as the subfamily Ecnominae Ulmer, 1903 within Hydropsychidae. It was subsequently transferred to the Polycentropodidae by Ulmer (1907) and to the Psychomyiidae by Ulmer (1910a) before it was elevated to family rank by Lepneva (1970).

In addition to seven extant genera the family includes one genus, *Archaeotinodes* Ulmer, 1912, of exclusively extinct species. The monotypic type genus of the family, *Ecnomus* McLachlan, 1864, was first erected for the species *Philopotamus tenellus* Rambur, 1842 described from France. The genus is now the largest in the family based on number of species, and is represented in all faunal regions except in the New World. Of the about 275 species in the genus 68 have been described from the Afrotropical Region, 61 from Oceania, 135 from the Oriental Region, and 15 species from the Palearctic Region. The genus *Psychomyiellodes* Mosely, 1931 is endemic to the Afrotropical Region and includes eight species. It was first described as a monotypic genus within Psychomyiidae based on *P. ungulata* from Sierra Leone and separated from other psychomyiid genera by possessing many non-genitalic character states not present in *Ecnomus*, e.g. a uniquely modified posterior apical spur of the hind legs (arrow in Fig. 1) resembling

*Corresponding author:
E-mail address: kjell.arne.johanson@nrm.se

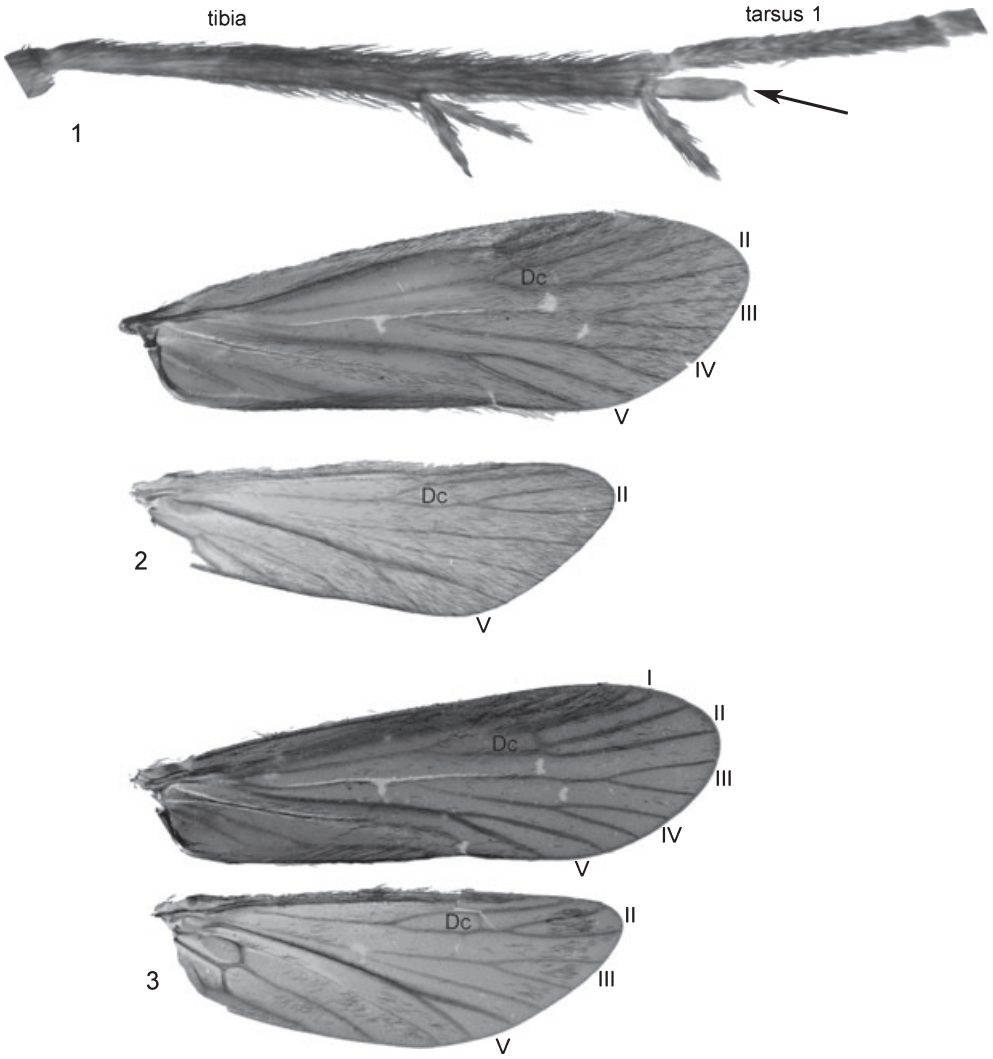

Fig. 1. *Psychomyiellodes excavatus*, hind leg with modified tibial spur (arrow).
Fig. 2. *Agmina parie*, right wings.
Fig. 3. *Parecnomina hamata*, right wings. Roman numbers refer to venation fork numbers; Dc, discoidal cell.

that in *Dipseudopsis* species (Dipseudopsidae). The genus *Ecnomina* Kimmins, 1953 was erected to accommodate the three Australian species in the ecnomines having no Fork 1 (as in Fig. 2) in the forewings and presence of both Dc and Fork 3 in the hind wings (Fig. 3). This genus now comprises 11 species and has previously been recorded also from New Zealand (Wise, 1958) and New Caledonia (Ward and Schefter, 2000). The genus *Austrotinodes* Schmid, 1955 was described to include the two species *A. latior* Schmid, 1955 and *A. angustior* Schmid, 1955 from Chile, the former subsequently synonymized with *A. talcanus* (Navás, 1934). The genus was established due to its "primitive" wing venation compared with that seen in *Tinodes* (Psychomyiidae), the genus to which it was considered most similar. About 35 species have been described in *Austrotinodes* and it has been recorded from most parts of the Neotropical region as far north as Mexico (Flint and Denning, 1989). The genus *Parecnomina* Kimmins, 1957 is widely recorded from the Afrotropical region, and includes seven described species. It was considered distinct from related Afrotropical genera based on two character states in the hind wings, i.e. presence of Dc and Fork 3 (Fig. 3). Ward and Schefter (2000) described the New Caledonian endemic genus *Agmina* to accommodate 19 novel New Caledonian ecnomid species that they characterized by four proposed synapomorphies, i.e. absence of nygma in Fork 2 of the forewings, absence of Fork 3 in the hind wings (Fig. 2), presence of sternal processes on the abdominal segment IX, and the

presence of phallic struts. Eight more species were described in the genus by Ward (2003). Neboiss (2002) separated the Australian ecnomid genus *Ecnomina sensu lato* (*s.l.*) into *Ecnomina sensu stricto* (*s.s.*) and a new genus *Daternomina*, based on states of four characters in the wings (forewing Fork 2 without nygma; forewing Fork 3 long, with short stalk; and narrow hind wings with almost straight anal veins) and one character in the female genitalia (sternite VIII forming an elongate meso-ventral lobe). Based on Australian material, Cartwright (2008) revised the genera *Ecnomina* and *Daternomina* and listed 16 species in *Daternomina* and 36 in *Ecnomina* but was not able to distinguish these genera morphologically based on male characters. He also indicated that "*Ecnomina*" *krokale* belongs to a new genus under description.

Phylogenetic analyses

The first hypothesis regarding the relationship between the Ecnomidae genera was presented by Flint (1973) who, based on few morphological characters, grouped *Psychomyiellodes* and *Ecnomus* as sister groups and *Parecnomina* as the sister group to (*Psychomyiellodes* + *Ecnomus*). These three genera formed the sister group to (*Ecnomina* + *Austrotinodes*).

Almost 25 years later, Li and Morse (1997) performed a parsimony analysis of the Ecnomidae genera, excluding *Agmina* and *Daternomina*, based on 13 morphological characters with Hydropsychidae and Polycentropodidae as outgroups. Their analysis generated three most parsimonious trees before successive weighting (*sensu* Farris, 1969). A single most parsimonious tree was recovered after successive weighting based on the rescaled consistency indices of each character. Their tree is shown in Fig. 4A. After analysing their character matrix by identical methods we found eight most parsimonious trees of 17 steps before successive weighting, and three trees were retained after successive weighting (Fig. 4B), one of which was identical to the one in Li and Morse (1997), and another of which left Ecnomidae paraphyletic as Polycentropodidae were included.

Holzenthal et al. (2007a) included the two ecnomid genera *Ecnomus* and *Austrotinodes* in their analysis of the relationship between Trichoptera families. Their Bayesian analysis recovered a monophyletic Ecnomidae with 100% posterior probability, but the monophyly of two included *Ecnomus* species was not supported by high posterior probabilities.

The current study aims to evaluate the validity of Ecnomidae because most of the genera in this family were generally vaguely defined and not always based on the presence of derived character states. In addition, due to the fact that previous phylogenetic analyses excluded many genera in the family, a new analysis with all genera included and broad character sampling is required in order to provide a more updated hypothesis of generic relationships, and to provide a more rigorous test of the monophyly of existing genera.

Material and methods

Sampling and specimens

Our analysis was based on recently collected specimens, and included most biogeographical areas. One genus, *Ecnomus*, was weakly represented by six described and 12 undescribed species out of about 275 previously described species (5%). These 18 species represent the genus as they cover the biogeographical regions where the genus exists, and a high diversity in morphology

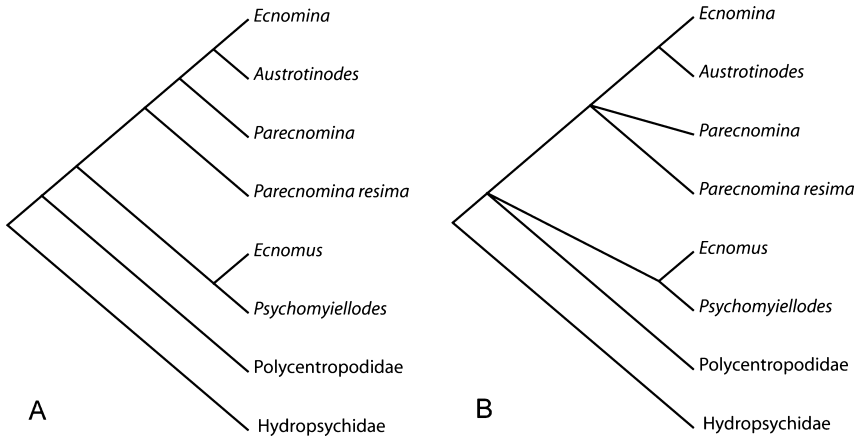

Fig. 4. Phylogeny of Ecnomidae based on morphological data provided by Li and Morse (1997): (A) the original shortest tree after successive reweighting as published by Li and Morse (1997); (B) strict consensus tree of three most parsimonious trees after successive reweighting following the same procedure as by Li and Morse (1997).

found in the genus. Fresh material of other species was unavailable for this study. "*Ecnomina*" *krokale* was included to test the hypothesis of Cartwright (2008) that it belongs to an undescribed genus. All included specimens were collected in the years 2000–2006 (Table 1) and stored in 80% alcohol at −20 °C. Vouchers and DNA extractions are deposited at the Entomology Department, Swedish Museum of Natural History (NHRS). DNA was extracted from the abdomen in adult specimens or the right hind leg in larvae, using the Qiagen DNeasy Tissue Extraction Kit (Qiagen Inc., Valencia, CA, USA) and gene regions were amplified using the polymerase chain reaction (PCR). Samples from described and undescribed species of Ecnomidae were included in the analysis. The putatively closely related families Polycentropodidae, Dipseudopsidae, Psychomyiidae, Hydropsychidae, and Philopotamidae (Kjer et al., 2001) were included as potential sister taxa to the Ecnomidae. The distantly related *Moruya charadra* (Hydrobiosidae) was chosen to represent the outgroup.

Molecular methods

Fragments of four protein-coding genes were sequenced: the mitochondrial gene cytochrome oxidase I (COI) (658 bp), and the nuclear elongation factor-1α (EF-1α) (1099 bp), RNA polymerase-II (POL-II) (772 bp), and CAD (850 bp). The genes COI and EF-1α have successfully been used for phylogenetic analyses of Trichoptera (Kjer et al., 2001, 2002; Holzenthal et al., 2007a; Johanson, 2007; Espeland et al., 2008; Johanson and Keijsner, 2008; Malm and Johanson, 2008; Johanson et al., in press). Fragments of the CAD and POL-II genes have not previously been used for phylogenetic analyses within Trichoptera but CAD was considered a useful gene for recovering both deep and shallow divergences in beetles while POL-II performed well for shallow recoveries and poorly for deep recoveries (Wild and Maddison, 2008). Within Apiformes (Hymenoptera) the CAD gene fragment was considered more informative than the POL-II gene fragment (Danforth et al., 2006). The primers used in this analysis are listed in Table 2. Sequences were complete for all taxa in the POL-II partition. In the CAD partition the sequence of "*Ecnomina*" *krokale* (EK3) starts at bp 25, and the sequence of taxon *Ecnomus* sp. 8 stops 12 bp before the end compared with sequences from the other taxa. In the partition EF-1α taxon *Ecnomina* sp. 1 lacks the first 7 bp. In the COI partition *Ecnomus* sp. 9 lacks the first 6 bp. In addition, individual *Hydrobiosella uncinata*, dipseudopsid genus indet sp. 1 and *Tinodes* sp. (EW2) lack sequences for CAD, and *Parecnomina hamata* lacks sequences for POL-II.

Loci were amplified using Hot-start RTG PCR Beads (GE Healthcare Lifesciences, Piscataway, NJ, USA). Each 25-μL reaction contained 1 μL of 10 μM primer pair mix (× 2), 2 μL of template and 21 μL of water. Reaction mixtures were heated to 95 °C for 5 min, followed by 40 cycles of 30 s at 95 °C, 30 s at a specific annealing temperature, and 50 s at 72 °C, and then a final extension of 8 min at 72 °C. Annealing temperature was set to 56 and 52 °C for EF-1α (first and second half, respectively) and to 50–52 °C for COI, CAD, and POL-II. PCR products were visualized by ultraviolet light on a 0.8% agarose gel after electrophoresis and were purified using EXOSAP (GE Healthcare Lifesciences). Gene regions were sequenced with the same primers as in the PCRs using the BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Each sequencing reaction mixture, including 1 μL BigDye (Applied Biosystems), 1 μL of 1.6 μM primer and 2–3 μL of DNA template, ran for 96 °C (1 min) and then 25 cycles of 96 °C (30 s), 50 °C (15 s), and 60 °C (4 min). Sequencing reactions were purified using the DyeEx 96 kit (Qiagen Inc.,) and cycle sequencing reactions were run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Raw sequence data and contigs were viewed and assembled using the Pregap4 and Gap4 modules of the Staden package (Staden et al., 1998). Forward and reverse primers were used to sequence each region in both directions. EF-1α was amplified in two overlapping regions. Primer sequences were removed from the beginning of each sequence and sequence data were checked for accuracy by matching forward and reverse sequences for each gene region. Sequences of individual genes were aligned separately using the L-INS-I strategy in MAFFT v. 6 (Katoh et al., 2005) and a combined character matrix was produced in MacClade 4.0.8 (Maddison and Maddison, 2005). The COI partition included all 75 taxa and 353 parsimony-informative characters. *Dipseudopsis africana* and *Protodipseudopsis* sp. 1 (both Dipseudopsidae) lacked 3 bases at the same site in their sequences (bases 472–474). The EF-1α partition included 75 taxa and 381 parsimony-informative characters. The CAD partition included 72 taxa and 409 parsimony-informative characters. The POL-II partition included 74 taxa and 278 parsimony-informative characters. The combined matrix included 75 taxa and 3379 characters, 1421 of which were parsimony informative (42.0%).

Phylogenetic reconstruction

Due to absence of sequences in a few taxa for some partitions, the combined dataset had 3.0% missing data. It was analysed for the four genes separately and combined following two different procedures. An equally weighted maximum parsimony (MP) analysis was performed with PAUP*4.0b10 (Swofford, 1999) and TNT version 1.1 (Goloboff et al., 2006). In PAUP* the heuristic searches were performed using 10 000 random

Table 1
List of specimens with voucher codes, locality information, and GenBank accession number

Family	Species	Area	NHRS voucher	GenBank accession number			
				COI	EF-1α	POL-II	CAD
Ecnomidae	*Ecnomus continentalis*	AUS	EG9	FN179079	FN178857	FN178745	FN178969
Ecnomidae	*Ecnomus continentalis*	AUS	EK2	FN179095	FN178873	FN178760	FN178985
Ecnomidae	*Ecnomus tillyardi*	AUS	EH6	FN179083	FN178861	FN178749	FN178973
Ecnomidae	*Ecnomus cygnitus*	AUS	EH5	FN179082	FN178860	FN178748	FN178972
Ecnomidae	*Ecnomus atratus*	Vanuatu	EL8	FN179109	FN178887	FN178774	FN178999
Ecnomidae	*Ecnomus similis*	Ghana	EJ7	FN179092	FN178870	FN178758	FN178982
Ecnomidae	*Ecnomus tenellus*	Sweden	FJ1	FN179146	FN178925	FN178812	FN179035
Ecnomidae	*Ecnomus* sp. 1	AUS	EI2	FN179085	FN178863	FN178751	FN178975
Ecnomidae	*Ecnomus* sp. 2	AUS	EL5	FN179106	FN178884	FN178771	FN178996
Ecnomidae	*Ecnomus* sp. 3	AUS	EL1	FN179102	FN178880	FN178767	FN178992
Ecnomidae	*Ecnomus* sp. 4	AUS	EK7	FN179101	FN178879	FN178766	FN178991
Ecnomidae	*Ecnomus* sp. 5	AUS	EL2	FN179103	FN178881	FN178768	FN178993
Ecnomidae	*Ecnomus* sp. 6	AUS	EL3	FN179104	FN178882	FN178769	FN178994
Ecnomidae	*Ecnomus* sp. 7	AUS	EL4	FN179105	FN178883	FN178770	FN178995
Ecnomidae	*Ecnomus* sp. 8	Ghana	EJ4	FN179091	FN178869	FN178757	FN178981
Ecnomidae	*Ecnomus* sp. 9	Ghana	EJ9	FN179093	FN178871	FN178759	FN178983
Ecnomidae	*Ecnomus* sp. 10	Malaysia	GM4	FN391557	FN391564	FN391571	FN391578
Ecnomidae	*Ecnomus* sp. 11	Laos	GM5	FN391558	FN391565	FN391572	FN391579
Ecnomidae	*Ecnomus* sp. 12	Laos	GM3	FN391556	FN391563	FN391570	FN391577
Ecnomidae	*Psychomyiellodes excavatus*	Ghana	EJ3	FN179090	FN178868	FN178756	FN178980
Ecnomidae	*Psychomyiellodes* sp. 1	Ghana	EJ2	FN179089	FN178867	FN178755	FN178979
Ecnomidae	*Parecnomina hamata*	Ghana	EK1	FN179094	FN178872	–	FN178984
Ecnomidae	*Daternomina* sp. 1	AUS	EH2	FN179080	FN178858	FN178746	FN178970
Ecnomidae	*Daternomina* sp. 1	AUS	EK5	FN179098	FN178876	FN178763	FN178988
Ecnomidae	*Daternomina* sp. 2	AUS	EL7	FN179108	FN178886	FN178773	FN178998
Ecnomidae	*Daternomina* sp. 2	AUS	EP8	FN179129	FN178907	FN178794	FN179019
Ecnomidae	*Daternomina* sp. 3	AUS	GM7	FN391560	FN391567	FN391574	FN391581
Ecnomidae	*Daternomina* sp. 4	AUS	GM8	FN391561	FN391568	FN391575	FN391582
Ecnomidae	"*Ecnomina*" *krokale*	AUS	EH3	FN179081	FN178859	FN178747	FN178971
Ecnomidae	"*Ecnomina*" *krokale*	AUS	EK3	FN179096	FN178874	FN178761	FN178986
Ecnomidae	"*Ecnomina*" *krokale*	AUS	GM6	FN391559	FN391566	FN391573	FN391580
Ecnomidae	*Ecnomina thinotes*	AUS	EP2	FN179123	FN178901	FN178788	FN179013
Ecnomidae	*Ecnomina thinotes*	AUS	EP3	FN179124	FN178902	FN178789	FN179014
Ecnomidae	*Ecnomina* sp. 3	AUS	EK6	FN179099	FN178877	FN178764	FN178989
Ecnomidae	*Ecnomina* sp. 3	AUS	EO9	FN179121	FN178899	FN178786	FN179011
Ecnomidae	*Ecnomina* sp. 3	AUS	EK4	FN179097	FN178875	FN178762	FN178987
Ecnomidae	*Ecnomina* sp. 4	AUS	EP4	FN179125	FN178903	FN178790	FN179015
Ecnomidae	*Ecnomina* sp. 5	AUS	EK8	FN179100	FN178878	FN178765	FN178990
Ecnomidae	*Ecnomina* sp. 6	AUS	EP1	FN179122	FN178900	FN178787	FN179012
Ecnomidae	*Ecnomina* sp. 6	AUS	EP6	FN179127	FN178905	FN178792	FN179017
Ecnomidae	*Ecnomina* sp. 6	AUS	EL6	FN179107	FN178885	FN178772	FN178997
Ecnomidae	*Ecnomina* sp. 6	AUS	EP7	FN179128	FN178906	FN178793	FN179018
Ecnomidae	*Agmina parie*	NC	EM8	FN179116	FN178894	FN178781	FN179006
Ecnomidae	*Agmina parie*	NC	EO7	FN179119	FN178897	FN178784	FN179009
Ecnomidae	*Agmina diriwi*	NC	EM6	FN179114	FN178892	FN178779	FN179004
Ecnomidae	*Agmina mariae*	NC	EO8	FN179120	FN178898	FN178785	FN179010
Ecnomidae	*Agmina hastata*	NC	FM8	FN391555	FN391562	FN391569	FN391576
Ecnomidae	*Agmina* sp. 1	NC	EM9	FN179117	FN178895	FN178782	FN179007
Ecnomidae	*Austrotinodes triangularis*	Chile	EM1	FN179111	FN178889	FN178776	FN179001
Ecnomidae	*Austrotinodes recta*	Chile	EM3	FN179113	FN178891	FN178778	FN179003
Ecnomidae	*Austrotinodes* sp. 1	Chile	EM2	FN179112	FN178890	FN178777	FN179002
Ecnomidae	*Austrotinodes* sp. 2	Chile	EL9	FN179110	FN178888	FN178775	FN179000
Ecnomidae	Gen. nov. 1 sp. nov. 1	AUS	EH8	FN179084	FN178862	FN178750	FN178974
Ecnomidae	Gen. nov. 2 sp. nov. 1	NC	EM7	FN179115	FN178893	FN178780	FN179005
Dipseudopsidae	*Peudoneureclipsis halongensis*	Laos	FR5	FN179147	FN178926	FN178813	FN179036
Dipseudopsidae	*Dipseudopsis africana*	Ghana	EI5	FN179086	FN178864	FN178752	FN178976
Dipseudopsidae	*Protodipseudopsis* sp. 1	Ghana	EI8	FN179087	FN178865	FN178753	FN178977
Dipseudopsidae	Genus indet. sp. 1	Vietnam	EW1	FN179141	FN178920	FN178807	–
Psychomyiidae	*Zelandoptila moselyi*	NZ	BR2	FN179045	FN178822	FN178711	FN178936
Psychomyiidae	*Lype phaeopa*	Germany	ED1	FN179077	FN178855	FN178743	FN178967
Psychomyiidae	*Tinodes waeneri*	Corsica	CC6	FN179050	FN178828	FN178716	FN178940

Table 1
(*Continued*)

Family	Species	Area	NHRS voucher	GenBank accession number			
				COI	EF-1α	POL-II	CAD
Psychomyiidae	*Tinodes* sp.	Vietnam	EW2	FN179142	FN178921	FN178808	–
Psychomyiidae	*Tinodes* sp.	Vietnam	EW3	FN179143	FN178922	FN178809	FN179032
Hydropsychidae	*Aoteapsyche philpotti*	NZ	BO8	FN179044	FN178822	FN178710	FN178935
Hydropsychidae	*Caledopsyche phallaina*	NC	CJ2	FN179068	FN178846	FN178734	FN178958
Hydropsychidae	*Asmicridea edwardsii*	AUS	EO5	FN179118	FN178896	FN178783	FN179008
Hydropsychidae	*Synoestropsis grisoli*	FRG	EE3	FN179078	FN178856	FN178744	FN178968
Hydropsychidae	*Pseudoleptonema quinquefasciata*	Laos	FT8	FN179148	FN178927	FN178814	FN179037
Hydropsychidae	*Polymorphanisus similis*	Ghana	EI9	FN179088	FN178866	FN178754	FN178978
Polycentropodidae	*Neureclipsis bimaculata*	Sweden	E2	FN179039	FN178816	FN178704	FN178929
Polycentropodidae	*Polycentropus irroratus*	Norway	AD7	FN179038	FN178815	FN178703	FN178928
Polycentropodidae	*Polyplectropus* sp. 1	NC	G8	FN179040	FN178817	FN178705	FN178930
Polycentropodidae	*Polyplectropus* sp. 2	NC	I2	FN179041	FN178818	FN178706	FN178931
Philopotamidae	*Hydrobiosella uncinata*	NC	BX2	FN179047	FN178825	FN178713	–
Hydrobiosidae	*Moruya charadra*	AUS	DC6	FN179076	FN178854	FN178742	FN178966

Table 2
Primers used

Gene	Primer	Primer sequence (5′–3′)	Source
COI	HCO2198 (forward)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
	LCO1490 (reverse)	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
CAD	1028r (reverse)	TTRTTNGGNARYTGNCCNCCCAT	Wahlberg and Wheat (2008)
	743nF (forward)	GGNGTNACNACNGCNTGYTTYGARCC	Wahlberg and Wheat (2008)
EF-1a	Lepto-IF (forward)	TTCGTNCCNATCTCAGGNTGGC	Johanson and Malm (in press)
	aF (forward)	ATCGAGAAGTTCGAGAARGARGC	Kjer et al. (2001)
	aIntR (reverse)	CCAYCCCTTGAACCANGGCAT	Malm and Johanson (2008)
	aR (reverse)	GGGAAYTCCTGGAARGAYTC	Kjer et al. (2001)
	M46.1 (forward)	GAGGAAATYAARAAGGAG	Whiting (2002)
POL-II	POLFOR2 (forward)	TGGGAYGSYAAAATGCCKCAACC	Modified from Danforth et al. (2006)
	POLREV2 (reverse)	TYYACAGCAGTATCRATRAGACCTTC	Modified from Danforth et al. (2006)
	LeptoF-ino (forward)	TRAARCCIAARCCIYTITGGAC	Johanson and Malm (in press)

addition sequence replicates with tree bisection–reconnection (TBR) branch swapping, gaps treated as missing, and branches collapsed if maximum branch length was zero. The analysis in TNT was performed with 50 random addition sequences followed by 10 000 iterations using the ratchet algorithm implemented by the following command string: "mult 50; rat = iter 10 000". Gaps were treated as missing data in all analyses. Jackknife values (Farris et al., 1996) were generated in TNT for evaluating nodal support using 1000 replicate searches and removal percentage set to 37%.

To evaluate group stability, a Bayesian analysis was conducted for the combined dataset. The sequences of the four genes were each separated into codon partitions and the best model of substitution for the 12 individual partitions (Table 3) were calculated in MrModeltest 2.2 (Nylander, 2004) and executed in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Analyses were performed with random starting trees without constraints. Two Markov chains were run simultaneously for 5 000 000 generations with sampling every 100 generations to ensure independence of samples. The first 8031 sampled trees were discarded as burn-in as standard deviation of split frequencies between the two runs was higher than 0.010 and following examination of the p-files in the

Table 3
Partitions, number of nucleotides, and models of nucleotide substitutions used in the Bayesian analysis for individual partitions

Partition	No. of nucleotides	Model
CAD codon 1	283	GTR + I + G
CAD codon 2	283	GTR + I + G
CAD codon 3	284	GTR + I + G
COI codon 1	219	GTR + I + G
COI codon 2	219	GTR + I + G
COI codon 3	220	GTR + G
EF-1α codon 1	366	GTR + I + G
EF-1α codon 2	366	GTR + I
EF-1α codon 3	367	GTR + I + G
POL-II codon 1	257	GTR + G
POL-II codon 2	257	HKY + I
POL-II codon 3	258	GTR + I + G

program Tracer 1.4 (Rambaut and Drummond, 2005). Groups receiving high support (≥ 95%) in both the Jackknife and Bayesian analyses were considered well supported.

Results

Monophyly of Ecnomidae

No MP of the partitioned and combined data recovered a monophyletic Ecnomidae, as *Zelandoptila* (Psychomyiidae) was included in the Ecnomidae in all analyses. In the 2000+ shortest MP trees based on the CAD gene sequences the Hydropsychidae, Psychomyiidae, Dipseudopsidae, and Polycentropodidae were also present in the Ecnomidae clade, and in all 17 shortest trees based on COI gene sequences alone, *Zelandoptila*, *Lype* (Psychomyiidae), Dipseudopsidae, and Polycentropodidae grouped together with Ecnomidae in a basal polytomy. In all 400 shortest trees from analysis of the EF-1α gene sequences and 33 shortest trees from analysis of the POL-II gene sequences Ecnomidae was monophyletic when *Zelandoptila* was included. MP analysis of the combined data set revealed six shortest trees (L = 13552, CI = 0.2105, RI = 0.5918, RC = 0.1246) in two equally large islands (Figs 5 and 6). In both islands the closest sister taxon to Ecnomidae with *Zelandoptila* is *Pseudoneureclipsis*.

Jackknife analysis of the combined data set produced a tree with monophyly of Ecnomidae including *Zelandoptila* having 93% support, and *Pseudoneureclispsis* as the sister taxon to Ecnomidae including *Zelandoptila*, with 97% support. No sister-group alternative to Ecnomidae + *Zelandoptila* + *Pseudoneureclipsis* received Jackknife support.

The majority rule consensus tree from the combined Bayesian analysis (Fig. 7) included both *Zelandoptila* and *Pseudoneureclipsis* in the Ecnomidae as in the MP analysis of EF-1α gene partition. The monophyly of this group is supported by 100% posterior probability. The following ten taxa were accepted as monophyletic ecnomid genera in the Bayesian analyses: *Agmina*, *Austrotinodes*, *Daternomina*, *Ecnomina*, "*Ecnomina*" *krokale*, *Ecnomus*, Gen. nov. 1, Gen. nov. 2, *Parecnomina*, and *Zelandoptila*. The Polycentropodidae form a monophyletic unit with 100% posterior probability and represent the sister group to Ecnomidae including *Zelandoptila* with *Pseudoneureclipsis* with 100% posterior probability.

Relationships among Ecnomidae genera

Except for *Ecnomus*, all proposed genera form monophyletic groups in all combined and partitioned analyses, except in the separate MP analysis of COI gene partition that leave *Agmina* in an unresolved basal polytomy. Within genera, almost all species group identically, including the two *Psychomyiellodes* species that always group together well inside *Ecnomus*. MP analysis of the combined data set retains four large monophyletic groups being identical in the two islands: (*Austrotinodes* + Gen. nov. 1); (*Agmina* + "*Ecnomina*" *krokale* + *Ecnomina* + Gen. nov. 2); (*Parecnomina* + *Ecnomus/Psychomyiellodes*); and (*Agmina* + "*Ecnomina*" *krokale* + *Ecnomina* + Gen. nov. 2 + *Zelandoptila*). The relationship between these groups differs in the two islands (Figs 5 and 6). The first three of these four groups are intact in the Bayesian analysis (Fig. 7) while the latter group is absent due to a topology with *Zelandoptila* near the Ecnomidae root.

Another major difference between the trees from the MP and Bayesian analyses is the topology of *Daternomina* and *Austrotinodes* + Gen. nov. 1, as is also reflected in the Jackknife indices (Figs 5 and 6) forming a polytomy with other genus groups. In the MP trees these genera are associated with the *Ecnomina* + *Agmina* + Gen. nov. 2 + "*Ecnomus*" *krokale* group, while in the Bayesian consensus trees they are associated with *Ecnomus/Psychomyiellodes* + *Parecnomina*.

The Chilean species of *Austrotinodes* form a monophyletic group that is sister group to a single species, Gen. nov. 1 sp. 1 from Australia. The group receives 100% posterior probability support from the Bayesian analysis and 98% Jackknife support. This configuration is also maintained in the MP POL-II and CAD partition trees. In the COI partition relationships among these taxa are uncertain. In the EF-1α partition these taxa form a paraphyletic group.

The group *Agmina* + "*Ecnomina*" *krokale* + *Ecnomina* + Gen. nov. 2 is supported with 100% posterior probability from the Bayesian analysis and strong Jackknife support. The topology among these four genera varies in the two MP tree islands and the consensus tree from the Bayesian analysis, which is reflected in weak support from the Jackknife analysis and low posterior probabilities from the Bayesian analysis.

The *Ecnomus/Psychomyiellodes* + *Parecnomina* group is monophyletic with weak support from the Jackknife analysis and 100% posterior probability from the Bayesian analysis. In the MP analysis the group forms a clade near the Ecnomidae root in island 1 (Fig. 5), and sister group to *Daternomina* in island 2 (Fig. 6) and in the Bayesian analysis (Fig. 7). The close relationship with *Daternomina* is supported by 100% posterior probability in the Bayesian analysis.

Zelandoptila groups with *Agmina* + "*Ecnomina*" *krokale* + *Ecnomina* + Gen. nov. 2 in the trees from the MP analysis with moderately high Jackknife support (87%), but forms a clade near the root in the Bayesian analysis. This basal topology is supported but with low posterior probability.

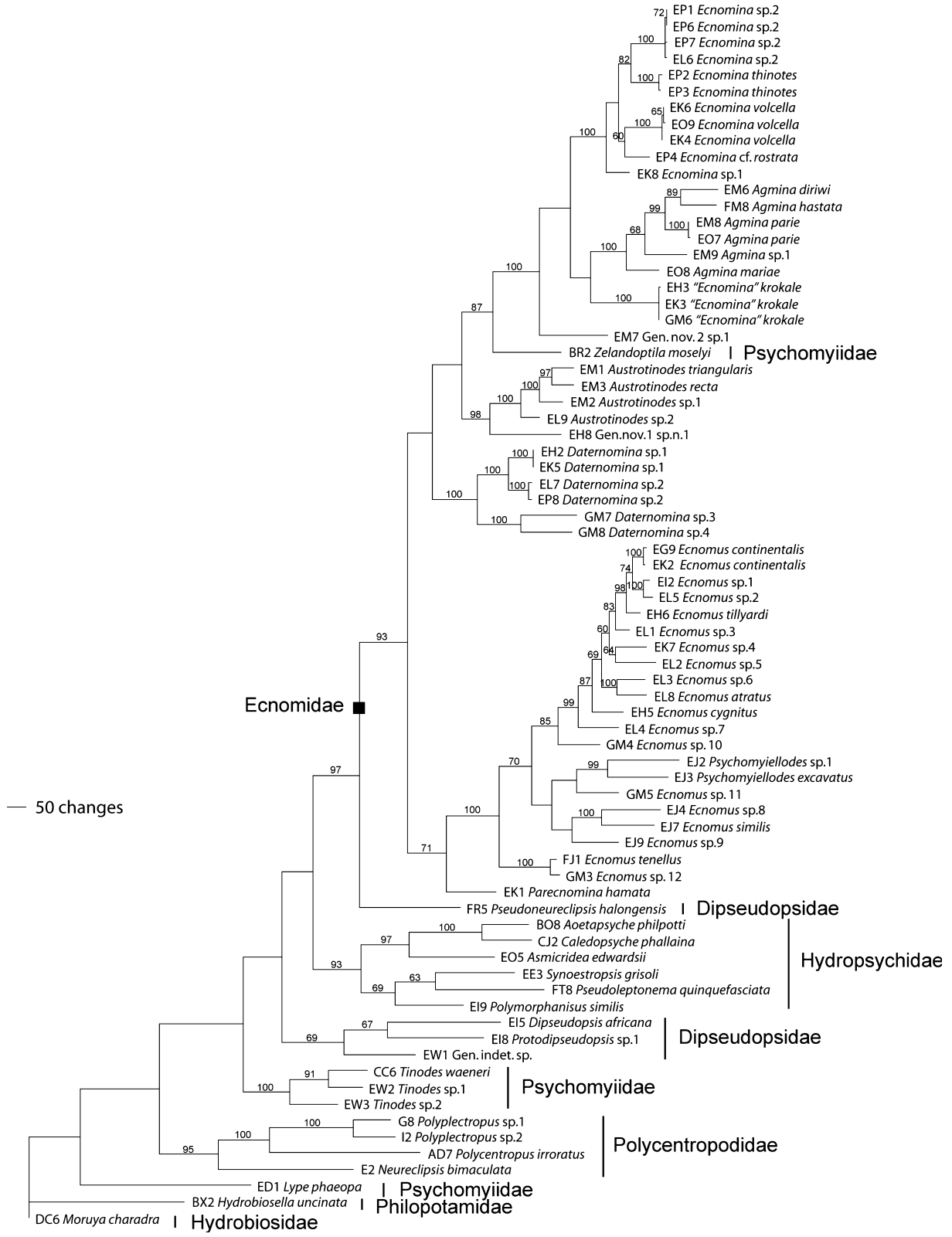

Fig. 5. Strict consensus of three most parsimonious trees in tree island 1 based on an unweighted combined data set of COI, EF-1α, CAD and POL-II sequences, generated in TNT 1.1 (Goloboff et al., 2006) and PAUP*4.0b10 (Swofford, 1999). Jackknife indices are given above branches based on 1000 replicate searches and 37% re-sampling frequency.

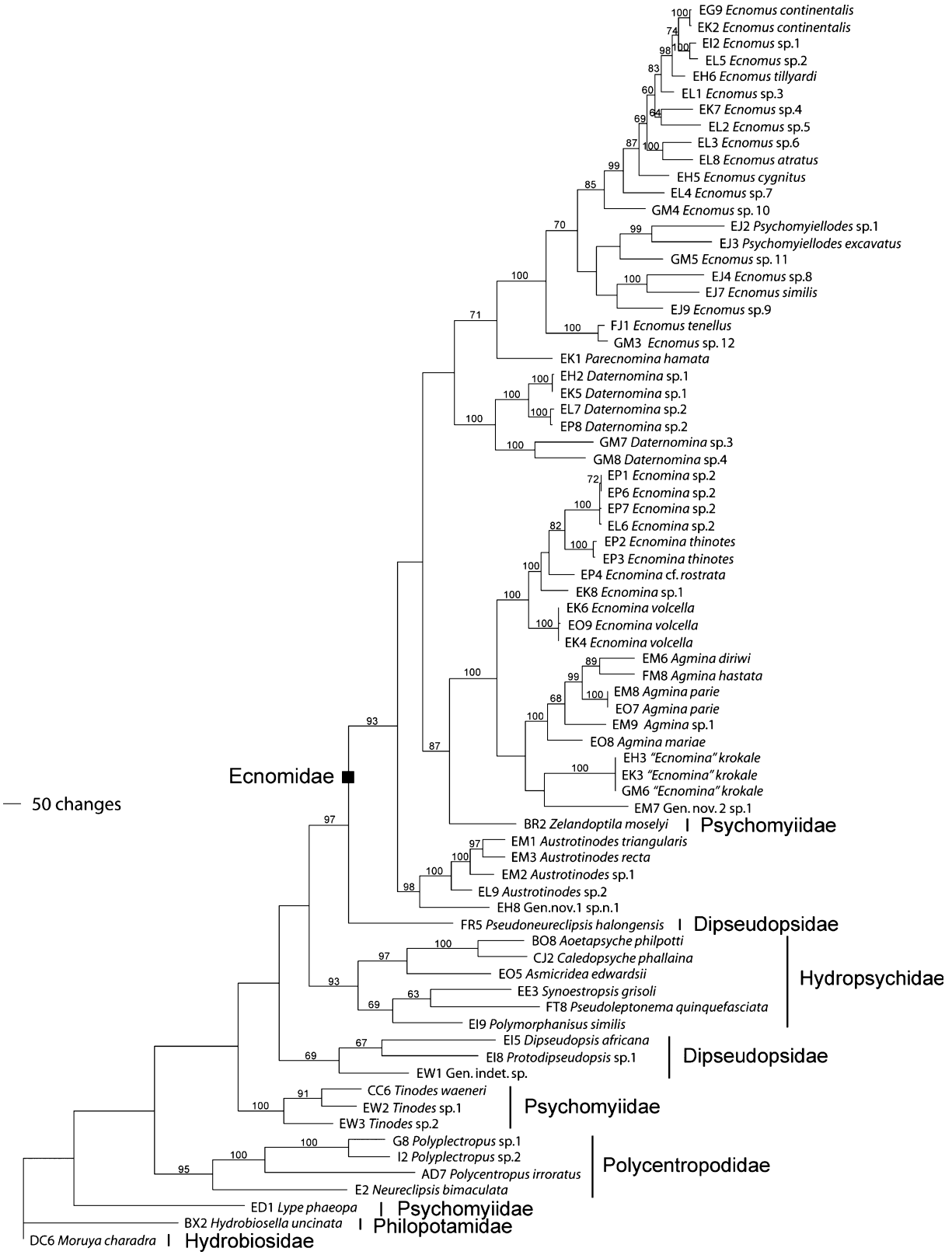

Fig. 6. Strict consensus of three most parsimonious trees in tree island 2 based on an unweighted combined data set of COI, EF-1α, CAD and POL-II sequences, generated in TNT 1.1 (Goloboff et al., 2006) and PAUP*4.0b10 (Swofford, 1999). Jackknife indices are given above branches based on 1000 replicate searches and 37% re-sampling frequency.

Fig. 7. Majority rule tree from 41 969 trees from the Bayesian analysis in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) of the combined data set of COI, EF-1α, CAD and POL-II sequences. Definition of partitions is given in the text. Numbers above each branch represent posterior probabilities.

Pseudoneureclipsis forms the sister group to Ecnomidae in the MP analysis, strongly supported in the Jackknife support consensus tree. The consensus tree from the Bayesian analysis groups this genus as sister group to *Austrotinodes* + Gen. nov. 1, a topology with 100% posterior probability.

Discussion and taxonomic implications

The genus *Daternomina* was established in 2002 to accommodate nine former Australian *Ecnomina* species characterized by possessing modified wing venation and female genitalia. Wing characters have traditionally been used to diagnose genera within Trichoptera. However, Johanson and Ward (2009) demonstrated that wing characters considered stable within a genus was strongly homoplastic among species in New Caledonian representatives of the genus *Polyplectropus* in the Polycentropodidae. Such characters are possibly less valuable than assumed for generic taxonomy in certain families, and the among-species stability of these wing characters is not fully outlined for ecnomids. Cartwright (2008) demonstrated that the wing characters assumed to separate *Daternomina* from *Ecnomina* varied among species in each of the genera, but the generic status of a modified *Daternomina* was retained due to the presence of stable character states in the female genitalia. Our study supports that *Daternomina* and *Ecnomina s.s.* are distantly related monophyletic groups. Cartwright (2008) also indicated that "*Ecnomina*" *krokale* is a separate, undescribed genus. Our study confirms that "*Ecnomina*" *krokale* belongs to a new genus and phylogenetically distinct from both *Ecnomina* and *Daternomina*.

The African monophyletic group *Psychomyiellodes* forms a clade within *Ecnomus* in all analyses. *Psychomyiellodes* species share unique morphological character states, such as presence of modified apical spur of the hind legs in males and laterad projecting lobes in the genitalia, and our results based on molecular data demonstrate that monophyly of these species is still maintained. However, as *Psychomyiellodes* species are well nested within a monophyletic *Ecnomus* it must be synonymized with *Ecnomus*.

This study supports that the Afrotropical endemic ecnomid genus *Parecnomina* is valid and forms the sister group to *Ecnomus*.

Both *Agmina* and *Austrotinodes* form separate monophyletic groups. We have not been able to determine the sister group to *Agmina* species within the (*Agmina* + "*Ecnomina*" *krokale* + *Ecnomina* + Gen. nov. 2) genus group.

The phylogenetic position of the New Zealand and Australian endemic genus *Zelandoptila* has been problematic. The genus was placed in different families in previous classifications, i.e. Hydroptilidae (Tillyard, 1924) and Psychomyiidae (McFarlane, 1964; Schmid, 1972), based on character states in the wings. Both wing shape and wing venation of the two species in the genus strongly resemble those found in members of Psychomyiidae genera, and not Ecnomidae. But as demonstrated for other Trichoptera groups, particularly in small species in groups inhabiting remote archipelagos/islands (Ulmer, 1910b; Marlier and Malicky, 1979; Johanson, 1997, 1998), the ancestral *Zelandoptila* might have developed reduced wing venation and modified the wing shape due to the reduction in body size. By accepting the MP approach, we recognize the genus as an ecnomid, a position that receives 100% Jackknife support. Alternatively, the Bayesian analysis places it as the sister group to Ecnomidae including *Pseudoneureclipsis* with 56% posterior probability.

The genus *Pseudoneureclipsis* was historically difficult to classify and was regarded as a Polycentropodidae (Ulmer, 1913, 1951; Gibbs, 1973; Malicky and Chantaramongkol, 1993), Psychomyiidae (Martynov, 1914, 1934), Hydropsychidae (Banks, 1916), or Dipseudopsidae (Li et al., 2001; Oláh and Johanson, in press). The results from analysing our molecular data show that *Pseudoneureclipsis* belongs to a clade that is separate from all of these families. If accepting the MP approach, it should be included in the Ecnomidae as the sister group to the remaining ecnomid genera, or in a distinct family, Pseudoneureclipsidae Ulmer, 1951, a position supported with high (97%) Jackknife support.

Further analyses with more genes and/or taxa are needed to establish the taxonomic status of both *Zelandoptila* and *Pseudoneureclipsis*.

Most of the initial Ecnomidae genera in the present study are restricted to the southern hemisphere, i.e. *Agmina*, *Daternomina*, *Ecnomina*, *Austrotinodes, Zelandoptila*, and *Parecnomina* and the three possibly new ecnomid genera. In addition, the widely distributed genus *Ecnomus* is also well represented in the southern continents. Thus, the family might form a group having originated in Gondwana.

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