

Molecular phylogeny of the Taeniapterini (Diptera: Micropezidae) using nuclear and mitochondrial DNA, with a reclassification of the genus *Taeniaptera* Macquart

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Abstract

DNA molecular data are used to generate a phylogeny for the micropezid subfamily Taeniapterinae. Thirty-two taeniapterine species were sampled, including 10 of the 20 New World genera recognized by Steyskal, as well as one genus formerly treated as a synonym of *Poecilotylus* Hennig (*Hemichaeta* Steyskal). Five species from the Micropezinae were included as outgroups. A total DNA dataset of 4705 bp, including mitochondrial genes (12S and cytochrome *c* oxidase I (COI)) and nuclear coding genes (*wingless* and CAD), was analysed using maximum parsimony and Bayesian inference. The genus *Taeniaptera* Macquart was found to be non-monophyletic with respect to the remainder of the Taeniapterini analysed here. *Taeniaptera* is restricted to the *Taeniaptera trivittata* Macquart species group, *Mitromyia* Cresson is resurrected to contain the *Taeniaptera grata* (Wulp) species group, and *Paragrallomyia* Hendel is resurrected to contain most species previously considered *Taeniaptera*. *Poecilotylus* is recognized as a paraphyletic group awaiting further research.

Keywords

Taeniapterinae; New World; Taxonomy; *Taeniaptera*; DNA; Maximum Parsimony; Bayesian Inference

Introduction

Most genera of Micropezidae are currently diagnosed using a few superficial characters, generally with a high level of variability and homoplasy. The tribe Taeniapterini, for example, is broken into genera based on little more than the shape of the palpus and the type of arista, with most species with a bare arista included in *Taeniaptera* Macquart (1835) on the basis of an “axe-shaped” palpus. These weak generic concepts need to be tested and either supported and clarified, or replaced by an alternative system of consistently diagnosable monophyletic taxa. We here use DNA data to reconsider the definition of the large genus *Taeniaptera* and related Taeniapterini.

Definition and diagnosis of Taeniapterinae and Taeniapterini

Taeniapterinae is the most diverse subfamily of Micropezidae, and has a global distribution (minus Antarctica), with the greatest diversity occurring in the New World tropics. The subfamily is considered monophyletic based on a suite of apomorphies (ocelli displaced forward; sternopleural (katepisternal) bristles in a dense vertical fan of bristle-like hairs; subscutellum vestigial, not convex; epandrium bilaterally compressed (male); and surstylus absent (male)) (McAlpine 1998). Taeniapterinae is usually divided into two tribes based on the length of cell cup and the length of vein CuA_2 relative to vein A_1+CuA_2 , with the short-celled genera (all Old World taxa and the following New World genera: *Mesoconius* Enderlein, 1922; *Zelatractodes* Enderlein, 1922; *Globopeza* Marshall 2005; *Metasphen* Frey, 1927; *Scipopus* Enderlein, 1922; *Pseudeurybata* Hennig, 1934; *Calosphen* Hennig, 1934; *Grallipeza* Rondani, 1850; *Planipeza* Marshall, 2008; *Cliobata* Enderlein, 1923; *Rainieria* Rondani, 1843; *Chaetotylus* Hendel, 1932; *Hoplocheiloma* Cresson, 1926) being placed in the Rainieriini of Cresson (1938) (=Grallipezini of Aczél 1951 and subsequent authors), and the exclusively New World long-celled genera (*Taeniaptera*; *Hemichaeta* Steyskal, 1968; *Parasphen* Enderlein, 1922; *Ptilosphen* Enderlein, 1922; *Tenthes* Cresson, 1930; *Grallomyia* Rondani, 1850; *Poecilotylus* Hennig, 1934; *Plocoscelus* Enderlein, 1922; *Cardiacephala* Macquart, 1843) placed in the Taeniapterini (Cresson 1938). We here consider the classification of the tribe Taeniapterini, with a focus on the large genus *Taeniaptera* and its close relatives. The New World Micropezidae have been studied by some of the leading dipterists of the 20th Century (Cresson 1938, Hennig 1934, 1935a,b, 1936, Aczél 1951, Steyskal 1967, 1968), yet several generic concepts are considered confluent and in need of redefinition; *Cliobata* and *Rainieria*; *Calosphen* and *Grallipeza*; *Pseudeurybata* and *Scipopus*; and, as will be discussed in this study, *Taeniaptera* and *Poecilotylus* (Marshall 2010). *Taeniaptera* (*sensu* Steyskal 1968) is currently the most diverse of these genera and one of the most problematic taxonomically. Originally erected by Macquart (1835) and redefined by Hennig (1934), this genus included 40 species in Steyskal's (1968) Catalog of Diptera South of the United States. A further four species have since been added (Albuquerque 1980, 1981; Steyskal 1986) without modification of Hennig's definition of the genus. Marshall (2010) followed Hennig (1934) in differentiating *Poecilotylus* and *Taeniaptera* by the shape of the maxillary palpus and the frontal vitta, but added wing characters to the diagnoses and expressed doubt that these diagnoses reflected real monophyletic groups. The apparent dearth of consistent morphological characters to define Taeniapterinae genera, possibly in part due to convergence on similar Hymenoptera models, suggests that the classification of this group could be improved with the use of additional, non-morphological character sets. Molecular data have proven useful for understanding generic relationships in other acalyptate families (Conopidae, Gibson et al. 2010; Sphaeroceridae, Kits et al. 2013; Diopsidae, Baker et al. 2001); we therefore set out to find nuclear and mitochondrial DNA characters of possible use in testing and defining generic boundaries within the Taeniapterini.

Materials and methods

Taxon sampling

Ten of the 20 New World taeniapterine genera recognized by Steyskal (1967) (11 of the 23 New World taeniapterine genera recognized by Marshall, 2010; Marshall & Jackson, 2014) including six of nine Taeniapterini genera were sequenced, with a particular focus on species of *Taeniaptera* and *Poecilotylyus* (Table 1). *Cardiacephala*, *Parasphen*, and *Tenthes* were not included due to a lack of suitably preserved material. *Cardiacephala* is likely closely allied with *Plocoscelus*, and *Parasphen* is very similar to *Ptilosphen*, but *Tenthes* shows no obvious affinities to other genera. Both genera of Micropezinae (*Micropeza* Meigen, 1800; *Cryogonus* Cresson, 1926) were included as outgroups.

DNA extraction and amplification

Total genomic DNA was extracted, amplified and sequenced following protocols from Gibson et al. (2010); however amplification reactions were carried out in 50 μ L reactions, and Gibson et al. halved the volume of each component listed compared to our protocol. Primers and polymerase used for each gene sequenced can be found in Table 2.

DNA sequence editing and alignment

Sequence chromatograms were edited and contigs built using BioEdit ver. 7.0.9.0 (Hall 1999).

Sequence alignments for 12S rRNA were performed in ClustalX2 (Larkin et al. 2007) using the default settings. Preliminary alignments for COI, *wingless* and CAD were performed in ClustalX2 and alignments were then hand-aligned based on amino acid codons using MacClade ver. 4.06 (Maddison et al. 2003). For *wingless*, a 128 bp unalignable intron was identified at positions 378–506 and excised prior to analysis. No introns were found in the region of CAD sequenced (Moulton & Wiegmann 2004).

Final sequences were concatenated using TaxonDNA (Vaidya et al. 2011) and formatted for analysis.

Concatenated molecular dataset parsimony analysis

PAUP* 4.0 beta (Swofford 2003) was used to perform parsimony analyses. Each gene was partitioned and analysed individually, in addition to a total, concatenated analysis. Sequences were added randomly, and a heuristic search using TBR branchswapping for 1000 repetitions. Strict consensus trees were constructed from the most parsimonious trees returned by the analysis, and bootstrap (1000 resampling repetitions, each using 10 random addition sequence repetitions) and jackknife values (deletion frequency = 0.33, 1000 resampling repetitions, each using 10 random addition sequence

Table 1. Species, collection data, unique specimen identifiers and GenBank Accession numbers for genes included in this study.

Species	Collection data	Unique specimen identifier	GenBank accession No.		
			12S	COI	wingless CAD
<i>Calosphen auristrigatus</i> group	VEN: Estado Carabobo: Rancho Grande Trails 10°20'59"N, 67°41'04"W 1070m; ix-14/2008 E. Fisher	TAE091	KM287332	KM287292	KM287196 n/a
<i>Cryogonus formicarius</i> (Rondani, 1863)	CHILE: V Region, Olmue On dead branch w. leaves 22 Nov. 2006. S.A. Marshall	TAE087	KM287333	KM287293	n/a KM287255
<i>Globopeza venezuelensis</i> Marshall, 2004	VEN, Estado Carabobo: Portachuelo 10°20'51"N, 67°41'16"W 1143m; ix-13-15/2008 M.D. Jackson	TAE092	KM287335	n/a	KM287198 KM287257
<i>Globopeza venezuelensis</i>	VEN: Estado Carabobo: Rancho Grande Trails 10°20'59"N, 67°41'04"W 1070m; ix-14/2008 E. Fisher	TAE096	KM287334	n/a	KM287197 KM287256
<i>Gnallipeza "glypha"</i>	CR: Horquetas 12K NW Ran. Aves 700m 22-27-ii-05 S.A. Marshall Collected into 95% EtOH	TAE090	KM287337	KM287295	KM287200 KM287259
<i>Gnallipeza "octa"</i>	CR: San Jose, Tarrazu, San Carlos, Reserva Riosparaiso, Albergue Pecari, 405m, 9°33'53"N 84°07'32"W, 1° forest 22-26 Feb 2006, S.A. Marshall	TAE089	KM287336	KM287294	KM287199 KM287258
<i>Gnallipeza "tapanti"</i> group	VENEZUELA: Estado Lara; Trail into Yacambu N.P. 9°41'59"N, 69°38'52"W 1904m, ix-05/2008 M.D. Jackson	TAE103	KM287338	KM287296	KM287201 KM287260
<i>Gnallipeza vicina</i> Hennig, 1934	CR: Puntoscenas Peninsula Osa, Agua Buena 11/8/01 dung pitfalls, primary forest S.A. Marshall	TAE088	KM287339	KM287297	n/a KM287261
<i>Gnallomyia tarsata</i> (Wiedemann, 1830)	Peru: Madre de Dios: Los Amigos Biol. Stn 2-14 June 2006 Klymko & Paiero	TAE078	KM287341	KM287299	KM287202 KM287263
<i>Gnallomyia tarsata</i>	BOL: Heath River Wildlife Centre 25 Apr-11 May 2007 S.M. Paiero	TAE079	KM287340	KM287298	n/a n/a
<i>Gnallomyia tarsata</i> group	CR: Dominical Hacienda Baru 22 Feb 08 S.A. Marshall	TAE107	n/a	n/a	n/a KM287262
<i>Hemichaeta scutellata</i> (Cresson, 1930)	CR: Dominical Hacienda Baru 22 Feb 08 S.A. Marshall	TAE101	n/a	KM287300	n/a KM287264
<i>Hoplocheiloma toliana</i> Gmelin, 1970	CUBA: Santiago Botanical Garden 27 Nov 2005 S.A. Marshall	TAE104	KM287342	n/a	n/a n/a

<i>Micropeza (Micropeza)</i> sp.	Argentina: Jujuy: Calilegua National Park, Ranger Headquarters; 12.xi.2008 23°42'00"S 64°52'00"W 1081m: J. Skevington	TAE099	KM287343	KM287301	n/a	KM287265
<i>Micropeza (Micropeza)</i> sp.	Argentina: Tucuman: Parque Sierra de San Javier, Rio Muerto trail; sweeps; 666m 4.xi.2008: JF Gibson 26°47'16"S 65°19'53"W	TAE100	KM287346	KM287304	n/a	KM287266
<i>Micropeza (Neriocephalus)</i> sp.	VEN: Estado Lara: Creek near Sabana Grande 9°44'02"N, 69°40'06"W 1282m; ix-04/2008 M.D. Jackson	TAE085	KM287345	KM287303	n/a	n/a
<i>Micropeza (Neriocephalus)</i> sp.	VEN: Estado Lara: Sanare Gravel Pit; near river valley 9°49'16"N 6°37'33"W 964m; ix-08-2008 M.D. Jackson	TAE086	KM287344	KM287302	n/a	n/a
<i>Plocoscelus harenosus</i> Cresson, 1930	CR: San Jose, Tarrazu, San Carlos, La Virgen, 821 m, 9°34'50"N 84°07'51"W, 2° forest, 25 Feb 2006, S.A. Marshall	TAE057	KM287347	KM287305	KM287203	KM287267
<i>Plocoscelus harenosus</i> group	CR: Osa San Pedrillo 13 Aug 2001 S.A. Marshall	TAE065	KM287349	KM287307	KM287205	KM287269
<i>Plocoscelus harenosus</i> group	CR: Puntarenas OSA, Agua Buena 11.8.01 Dung Pans M. Buck	TAE069	KM287348	KM287306	KM287204	KM287268
<i>Poecilolytus</i> "2FOnohd"	BOL: Depto. La Paz, Heath River Wildlife Centre, 12°40'S, 68°42'W. 28 Apr-11 May 2007. S.A. Marshall	TAE050	KM287351	KM287309	n/a	KM287271
<i>Poecilolytus paraguayensis</i> (Enderlein, 1922)	BOL: Depto. La Paz, Heath River Wildlife Centre, 12°40'S, 68°42'W. 28 Apr-11 May 2007. S.A. Marshall	TAE046	KM287350	KM287308	KM287206	KM287270
<i>Poecilolytus trifasciatus</i> (Wiedemann, 1830)	CR: Dominical Hacienda Baru 22 Feb 08 S.A. Marshall	TAE106	KM287352	KM287310	KM287207	n/a
<i>Ptilosphen cyaneiventris</i> (Macquart, 1846)	VEN, Carabobo; Henri Pirtier National Park, Portachuelo Pass 13.ix.2008: 10°20'51"N, 67°41'16"W J. Skevington 1143m	TAE084	KM287353	KM287311	KM287208	KM287272
<i>Scipopus (Phaeopterina)</i> sp.	VEN, Estado Carabobo: Portachuelo 10°20'51"N, 67°41'16"W 1143m: ix-13-15/2008 M.D. Jackson	TAE095	KM287354	KM287312	n/a	KM287273
<i>Scipopus</i> sp.	ARGENTINA: JUJUY: Calilegua National Park, Ranger Headquarters; 12-xi-2008 23°42'00"S 64°52'00"W 1081m J. Skevington	TAE111	KM287356	KM287314	KM287210	KM287275
<i>Scipopus</i> sp.	CR: San Jose, Tarrazu, San Carlos, Reserva Riosparaiso, Albergue Pecari, 405m, 9°33'53"N 84°07'32"W, primary, 22-26 Feb 2006, S.A. Marshall	TAE112	KM287355	KM287313	KM287209	KM287274
<i>Taeniaptera albibasis</i> (Enderlein, 1922)	BOL: Depto. La Paz, Heath River Wildlife Centre, 12°40'S, 68°42'W, 28 Apr-11 May 2007. S.A. Marshall	TAE009	KM287357	KM287315	KM287211	KM287276
<i>Taeniaptera albitarsis</i> (Enderlein, 1922)	Peru: Madre de Dios: Los Amigos Biol Strn, 2-14 vi. 2006 S. Paiero & J. Klymko	TAE017	KM287358	KM287316	KM287212	KM287277

(Continued)

Table 1. (Cont.)

Species	Collection data	Unique specimen identifier	GenBank accession No.		
			12S	COI	wingless CAD
<i>Taeniaptera aliacea</i> group	BOL: Depto. La Paz, Heath River Wildlife Centre, 12°40'S, 68°42'W; 28 Apr-11 May 2007. S.M. Paiero	TAE020	KM287366	n/a	n/a
<i>Taeniaptera angulata</i> (Loew, 1866)	Peru: Madre de Dios: Los Amigos Biol Stn, 2-14 vi 2006 Klymko & Paiero	TAE001	KM287359	KM287317	KM287213
<i>Taeniaptera annulata</i> (Fabricius, 1787)	CR: Corcovado NP San Pedrillo Stn. 12-14/8/01 M. Buck Compost Pans	TAE003	KM287360	KM287318	KM287214
<i>Taeniaptera balzapambana</i> group	ECU: Prov. Pichincha, Bellavista Cloud Forest Res. 001'13"S 78°40'30"W; 2200m, 9-13 May 2009 S.A. Marshall	TAE109	n/a	KM287325	KM287217
<i>Taeniaptera balzapambana</i> group	Peru: Madre de Dios: Los Amigos Biol Stn, 2-14 vi 2006 S. Paiero & J. Klymko	TAE052	KM287367	KM287326	KM287218
<i>Taeniaptera balzapambana</i> group	CR: Horquetas 12K NW Rara Aves 700m 22-27-ii-05 S.A. Marshall Collected into 95% EtOH	TAE053	KM287368	KM287327	KM287219
<i>Taeniaptera balzapambana</i> group	CR: Horquetas 12K NW Rara Aves 700m 22-27-ii-05 S.A. Marshall Collected into 95% EtOH	TAE081	KM287369	KM287328	KM287220
<i>Taeniaptera gorgonae</i> Hennig, 1934	CR: 1000m on road to Rios Paraisos 15 Apr 06 S.A. Marshall	TAE007	KM287361	KM287319	KM287215
<i>Taeniaptera gratula</i> Steyskal, 1967	PERU: Manu National Park x. 2006 J. Skevington	TAE093	KM287362	KM287320	n/a
<i>Taeniaptera ichneumonea</i> Brauer, 1885	CR: Puntarenas, Ecologue, San Luis de Monteverde, 1-24 Mar 2007, sendero espajito, malaise, P.D. Careless	TAE043	KM287363	KM287321	KM287216
<i>Taeniaptera lasciva</i> (Fabricius, 1798)	Venezuela: Aragua: PN H. Pirtier, Rancho Grande, Portachuelo; 1143m: Malaise 10°20'51"N, 67°41'16"W 13-14.ix.2008: E. Fisher, J. Skevington, M. Jackson	TAE094	n/a	KM287324	n/a
<i>Taeniaptera lasciva</i>	VENEZUELA, Estado Aragua: Overgrown yard near river 10°28'31"N 67°36'19"W 205m; ix-12/2008 M.D. Jackson	TAE098	KM287365	KM287323	n/a

<i>Taeniptera lasciva</i>									
	VENEZUELA, Estado Aragua: Overgrown yard near river 10°28'31"N 67°36'19"W 205m; ix-12/2008 M.D. Jackson	TAE097	KM287364	KM287322	n/a				KM287283
<i>Taeniptera thieni</i> (Enderlein, 1922)	CR: Rios Paraiso 12-15 Apr 06 S.A. Marshall	TAE010	KM287370	KM287329	KM287221				KM287290
<i>Taeniptera trivittata</i> Macquart, 1835	USA: SC: Georgetown Co. Hobcaw Barony, Belle Baruch Marine Field Lab, S.A. Marshall	TAE005	KM287371	KM287330	KM287222				KM287291
<i>Taeniptera vulgata</i> Hennig, 1934	n/a	TAE002	KM287372	KM287331	KM287223				n/a

Table 2. Oligonucleotide primers used for PCR amplification.

Gene locus	Primer name	Sequence (5'→3')	3' location	Genbank accession No.	Reference	Polymerase	
12S rRNA	12Sh	GACCAAAATTGGTGCCAGCAGT	14776	NC_001322.1	Simon et al. (1994)	<i>Taq</i>	
	12Sbi	AAGAGCGACGGCGGATGTGT		NC_001322.1	Simon et al. (1994)	<i>Taq</i>	
COI	COI HEB F	GGTCAACAAATCATAAAGATAT	1514	NC_001322.1	Folmer et al. (1994)	<i>Taq</i>	
	COI HEB R	TAAACTTCAGGGTGACCAAAAAATCA	2173	NC_001322.1	Folmer et al. (1994)	<i>Taq</i>	
	COI PAT	TCCAATGCACATAATCTGCCATATTA	3014	NC_001322.1	Simon et al. (1994)	<i>Taq</i>	
	COI JERRY	CAACATTATTTTGATTTTTTGGG	2183	NC_001322.1	Simon et al. (1994)	<i>Taq</i>	
<i>wingless</i>	LEP Wg1 (S)	GARTGYAARTGYCAVGGYATGTCTGG	1136	M17230.1	Brower et al. (1998)	ExTaq	
	POMP Wg2R	ACTGCGCAGCACCAATGGAAATGTGCA	1774	M17230.1	Pilgrim et al. (2008)	ExTaq	
	1099-1118 (S)	GAAATGCGNCARGARTGYAA	1118	M17230.1	Baker et al. (2001)	ExTaq	
	1597-1617 (A)	ATTTTTCRCAAAARCTTGG	1617	M17230.1	Baker et al. (2001)	ExTaq	
	1147-1166 (S)	GTTAGAACWITYGTGGATGCG	1166	M17230.1	Baker et al. (2001)	ExTaq	
	1756-1775 (A)	ACYTQRCARCACCARTGRAA	1775	M17230.1	Baker et al. (2001)	ExTaq	
	Wing-Dipt-7430R	CGACACCGATCGATGTGTC	1703	M17230.1	Gibson et al. 2011)	ExTaq	
	Wing-Dipt-6803F	GCGTTTGGCTAACTTCCG	1181	M17230.1	Gibson et al. 2011)	ExTaq	
	Wing-Dipt-1505R	CCRTGACGTCCNCGTCC	1505	M17230.1	Gibson et al. 2011)	ExTaq	
	Wing-Dipt-1505F	GGACNGGACGTCARGG	1505	M17230.1	Gibson et al. 2011)	ExTaq	
	Wing-Dipt-1393R	RGATGCCGATCGTATG	1393	M17230.1	Gibson et al. 2011)	ExTaq	
	CAD	581F2	GGWGGWCAAAACWGCWYTMAYTYGGG	1507	X04813.1	Moulton et al. (2004)	ExTaq
		843R	GCTTYTGRAANGCYCTCRAA	2393	X04813.1	Moulton et al. (2004)	ExTaq
806F		GTNGTNAARATGCCNMGNMTGGGA	2287	X04813.1	Moulton et al. (2004)	ExTaq	
1124R		CAINCGNGARAAAYTTRAAACGATTYTC	3227	X04813.1	Moulton et al. (2004)	ExTaq	
1057F		GTNTCNACNGAYTAYGAYATGTG	3020	X04813.1	Moulton et al. (2004)	ExTaq	
1278R		TCRTNTTYTTWGCRTAAYATGCAT	3694	X04813.1	Moulton et al. (2004)	ExTaq	

repetitions) were calculated. The full datasets and PAUP* 4.0 scripts used are available from the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.nn20r>). Results of PAUP* 4.0 analyses were confirmed using a New Technologies Search including Sect. Search, Ratchet, Drift, and Tree Fusing with trees generated by randomly adding sequences in TNT: Tree Analysis Using New Technology (Goloboff et al., 2008).

Bayesian inference analysis

The total concatenated molecular dataset was analysed using Bayesian inference. Data were divided into four partitions (12S, COI, *wingless*, CAD). MrModeltest ver. 2.3 (Nylander 2004) was used to calculate evolution models of each gene partition in the analysis under Akaike Information Criterion (AIC) (12S, COI, CAD = GTR+I+G; *wingless* = K80+I+G). Bayesian analysis was performed using Mr. Bayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) submitted remotely to the Cyberinfrastructure for Phylogenetic Research Science Gateway V. 3.3 (<http://www.phylo.org/index.php/portal/>). The Monte Carlo Markov Chain (MCMC) method was used with the default analysis settings. Four chains (three hot, one cold) were run simultaneously for 1×10^7 generations, with tree sampling occurring every 1000 generations. The first 2500 sample generations were discarded as burn-in. The full dataset and MrBayes command block used are available from the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.nn20r>).

Results

12S rRNA

Sequence data were acquired for 41 taxa. After alignment, a total of 544 bp were analysed, of which 195 (35.8%) were parsimony informative. Parsimony analysis returned 29 most parsimonious trees (726 steps, CI=0.474, RI=0.646), from which a strict consensus tree was constructed (Fig. A1 in the Appendix).

COI

Sequence data were acquired for 40 taxa. After alignment a total of 1495 bp were analysed, of which 540 (36.1%) were parsimony informative. Parsimony analysis returned 15 most parsimonious trees (3206 steps, CI=0.289, RI=0.483), from which a strict consensus tree was constructed (Fig. A2 in the Appendix).

wingless

Sequence data were acquired for 31 taxa, however the sequence length varied widely between specimens, and a paralog of *wingless* was found to have been sequenced for Micropezinae. The three micropezine taxa were excluded from the analysis and *Globopeza venezuelensis* was selected as the outgroup taxon. After alignment, a total of 556 bp were analysed for 28 taxa, of which 135 (24.3%) were parsimony informative. Parsimony analysis returned 24 most parsimonious trees (418 steps, CI=0.583,

RI=0.668), with which a strict consensus tree was constructed (Fig. A3 in the Appendix).

CAD

Sequence data were acquired for 37 taxa, with a total of 2110 bp analysed, of which 713 (33.8%) were parsimony informative. Parsimony analysis returned four most parsimonious trees (3131 steps, CI=0.382, RI=0.515), from which a strict consensus tree was constructed (Fig. A4 in the Appendix).

Concatenated molecular dataset

DNA sequence data were included from a total of 45 taxa, combining for a total of 4705 bp, 1583 (33.6%) of which were parsimony informative. Maximum parsimony analysis returned 72 most parsimonious trees (7650 steps, CI=0.382, RI=0.515), from which a strict consensus tree was constructed (Fig. 1).

Bayesian inference analysis

The MCMC chains reached stationarity (split frequencies <0.05) by 436 000 generations (final standard deviation of split frequencies 0.0240, all parameter estimates asymptotic). A total of 7500 generation samples were used to create a majority-rule consensus tree with branch length estimates and posterior probabilities for each node mapped (Fig. 2).

Discussion

Despite weak backbone congruency between gene trees, *Grallomyia* was consistently returned as the sister group of a clade, hereafter referred to as *Taeniptera* s.s., containing the generic type species *Taeniptera trivittata* as well as *T. lasciva*. A clade containing *T. angulata*, *T. albibasis*, *T. vulgata*, *T. annulata*, *T. gorgonae* and *T. thiemi*, all of which were considered by Hennig (1934) and Steyskal (1968) to be *Taeniptera*, was returned separately and is hereafter referred to as the *Taeniptera annulata* clade. When the total molecular dataset was analysed, *Hemichaeta* was returned as the sister group to *Grallomyia*+*Taeniptera* s.s., further complicating “*Taeniptera*”.

The placement of *Hemichaeta* and *Grallomyia* in these analyses renders *Taeniptera* non-monophyletic, leaving two possible solutions: synonymize *Hemichaeta* and *Grallomyia* with *Taeniptera*, or restrict *Taeniptera* to *Taeniptera* s.s. and recognize the *Taeniptera annulata* clade as a separate genus. A broad definition of *Taeniptera* encompassing *Hemichaeta* and *Grallomyia* would result in a taxon that is very difficult to identify morphologically due to a suite of conflicting characters (shape of the maxillary palpus — parallel-sided in *Hemichaeta*, axe-shaped in *Taeniptera* and *Grallomyia*; wing cell r_{4+5} — open to wing margin in the *Taeniptera annulata* clade and *Grallomyia*, closed prior to wing margin in *Hemichaeta* and *Taeniptera* s.s.; length of the sclerotized portion of the distiphallus apical to the phallic bulb — less than 50% of the

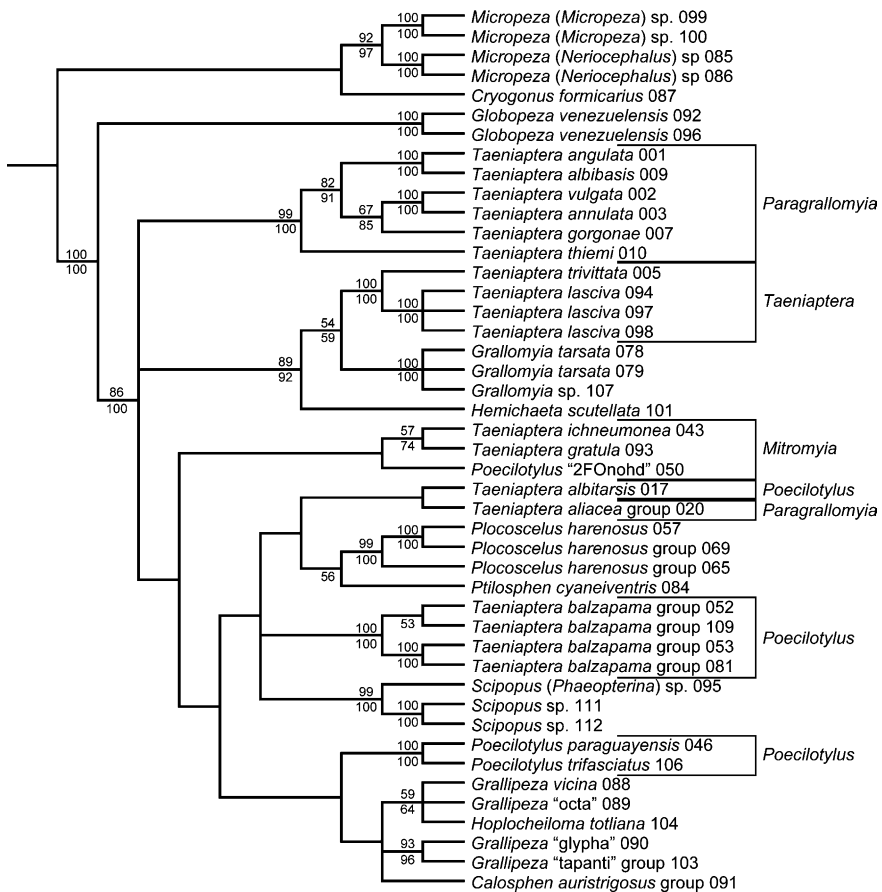


Fig. 1. Strict Consensus Tree for the Taeniapterini from concatenated DNA sequence dataset of four genes (12S, COI, *wingless*, CAD) (constructed from 72 most parsimonious trees; tree length=7650 steps, CI=0.382, RI=0.515). Nomenclature of taxon labels follows current published generic concepts (Steyskal, 1968; Marshall, 2010; Marshall & Jackson, 2014). Numbers following taxon name indicate unique specimen identifiers for this study. New generic classifications proposed as a result of this study are indicated by labeled brackets. Numbers above nodes represent Bootstrap support, numbers below nodes represent Jackknife support.

length of the basal distiphallus in the *Taeniaptera annulata* clade and *Grallomyia*, greater than 50% of the length of the basal distiphallus in *Hemichaeta* and *Taeniaptera* s.s.).

Alternatively, *Taeniaptera* can be restricted to the *Taeniaptera trivittata* clade, requiring recognition of the species in the *Taeniaptera annulata* group as a separate genus. Hendel (1933) already provided a name for that group when he erected *Paragrallomyia* as a subgenus of *Grallomyia*, designating *Taeniaptera annulata* (as *Grallomyia*) as the type species. The elevation of *Paragrallomyia* to the generic level is the most acceptable option based on the taxa considered in this paper, since it breaks the former genus into two diagnosable clades, each with an existing name, while leaving the easily diagnosed taxa *Hemichaeta* and *Grallomyia* as separate genera. *Paragrallomyia* can be recognized

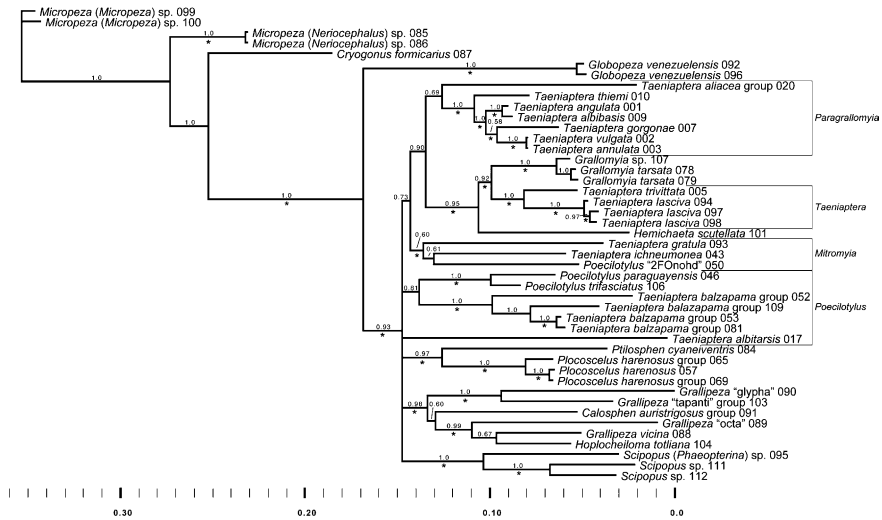


Fig. 2. Majority Rule Consensus cladogram from Bayesian Markov Chain Monte Carlo analysis of the Taeniapterini from concatenated DNA sequence dataset (12S, COI, *wingless*, CAD), including branch lengths, RI=0.483). Nomenclature of taxon labels follows current published generic concepts (Steyskal, 1968; Marshall, 2010; Marshall & Jackson, 2014). Numbers following taxon name indicate unique specimen identifiers for this study. New generic classifications proposed as a result of this study are indicated by labeled brackets. Numbers at each node represent posterior probabilities. *Nodes recovered in the maximum parsimony analysis (Fig. 1).

morphologically by an axe-shaped maxillary palpus, wing cell r_{4+5} open to the wing margin, and by the presence of at least one dorsocentral bristle.

Hemichaeta scutellata is currently the only species placed in *Hemichaeta* (Marshall & Jackson 2014). A relationship between *Hemichaeta* and *Taeniaptera* is suggested by some morphological characters including wing venation (R_{4+5} and M_{1+2} converging well before the wing margin, forming a closed cell r_{4+5}) and distiphallus proportions and shape (portion distal to the phallic bulb more than 50% length of the segment basal to the phallic bulb and strongly hooked towards the posterior). Neither of these character states are present in *Grallomyia* (wing cell r_{4+5} open to the wing margin; distiphallus beyond bulb much shorter than basal portion, not strongly hooked towards the posterior). *Grallomyia* is currently monotypic, although at least one further species awaits description. Marshall (2010) recognized *Grallomyia* as a distinct genus in his key to Neotropical Micropezidae, and we support that nomenclatural decision here.

Our data show that, as expected, some species previously considered to be in *Taeniaptera* are misplaced. Among the species included in this analysis, *Taeniaptera albitarsus* was consistently found to be widely separated from the *Taeniapteral* *Paragrallomyia* clade despite a superficial similarity to *Paragrallomyia* species. We propose a transfer of *Taeniaptera albitarsus* to *Poecilotyus* to reflect this relationship, while at the same time recognizing that *Poecilotyus* is currently a paraphyletic assemblage of unplaced species and is in need of revision. The *Taeniaptera aliacea* group specimen we

included was returned outside of *Taeniptera/Paragrallomyia* in the maximum parsimony analysis, but as sister to *Paragrallomyia* using Bayesian inference. Until this species complex can be further studied, we propose retaining it in *Paragrallomyia*.

Taeniptera gratula was also recovered outside of the *Taeniptera/Paragrallomyia* clade, along with *Taeniptera ichneumonea* and a similar undescribed species. These species, along with *Taeniptera grata* Wulp, 1897, *T. wulpi* Steyskal, 1967 and *T. feei* Steyskal 1986 form a distinctive clade that keys out with *Poecilotylus* in Marshall (2010); however, there is an available name that applies to this clade, *Mitromyia* Cresson 1930 (type species *Mitromyia conifer* Cresson 1930 = *Calobata grata* Wulp 1897). Steyskal (1967, 1986) considered *Taeniptera grata*, *T. gratula*, *T. wulpi*, and later *Taeniptera feei* to form a well-defined group characterized by unusual posterodorsal humps on the head, as well as synapomorphic male genital characters (Steyskal 1986), and stated “Should further investigation of the large and multifarious genus *Taeniptera* lead to means of defining and delimiting distinct genera, then *Mitromyia* may be found to deserve generic rank” (1986: p. 175). We agree, and therefore resurrect *Mitromyia* to include *Taeniptera grata*, *T. gratula*, *T. wulpi*, *T. feei* and *T. ichneumonea*.

Poecilotylus sensu Marshall (2010) was recovered as paraphyletic, as predicted by Marshall (2010), including two or three distinct species groups depending on analysis method. *Poecilotylus* is currently a convenient repository for Taenipterini that do not fit into defined monophyletic taxa such as *Taeniptera* ss, *Paragrallomyia*, *Grallomyia*, *Hemichaeta*, *Plocoscelus*, or *Ptilosphen*. All nomenclatural changes proposed as a result of these analyses can be found in Table 3.

The *Grallipeza* species included in these analyses displayed considerable variation in their phylogenetic positions. The four species included in this analysis represent disparate species groups of what is currently considered *Grallipeza*, a genus currently undergoing a full revision to complement Marshall’s Caribbean clade revision (2013). *Grallipeza* and *Calosphen* together represent a taxonomic mire similar to the *Taeniptera/Poecilotylus* problem examined in this study, as neither genus is currently adequately defined or consistently diagnosable.

Our data do not support the tribal classifications proposed by Cresson (1938) and Aczél (1951), with the Taenipterini rendering the Rainieriini/Grallipezini paraphyletic, however they remain a convenient shorthand for the division of the subfamily Taenipterinae into two phenetic groups based on distinctive wing venation characters. We suggest the retention of these tribes for now, with the full expectation that further phylogenetic analysis of the subfamily will provide additional evidence that one or both tribes are an artificial group.

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Table 3. Changes to generic nomenclature of the Taeniapterini based on phylogenetic relationships.

Mitromyia Cresson, 1930 resurrected from synonymy
Mitromyia grata (Wulp, 1897) (type species)
Mitromyia gratula (Steyskal, 1967) comb.n.
Mitromyia ichneuomoneus (Brauer, 1885) comb.n.
Mitromyia munda (Wulp, 1897) comb.n.
Mitromyia wulpi (Steyskal, 1967) comb.n.

Paragrallomyia Hendel, 1933 stat.n., resurrected from synonymy
Paragrallomyia aeripennis (Enderlein, 1922) comb.n.
Paragrallomyia albibasis (Enderlein, 1922) comb.n.
Paragrallomyia aliacea (Albuquerque, 1980) comb.n.
Paragrallomyia angulata (Loew, 1866) comb.n.
Paragrallomyia annulata (Fabricius, 1787) (type species)
Paragrallomyia caerulescens (Macquart, 1846) comb.n.
Paragrallomyia continentalis (Hennig, 1934) comb.n.
Paragrallomyia dilutimacula (Enderlein, 1922) comb.n.
Paragrallomyia diversicolor (Macquart, 1855) comb.n.
Paragrallomyia feei (Steyskal, 1986) comb. n.
Paragrallomyia gorgonae (Hennig, 1934) comb.n.
Paragrallomyia inornata (Hennig, 1934) comb.n.
Paragrallomyia inpai (Albuquerque, 1980) comb.n.
Paragrallomyia latifascia (Wulp, 1897) comb.n.
Paragrallomyia lauta (Cresson, 1930) comb. n.
Paragrallomyia longifurca Hendel, 1933
Paragrallomyia mediofusca (Hennig, 1934) comb.n.
Paragrallomyia nigritarsis (Macquart, 1848) comb.n.
Paragrallomyia parens (Cresson, 1926) comb.n.
Paragrallomyia planitibia (Enderlein, 1922) comb.n.
Paragrallomyia platycnema (Loew, 1866) comb.n.
Paragrallomyia postannulus (Enderlein, 1922) comb.n.
Paragrallomyia ruffifacies (Macquart, 1851) comb.n.
Paragrallomyia seiuncta (Czerny, 1931) comb.n.
Paragrallomyia simillima (Hendel, 1922) comb.n.
Paragrallomyia strigata (Enderlein, 1922) comb.n.
Paragrallomyia teresacristinae (Albuquerque, 1981) comb.n.
Paragrallomyia thiemii (Enderlein, 1922) comb.n.
Paragrallomyia tibialis (Macquart, 1843) comb.n.
Paragrallomyia vittipennis (Coquillett, 1902) comb.n.
Paragrallomyia volens (Cresson, 1926) comb.n.
Paragrallomyia vulgata (Hennig, 1934) comb.n.

Poecilotylyus Hennig, 1934
Poecilotylyus albitarsis (Enderlein, 1922) comb.n.
Poecilotylyus balzapambana (Enderlein, 1922) comb.n.
Poecilotylyus bistrigatus (Enderlein, 1922)
Poecilotylyus buscki (Cresson, 1930)
Poecilotylyus egregius (Hennig, 1934)
Poecilotylyus leucomelas (Walker, 1852)
Poecilotylyus lividisocatus (Enderlein, 1922)
Poecilotylyus luridilabris (Enderlein, 1922)

Table 3. (Cont.)

<i>Poecilotylus minor</i> (Hennig, 1934)	
<i>Poecilotylus obliquus</i> (Fabricius, 1805)	
<i>Poecilotylus paraguayensis</i> (Enderlein, 1922)	
<i>Poecilotylus pictus</i> Hennig 1937	
<i>Poecilotylus stellatus</i> (Wulp, 1897)	
<i>Poecilotylus testaceus</i> (Fabricius, 1805)	
<i>Poecilotylus tricolorus</i> (Enderlein, 1922)	
<i>Poecilotylus trifasciatus</i> (Wiedemann, 1830)	
<i>Poecilotylus varius</i> (Wiedemann, 1830) (type species)	
<hr/>	
<i>Taeniaptera</i> Macquart, 1835	
<i>Taeniaptera lasciva</i> (Fabricius, 1798)	
<i>Taeniaptera nigriceps</i> Hennig 1934	
<i>Taeniaptera trivittata</i> Macquart 1835 (type species)	
<i>Taeniaptera vulpes</i> Cresson 1926	

regarding evolution of the Diptera. Funding for this project was provided by NSERC Discovery Grants to S.A.M. and J.H.S., a US National Science Foundation Assembling the Tree of Life grant (EF-0334948) to B.M. Wiegmann, funding to J.H.S. from Agriculture and Agri-Food Canada and a North American Dipterists Society travel grant to M.D.J.

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Appendix

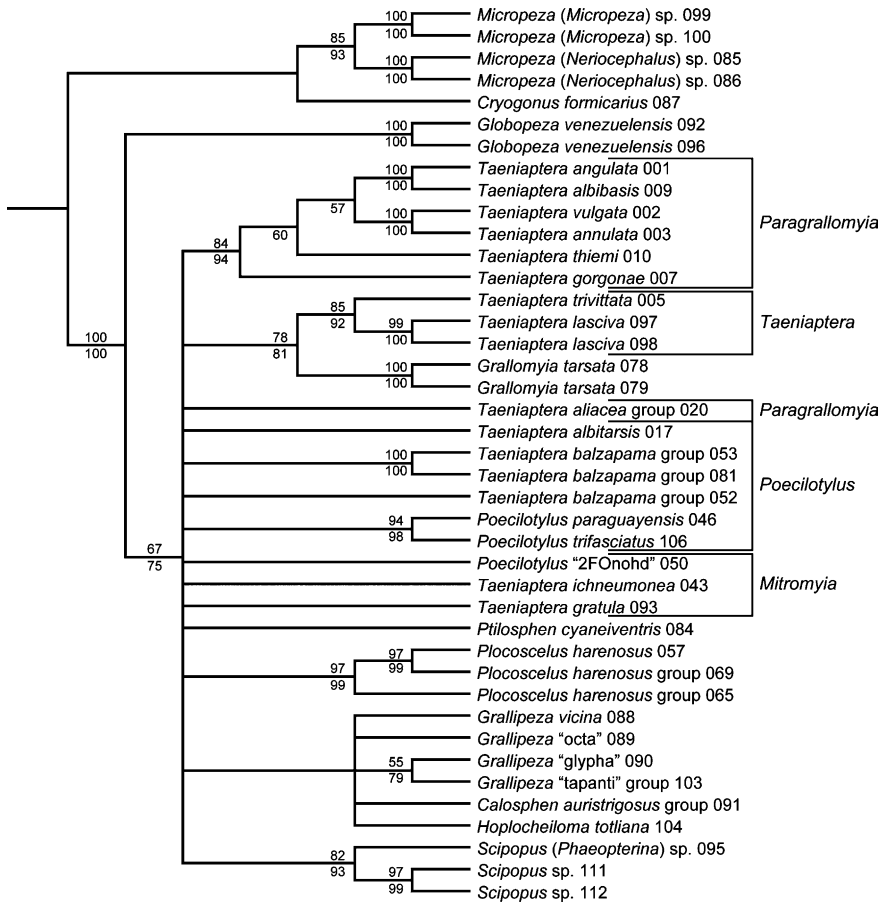


Fig. A1. Strict Consensus Tree for the Taeniopterini from 12S sequence data (constructed from 29 most parsimonious trees; tree length=726 steps, CI=0.474, RI=0.646). Nomenclature of taxon labels follows current published generic concepts (Steyskal, 1968; Marshall, 2010; Marshall & Jackson, 2014). Numbers following taxon name indicate unique specimen identifiers for this study. New generic classifications proposed as a result of this study are indicated by labeled brackets. Numbers above nodes represent Bootstrap support, numbers below nodes represent Jackknife support.

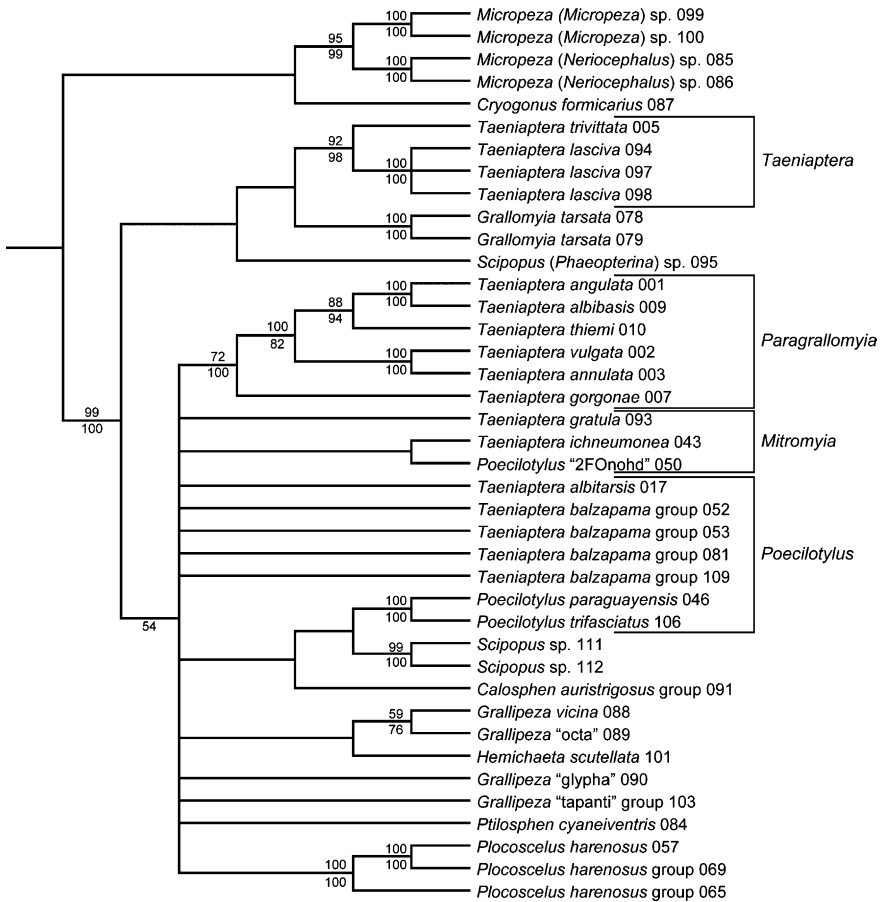


Fig. A2. Strict Consensus Tree for the Taeniapterini from COI sequence data (constructed from 15 most parsimonious trees; tree length=3206 steps, CI=0.289, RI=0.483). Nomenclature of taxon labels follows current published generic concepts (Steyskal, 1968; Marshall, 2010; Marshall & Jackson, 2014). Numbers following taxon name indicate unique specimen identifiers for this study. New generic classifications proposed as a result of this study are indicated by labeled brackets. Numbers above nodes represent Bootstrap support, numbers below nodes represent Jackknife support.

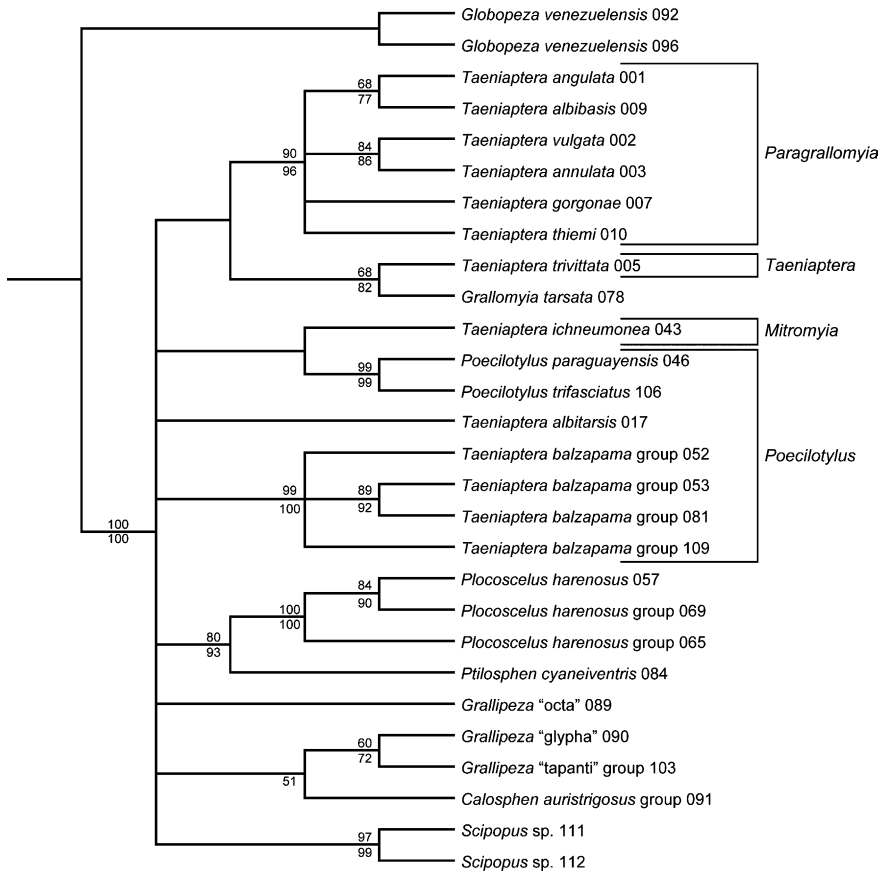


Fig. A3. Strict Consensus Tree for the Taeniapterini from *wingless* sequence data (constructed from 24 most parsimonious trees; tree length=418 steps, CI=0.584, RI=0.668). Nomenclature of taxon labels follows current published generic concepts (Steyskal, 1968; Marshall, 2010; Marshall & Jackson, 2014). Numbers following taxon name indicate unique specimen identifiers for this study. New generic classifications proposed as a result of this study are indicated by labeled brackets. Numbers above nodes represent Bootstrap support, numbers below nodes represent Jackknife support.

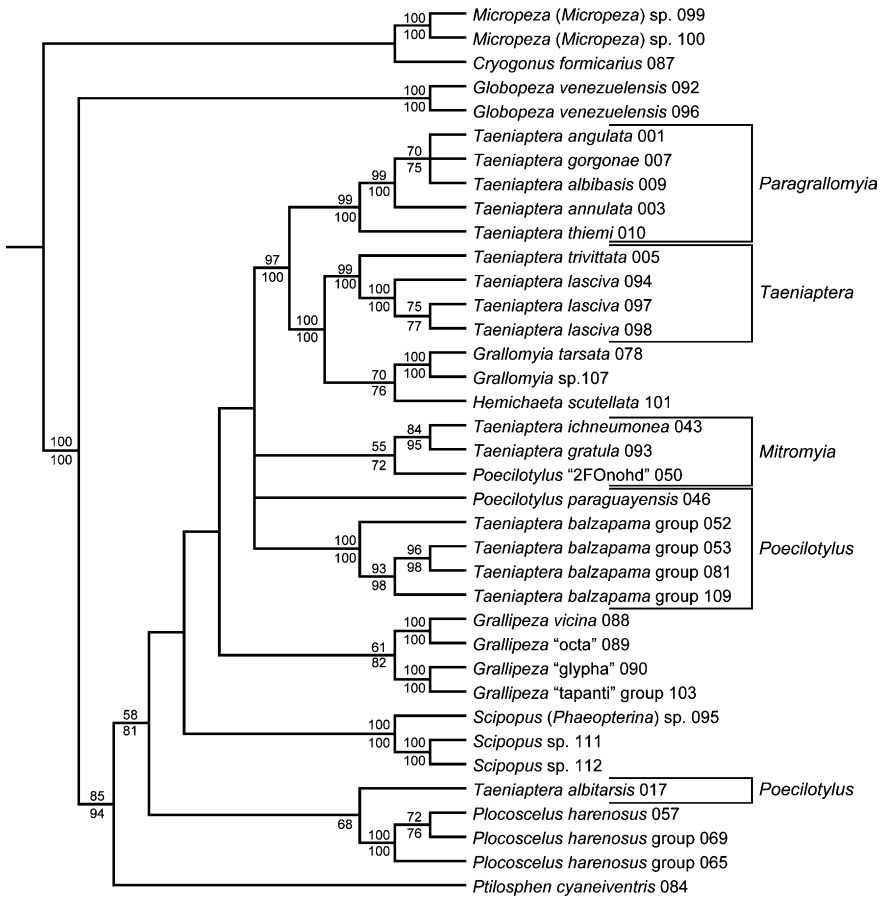


Fig. A4. Strict Consensus Tree for the Taeniapterini from CAD sequence data (constructed from 4 most parsimonious trees; tree length=3131 steps, CI=0.451, RI=0.540). Nomenclature of taxon labels follows current published generic concepts (Steyskal, 1968; Marshall, 2010; Marshall & Jackson, 2014). Numbers following taxon name indicate unique specimen identifiers for this study. New generic classifications proposed as a result of this study are indicated by labeled brackets. Numbers above nodes represent Bootstrap support, numbers below nodes represent Jackknife support.