

## Supplementary Material

### **Phylogenomics of endemic Australian Ulopinae (Hemiptera: Cicadomorpha: Cicadellidae)**

*Olivia Evangelista*<sup>A,\*</sup>, *Nikolai Tatarnic*<sup>B,C</sup> and *Keith Bayless*<sup>A,D</sup>

<sup>A</sup>Australian National Insect Collection, CSIRO, Canberra, ACT 2601, Australia

<sup>B</sup>Collections and Research Centre, Western Australian Museum, Locked Bag 49, Welshpool DC, Perth, WA 6106, Australia

<sup>C</sup>Centre for Evolutionary Biology, University of Western Australia, Crawley, WA 6009, Australia

<sup>D</sup>Australian Museum, Sydney, NSW 2010, Australia.

\*Correspondence to: Email: [olivia.evangelista@csiro.au](mailto:olivia.evangelista@csiro.au)

**Table S1.** Voucher information for specimens sequenced in this study.

Classification	Identification	Repository/ Accession	Voucher Code	Locality name	Coordinates	Date collected	Collector
CICADELLIDAE: Deltocephalinae: Eupelicini	<i>Paradorydium</i> sp. ♀	ANIC 20-006235	MEM704	AUS: NSW: Kosciuszko National Park	148.3153, -36.4417	Mar 2018	F. Encinas-Viso <i>et al.</i>
CICADELLIDAE: Ledrinae: Ledrini	<i>Ledropsis</i> sp. ♀	ANIC 20-006236	MEM680	AUS: NSW: Barren Grounds Nature Reserve	-34.6696, 150.7117	Jan–Feb2022	K. M. Bayless & O. Evangelista
CICADELLIDAE: Ulopiniae: Cephelelini	<i>Paracephaleus dobsonensis</i> ♀	ANIC 20-006237	MEM684	AUS: NSW: Kosciuszko National Park	-36.4457, 148.3149	Dec 2017	F. Encinas-Viso <i>et al.</i>
	<i>Paracephaleus aff. marginatus</i> ♀	ANIC 20-006238	MEM692	TAS: Melaleuca, Bathurst Harbour	-43.25, 146.1	Dec 1990	I.D. Naumann
	<i>Paracephaleus marginatus</i> ♀	WAM-E105705	MEM718	SA: Great Victoria Desert, Mamungari Conservation Park	-29.17, 129.46	Sep 2017	N. Tatarinic
	<i>Paracephaleus</i> sp. ♀	ANIC 20-006239	MEM700	WA: Stratton	-31.8743, 116.0449	Jul 2021	J. & F. Horst
CICADELLIDAE: Ulopiniae: Ulopinini	<i>Austrolopa botanica</i> <b>sp. nov.</b> <sup>A</sup> ♀	ANIC 20-006240	MEM698	AUS: ACT: Australian National Botanical Gardens	-35.2795, 149.1055	Sep–Oct 2020	K.M. Bayless
	<i>Austrolopa rotunda</i> <b>sp. nov.</b> <sup>A</sup> ♀	ANIC 20-011403	MEM720	ACT: Namadgi National Park, Corin Dam	-35.5318, 148.8362	Dec 2018	O. Evangelista
	<i>Austrolopa sublima</i> <b>sp. nov.</b> <sup>A</sup> ♂	ANIC 20-006241	MEM695	AUS: NSW: Kosciuszko National Park	-36.4393, 148.3243	Feb 2018	F. Encinas-Viso <i>et al.</i>
	<i>Microlopa</i> sp. ♀	ANIC 20-006242	MEM693	AUS: NSW: Kosciuszko National Park	148.3153, -36.4417	Mar 2018	F. Encinas-Viso <i>et al.</i>
	<i>Taslopa montana</i> <sup>B</sup> ♀	ANIC 20-006243	MEM696	AUS: NSW: Kosciuszko National Park	-36.4429, 148.3191	Dec 2017	F. Encinas-Viso <i>et al.</i>
MEMBRACIDAE: Centrotinae: Terentiini	<i>Acanthuchus</i> sp. ♀	ANIC 20-006244	MEM679	AUS: ACT: Black Mountain Reserve	-35.2795, 149.1055	Sep 2021	K.M. Bayless

<sup>A</sup>Data obtained from holotype, here designated.<sup>B</sup>Brachypterous specimen.

**Table S2.** Gene sampling and NCBI accession information for taxa included in the phylogenetic analyses.

Subfamily: Tribe	Genus or species	18S	28S	H2A	H3	ATP6	ATP8	COX1	COX2	COX3	CYTB	ND1	ND2	ND3	ND4	ND4L	ND5	ND6	rnl	rns	Genbank number
(1) Aetalioninae: Aetalionini	<i>Aetalion reticulatum</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	SRX2450080; SRX3144382
(1) Biturritiinae: Biturritiini	<i>Tropidaspis</i> sp.	X	X	X	X			X				X					X				SRR5988640
(2) Deltocephalinae: Eupelicini	<i>Paradorydium</i> sp. [MEM704]	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	SAMN37932137
(2) Eurymelinae: Macropsini	<i>Macropsis notata</i>			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	KX010420; MK056074.1; MK055993.1
(2) Ledrinae: Ledrini	<i>Ledropsis</i> sp. [MEM680]		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	SAMN37932215
(2) Megophthalminae: Agalliini	<i>Bergallia</i> sp.	X	X	X	X	X	X	X	X	X					X	X	X				SRR5988721
(2) Megophthalminae: Agalliini	<i>Sinoagallia serrata</i>	X	X	X	X			X	X	X	X			X	X						SRR5988666
(2) Ulopinae: Cephalelini	<i>Paracephaleus</i> aff. <i>marginatus</i> [MEM692]	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	SAMN37932317
(2) Ulopinae: Cephalelini	<i>Paracephaleus marginatus</i> [MEM718]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	SAMN37932422
(2) Ulopinae: Cephalelini	<i>Paracephaleus</i> sp. [MEM700]	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	SAMN37932446
(2) Ulopinae: Cephalelini	<i>Paracephalus dobsonensis</i> [MEM684]	X	X	X	X																SAMN37932286
(2) Ulopinae: Coloborrhinini	<i>Coloborrhis corticina</i>	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X		SRR5988703
(2) Ulopinae: Mesargini	<i>Mesargus hirsutus</i>	X	X	X	X			X	X	X	X										SRR5988608; MG813495
(2) Ulopinae: Mesargini	<i>Mesargus serrata</i>					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	MG813495.1
(2) Ulopinae: Ulopini	<i>Austrolopa botanica</i> <b>sp. nov.</b> [MEM698]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	SAMN37932475
(2) Ulopinae: Ulopini	<i>Austrolopa rotunda</i> <b>sp. nov.</b> [MEM720]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	SAMN37932476
(2) Ulopinae: Ulopini	<i>Austrolopa sublima</i> <b>sp. nov.</b> [MEM695]	X	X	X	X	X		X	X	X	X	X			X	X	X	X	X	X	SAMN37932477
(2) Ulopinae: Ulopini	<i>Microlopa</i> sp. [MEM693]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	SAMN37932484
(2) Ulopinae: Ulopini	<i>Taslopa montana</i> [MEM696]	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	SAMN37932485
(2) Ulopinae: Ulopini	<i>Ulopa reticulata</i>	X	X	X	X	X		X	X	X	X			X	X	X	X	X		X	SRR2051513; AF304576
(3) Melizoderinae: Melizoerini	<i>Melizoderes osborni</i>	X	X	X	X			X		X		X		X			X				SRR5988615
(4) Centrotinae: Terentiini	<i>Acanthuchus</i> sp. [MEM679]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	SAMN37932486
(4) Stegaspidinae: Stegaspidini	<i>Bocydium globulare</i>	X	X	X	X	X				X				X	X				X		SRR5988619

(1) Aetalionidae; (2) Cicadellidae; (3) Melizoderidae; (4) Membracidae.

**Table S3.** Reference sequences used to retrieve target loci from genome assemblies.

Reference set	NCBI Accession	Family	Subfamily	Tribe	Species
Mitofinder: Mitogenome	NC_026699	Aetalionidae	Aetalionidae	Darthulini	<i>Darthula hardwickii</i>
Mitofinder: Mitogenome	NC_045238	Cicadellidae	Deltocephalinae	Athysanini	<i>Abrus expansivus</i>
Mitofinder: Mitogenome	NC_073513	Cicadellidae	Deltocephalinae	Chiasmini	<i>Zahniserius cylindricus</i>
Mitofinder: Mitogenome	NC_050258	Cicadellidae	Deltocephalinae	Drabescini	<i>Drabescus ineffectus</i>
Mitofinder: Mitogenome	NC_050257	Cicadellidae	Deltocephalinae	Drabescini	<i>Roxasellana stellata</i>
Mitofinder: Mitogenome	NC_039560	Cicadellidae	Deltocephalinae	Macrostelini	<i>Macrosteles quadrimaculatus</i>
Mitofinder: Mitogenome	MK450366	Cicadellidae	Deltocephalinae	Paralimnini	<i>Paralaevicephalus gracilipenis</i>
Mitofinder: Mitogenome	NC_072998	Cicadellidae	Eurymelinae	Idiocerini	<i>Chinaocerus tubulatus</i>
Mitofinder: Mitogenome	ON510776	Cicadellidae	Eurymelinae	Idiocerini	<i>Idiocerus consimilis</i>
Mitofinder: Mitogenome	OQ331234	Cicadellidae	Eurymelinae	Macropsini	<i>Macropsis hainanensis</i>
Mitofinder: Mitogenome	MG813491	Cicadellidae	Ledrinae	Ledrini	<i>Ledra trigona</i>
Mitofinder: Mitogenome	NC_051527	Cicadellidae	Ledrinae	Ledrini	<i>Petaloccephala chlorophana</i>
Mitofinder: Mitogenome	MT610900	Cicadellidae	Ledrinae	Ledrini	<i>Tituria sagittata</i>
Mitofinder: Mitogenome	NC_072571	Cicadellidae	Megophthalminae	Agalliini	<i>Dryodurgades formosana</i>
Mitofinder: Mitogenome	NC_035685	Cicadellidae	Megophthalminae	Agalliini	<i>Japanagallia spinosa</i>
Mitofinder: Mitogenome	MG813495	Cicadellidae	Ulopiniae	Mesargini	<i>Mesargus serrata</i>
Mitofinder: Mitogenome	NC_060744	Membracidae	Centrotinae	Centrotini	<i>Anchon yunnanense</i>
Mitofinder: Mitogenome	NC_071378	Membracidae	Centrotinae	Centrotini	<i>Centrotus cornutus</i>
Mitofinder: Mitogenome	NC_060745	Membracidae	Centrotinae	Gargarini	<i>Gargara genistae</i>
Phyloherb: 18S	AY744777.1	Aetalionidae	Aetalioninae	Aetalionini	<i>Aetalion reticulatum</i>
Phyloherb: 18S	AY498432.1	Aetalionidae	Biturritinae	Biturritini	<i>Lophyraspis muscaria</i>
Phyloherb: 18S	U15148.1	Cicadellidae	Deltocephalinae	Athysanini	<i>Euscelidius variegatus</i>
Phyloherb: 18S	JX273234.1	Cicadellidae	Deltocephalinae	Macrostelini	<i>Macrosteles quadripunctulatus</i>
Phyloherb: 18S	AY498437.1	Cicadellidae	Deltocephalinae	Paralimnini	<i>Flexamia areolata</i>
Phyloherb: 18S	AY498406.1	Cicadellidae	Ledrinae	Ledrini	<i>Putoniessa rivularis</i>
Phyloherb: 18S	AY498407.1	Cicadellidae	Ulopiniae	Cephalelini	<i>Paracephaleus brunneus</i>
Phyloherb: 18S	AY498424.1	Membracidae	Darninae	Cymbomorphini	<i>Cymbomorpha</i> sp.
Phyloherb: 18S	U06477.1	Membracidae	Smiliinae	Ceresini	<i>Spissistilus festinus</i>
Phyloherb: 18S	AY498463.1	Membracidae	Smiliinae	Telamoni	<i>Telamona monticola</i>
Phyloherb: 28S	AF304662.1	Aetalionidae	Aetalioninae	Aetalionini	<i>Aetalion nervosopunctatum</i>
Phyloherb: 28S	GU123730.1	Cicadellidae	Deltocephalinae	Athysanini	<i>Eutettix pictus</i>
Phyloherb: 28S	JX845491.1	Cicadellidae	Deltocephalinae	Dorycephalini	<i>Dorycephalus baeri</i>
Phyloherb: 28S	AF304637.1	Cicadellidae	Deltocephalinae	Dorycephalini	<i>Paradorydium lanceolatum</i>
Phyloherb: 28S	GU123736.1	Cicadellidae	Deltocephalinae	Goniagnathini	<i>Goniagnathus guttulinervis</i>
Phyloherb: 28S	GU123733.1	Cicadellidae	Deltocephalinae	Paralimnini	<i>Flexamia areolata</i>
Phyloherb: 28S	GU123741.1	Cicadellidae	Deltocephalinae	Stenometopiini	<i>Kinonia elongata</i>
Phyloherb: 28S	AF304612.1	Cicadellidae	Ledrinae	Ledrini	<i>Putoniessa rivularis</i>
Phyloherb: 28S	AF304613.1	Cicadellidae	Ledrinae	Stenocotini	<i>Stenocotis depressa</i>
Phyloherb: 28S	AF304672.1	Cicadellidae	Ledrinae	Xerophloeini	<i>Proranus adpersipennis</i>
Phyloherb: 28S	AF304626.1	Cicadellidae	Ulopiniae	Cephalelini	<i>Paracephaleus brunneus</i>
Phyloherb: 28S	AF304576.1	Cicadellidae	Ulopiniae	Ulopiini	<i>Ulopa reticulata</i>
Phyloherb: 28S	AF304630.1	Membracidae	Centrotinae	Terentiini	<i>Ceraon vitta</i>
Phyloherb: 28S	KX924801.1	Membracidae	Darninae	Cymbomorphini	<i>Cymbomorpha</i> sp.
Phyloherb: H2A	JN797414.1	Aetalionidae	Aetalioninae	Aetalionini	<i>Aetalion reticulatum</i>
Phyloherb: H2A	KX924703.1	Aetalionidae	Biturritinae	Biturritini	<i>Lophyraspis scutellata</i>
Phyloherb: H2A	MW328801.1	Cicadellidae	Deltocephalinae	Macrostelini	<i>Balclutha punctata</i>

Reference set	NCBI Accession	Family	Subfamily	Tribe	Species
Phyloherb: H2A	MW328802.1	Cicadellidae	Deltocephalinae	Macrostelini	<i>Cicadulina bipunctata</i>
Phyloherb: H2A	MW328803.1	Cicadellidae	Deltocephalinae	Macrostelini	<i>Yamatotettix flavovittatus</i>
Phyloherb: H2A	MK400113.1	Cicadellidae	Ledrinae	Ledrini	<i>Petaloccephala chlorophana</i>
Phyloherb: H2A	MK400117.1	Cicadellidae	Ledrinae	Ledrini	<i>Tituria maculata</i>
Phyloherb: H2A	MK056048.1	Cicadellidae	Megophthalminae	Agalliini	<i>Dryodurgades lamellaris</i>
Phyloherb: H2A	MK056066.1	Cicadellidae	Megophthalminae	Agalliini	<i>Japanagallia</i> sp.
Phyloherb: H2A	JN797419.1	Melizoderidae	Melizoderinae	Melizoderini	<i>Melizoderes</i> sp.
Phyloherb: H2A	KX924644.1	Membracidae	Centrotinae	Boocerini	<i>Ischnocentrus niger</i>
Phyloherb: H2A	KX924650.1	Membracidae	Stegaspidae	Stegaspidini	<i>Stegaspis fronditia</i>
Phyloherb: H3	KX925045.1	Aetalionidae	Aetalionidae	Biturritini	<i>Lophyraspis scutellata</i>
Phyloherb: H3	AY744849.1	Aetalionidae	Aetalioninae	Aetalionini	<i>Aetalion reticulatum</i>
Phyloherb: H3	GU123798.1	Cicadellidae	Deltocephalinae	Bahitini	<i>Bahita</i> sp.
Phyloherb: H3	KR230250.1	Cicadellidae	Deltocephalinae	Chiasmini	<i>Chiasmus</i> sp.
Phyloherb: H3	JX845532.1	Cicadellidae	Deltocephalinae	Dorycephalini	<i>Dorycephalus baeri</i>
Phyloherb: H3	GU123823.1	Cicadellidae	Deltocephalinae	Pendarini	<i>Dorydiella</i> sp.
Phyloherb: H3	MK400112.1	Cicadellidae	Ledrinae	Ledrini	<i>Petaloccephala chlorophana</i>
Phyloherb: H3	MK400116.1	Cicadellidae	Ledrinae	Ledrini	<i>Tituria maculata</i>
Phyloherb: H3	KC753648.1	Cicadellidae	Ledrinae	Stenocotini	<i>Stenocotis depressa</i>
Phyloherb: H3	KC753647.1	Cicadellidae	Ledrinae	Thymbrini	<i>Putoniessa rivularis</i>
Phyloherb: H3	KC753649.1	Cicadellidae	Ledrinae	Xerophloeini	<i>Xerophloea peltata</i>
Phyloherb: H3	MH682028.1	Cicadellidae	Megophthalminae	Agalliini	<i>Austroagallia caboverdensis</i>
Phyloherb: H3	MK055969.1	Cicadellidae	Megophthalminae	Agalliini	<i>Dryodurgades lamellaris</i>
Phyloherb: H3	MK055987.1	Cicadellidae	Megophthalminae	Agalliini	<i>Japanagallia</i> sp.
Phyloherb: H3	JN797367.1	Melizoderidae	Melizoderinae	Melizoderini	<i>Llanquihuea</i> sp.
Phyloherb: H3	JN797368.1	Melizoderidae	Melizoderinae	Melizoderini	<i>Melizoderes</i> sp.
Phyloherb: H3	KX924982.1	Membracidae	Centrotinae	Boocerini	<i>Ischnocentrus niger</i>
Phyloherb: H3	KX924981.1	Membracidae	Darninae	Cymbomorphini	<i>Cymbomorpha</i> sp.
Phyloherb: H3	KX924983.1	Membracidae	Stegaspidae	Microcentrini	<i>Microcentrus caryae</i>
Phyloherb: H3	KX924988.1	Membracidae	Stegaspidae	Stegaspidini	<i>Stegaspis fronditia</i>
Phyloherb: H3	JN797326.1	Myerslopiidae	Myerslopiinae	Myerslopiini	<i>Myerslopiia</i> sp.

**Table S4.** Statistics of matrices used in phylogenetic analyses.

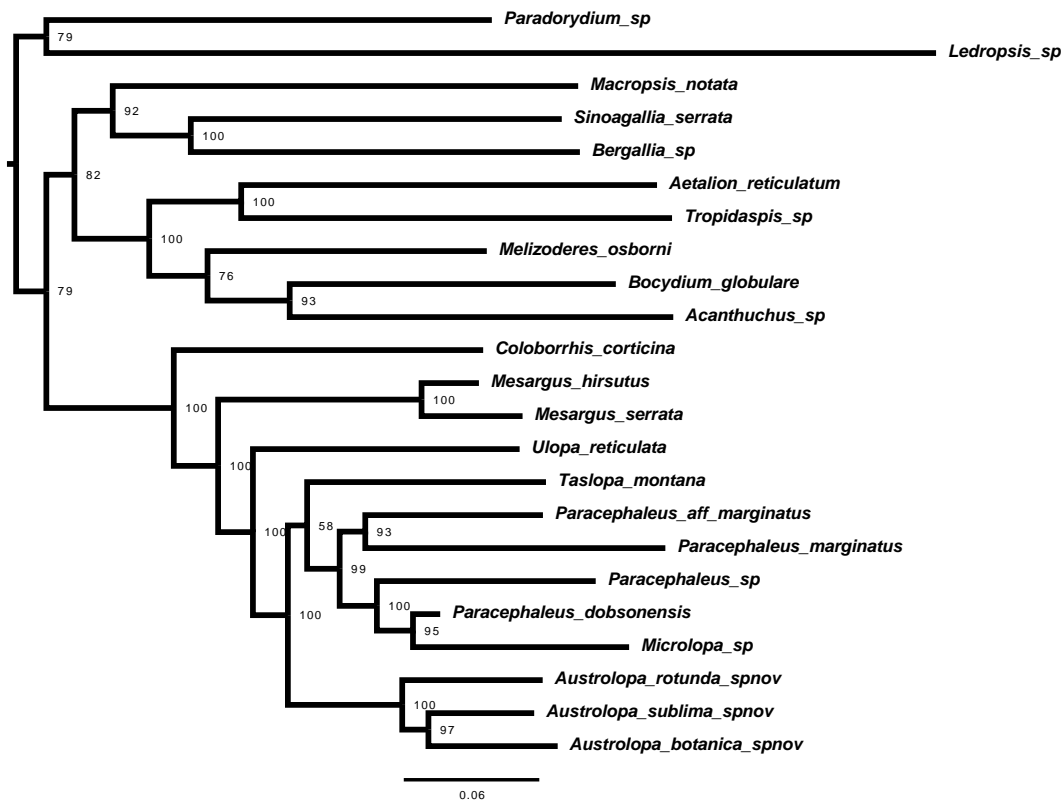
Matrix	Number of genes	Number of sites	Number of parsimony informative sites	Missing percentage	GC_Content
Matrix A	19	19 989 nucleotides	7 683	27.813	36.10%
Matrix B	15	4104 aminoacids	2 030	25.747	N/A
Matrix C	15	12312 [nucleotides], 4104 codons	6 444	25.747	N/A

**Table S5.** Statistics for the phylogenetic analyses.

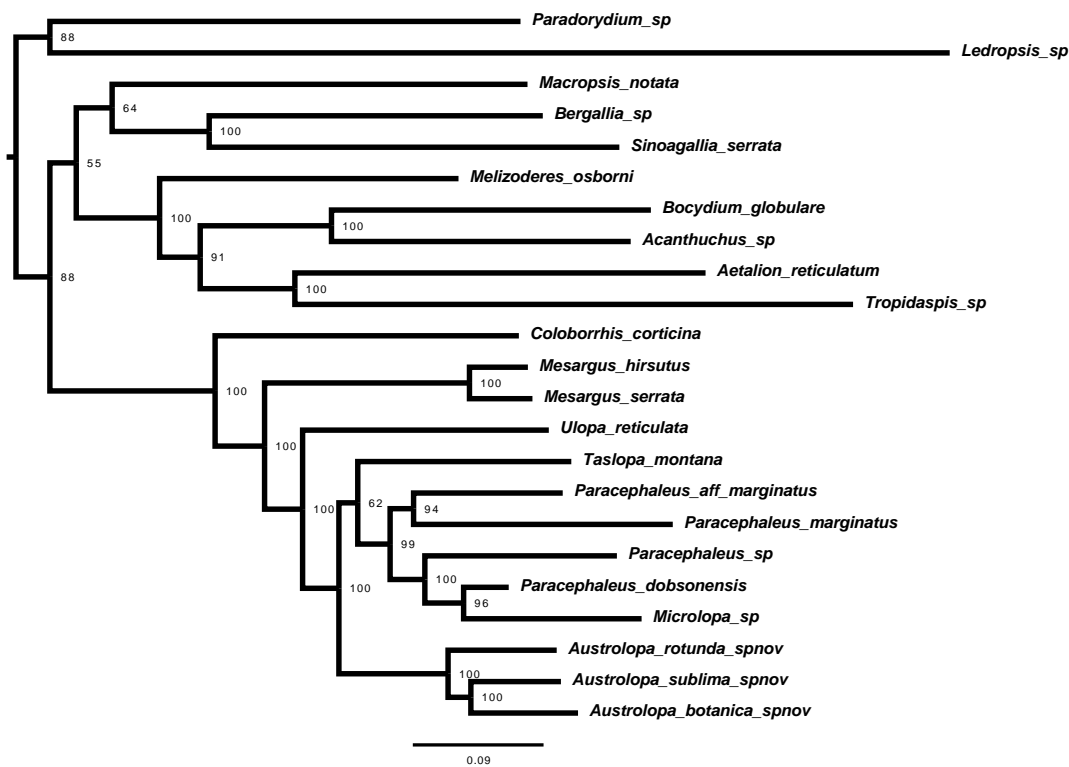
Matrix	Number of partitions	log-likelihood of consensus tree
Analysis 1	1	-162539.52
Analysis 2	15	-156155.75
Analysis 3	7	-156361.94
Analysis 4	49	-150234.64
Analysis 5	13	-150685.79
Analysis 6	1	-64397.699
Analysis 7	15	-116036.26

**Table S6.** Comparisons of statistical support for major nodes across the sensitivity analyses.

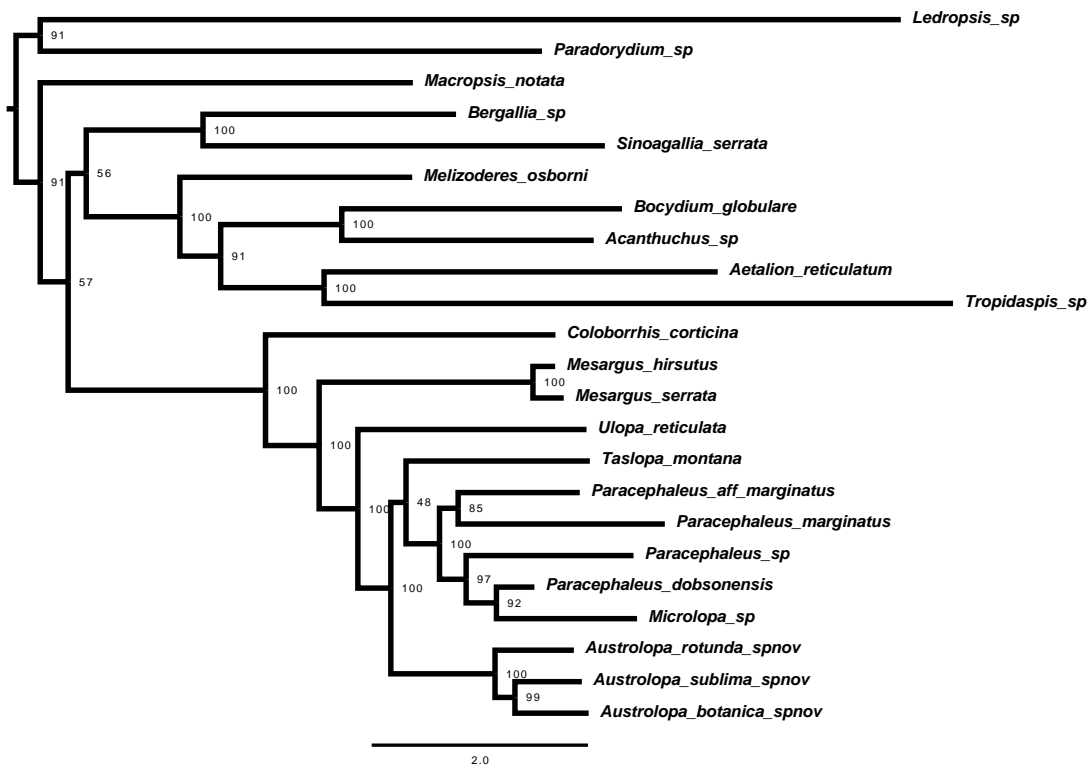
Relationships between membracoid/ulopine clades	Phylogenetic analyses performed							Concordance factors	
	1	2	3	4	5	6	7	gCF_N	sCF
Ulopinæ sister to Eurymelinae + Megophthalminæ + treehoppers	79	88	65	57	96	N/A	N/A	2	37
Megophthalminæ sister to treehoppers	82	55	47	N/A	44	N/A	N/A	4	35
Ulopinæ sister to treehoppers	N/A	N/A	N/A	N/A	N/A	28	57		
Ulopinæ monophyletic	100	100	100	100	100	98	100	13	49
Ulopa + Australian Ulopinæ	100	100	100	100	100	94	100	6	35
<i>Taslopa</i> sister to ' <i>Paracephaleus</i> ' and <i>Microlopa</i>	58	62	56	48	N/A	N/A	N/A		
<i>Austrolopa</i> sister to ' <i>Paracephaleus</i> ' and <i>Microlopa</i>	N/A	N/A	N/A	N/A	47	77	91	2	36
Monophyly of <i>Austrolopa</i>	100	100	100	100	100	93	100	19	59



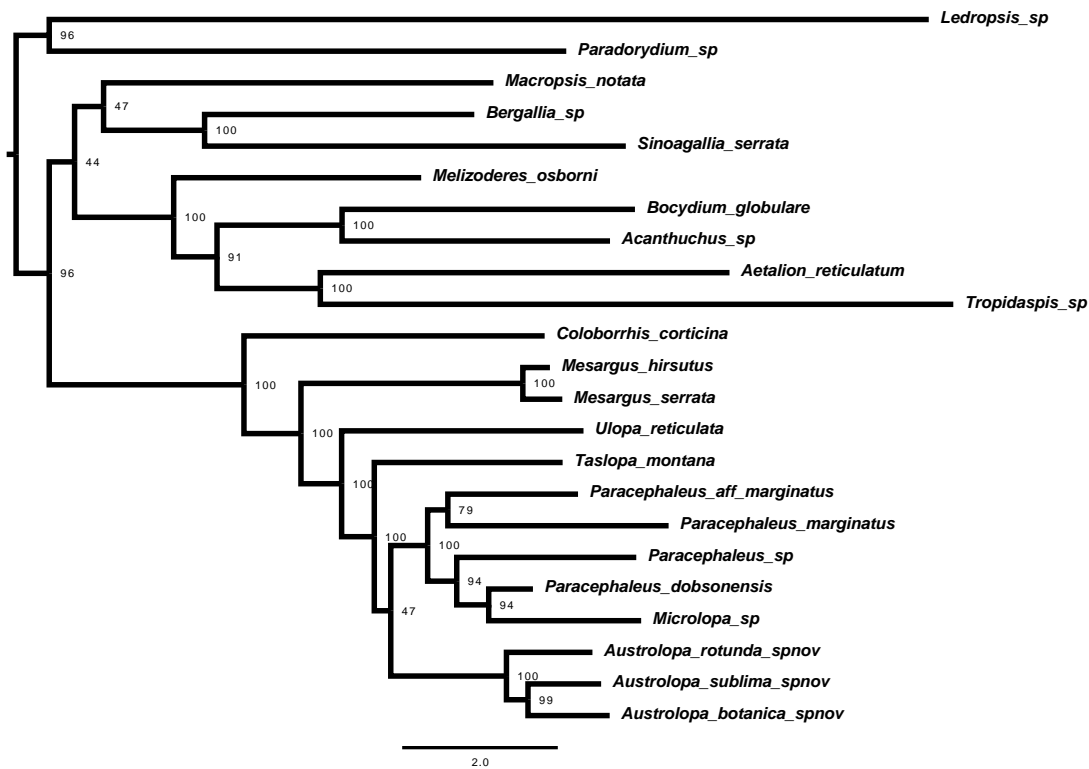
**Figure S1.** Analysis 1; Maximum likelihood consensus tree inferred from 19 genes, 23 membracoid taxa. Topologies were estimated in IQTREE (ver. 2.1.3) based on a dataset with one partition (Matrix A, Analysis 1). Branch support statistics are represented by 1000 rapid bootstrap pseudoreplicates. Scale bar represents substitutions per site. log-likelihood of consensus tree:  $-162539.524$ .



**Figure S2.** Analysis 2; Maximum likelihood consensus tree inferred from 19 genes, 23 membracoid taxa. Topologies were estimated in IQTREE (ver. 2.1.3) based on a dataset partitioned by gene (Matrix A, Analysis 2). Branch support statistics are represented by 1000 rapid bootstrap pseudoreplicates. Scale bar represents substitutions per site. log-likelihood of consensus tree:  $-156155.751$ .

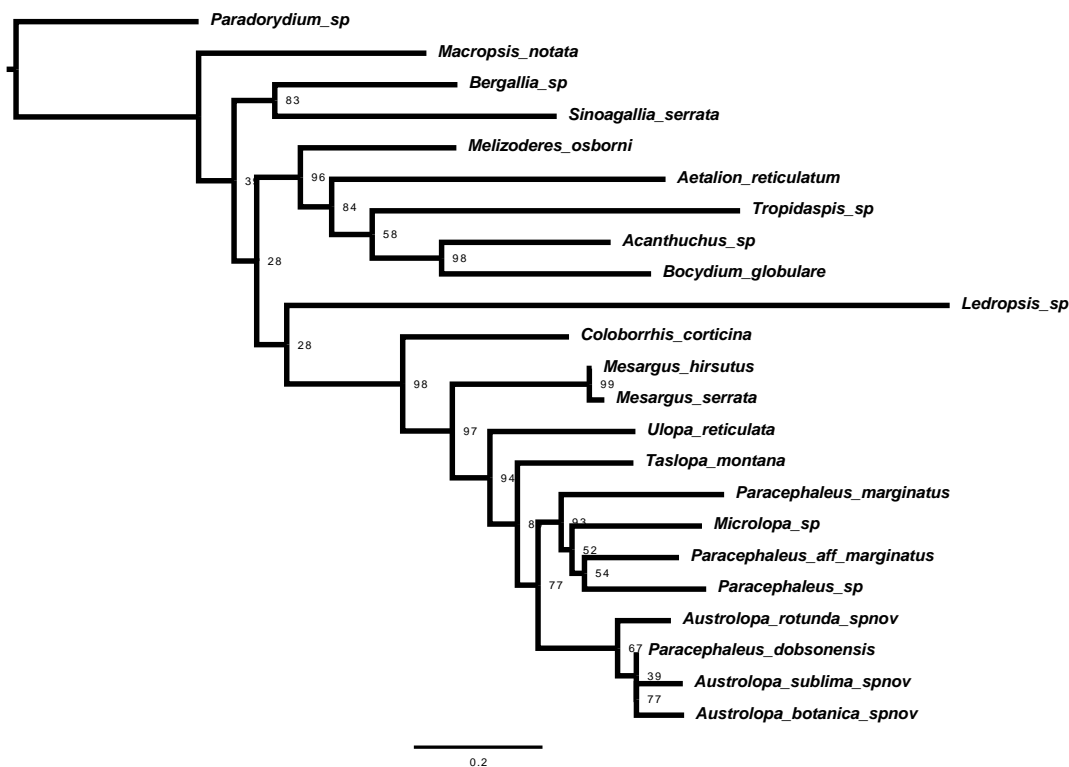


**Figure S3.** Analysis 4. Maximum likelihood consensus tree inferred from 19 genes, 23 membracoid taxa. Topologies were estimated in IQTREE (ver. 2.1.3) based on a dataset partitioned by gene by codon (Matrix A, Analysis 4). Branch support statistics -1000 rapid bootstrap pseudoreplicates. Scale bar represents substitutions per site. log-likelihood of consensus tree:  $-150234.636$ .

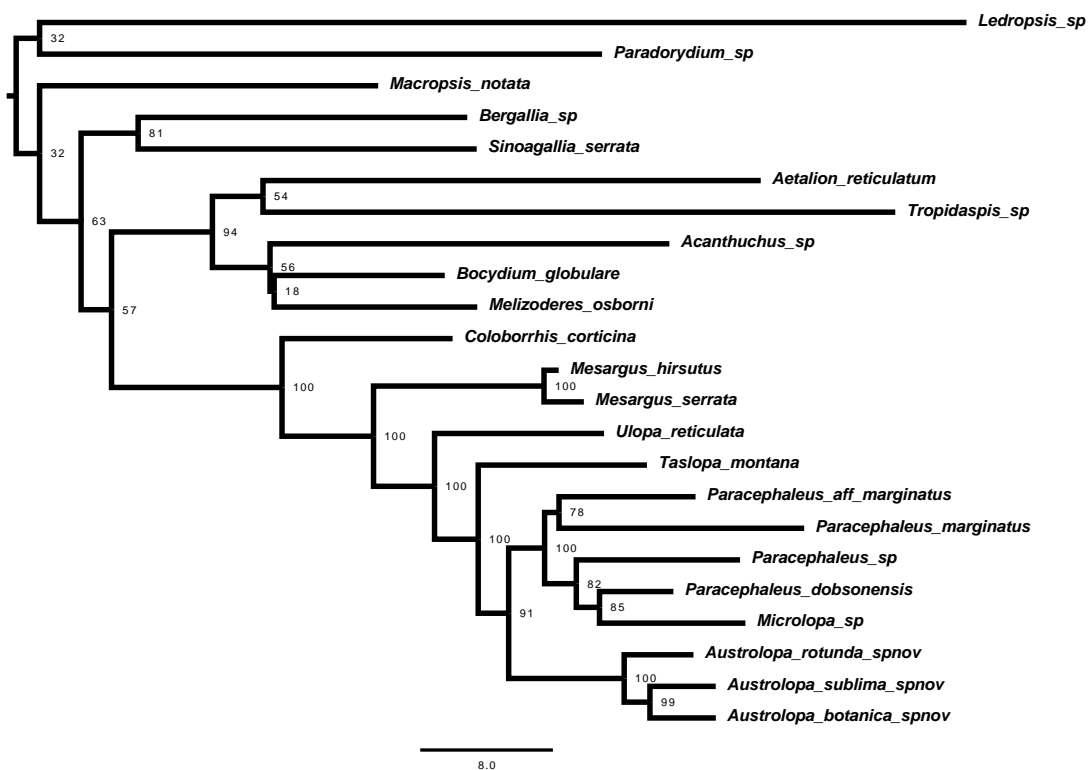


**Figure S4.** Analysis 5. Maximum likelihood consensus tree inferred from 19 genes, 23 membracoid taxa. Topologies were estimated in IQTREE (ver. 2.1.3) based on a dataset partitioned by gene by codon, with partitions merged into 13 metapartitions (Matrix A, Analysis 4). Branch support statistics -1000 rapid bootstrap pseudoreplicates. Scale bar represents substitutions per site. log-likelihood of consensus tree:  $-150685.789$ .





**Figure S5.** Analysis 6. Maximum likelihood consensus tree inferred from 15 protein coding genes translated to amino acids, 23 membracoid taxa. Topologies were estimated in IQTREE (ver. 2.1.3) based on a dataset in a single partition (Matrix B, Analysis 6). Branch support statistics -1000 rapid bootstrap pseudoreplicates. Scale bar represents substitutions per site. log-likelihood of consensus tree: -64397.699.



**Figure S6.** Analysis 7; Maximum likelihood consensus tree inferred from 5 genes, 23 membracoid taxa. Topologies were estimated in IQTREE (ver. 2.1.3) based on a dataset with partitioned by genes, with codon aware models (Matrix C, Analysis 7). Branch support statistics are represented by 1000 rapid bootstrap pseudoreplicates. Scale bar represents substitutions per site. log-likelihood of consensus tree: -116036.260.