

Off-target capture data, endosymbiont genes and morphology reveal a relict lineage sister to all other singing cicadas

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Supplementary Figures S1-19,
Tables S1 and S2



Figure S1. (A) *Derotettix mendosensis* male (PL 754) on salt bush, *Atriplex* sp., Río Negro Province, Argentina. (B) male dorsal view, (C) male side view, (D) male ventral view. Photos PŁ. See Table 1, Fig. 2 for exact locality.

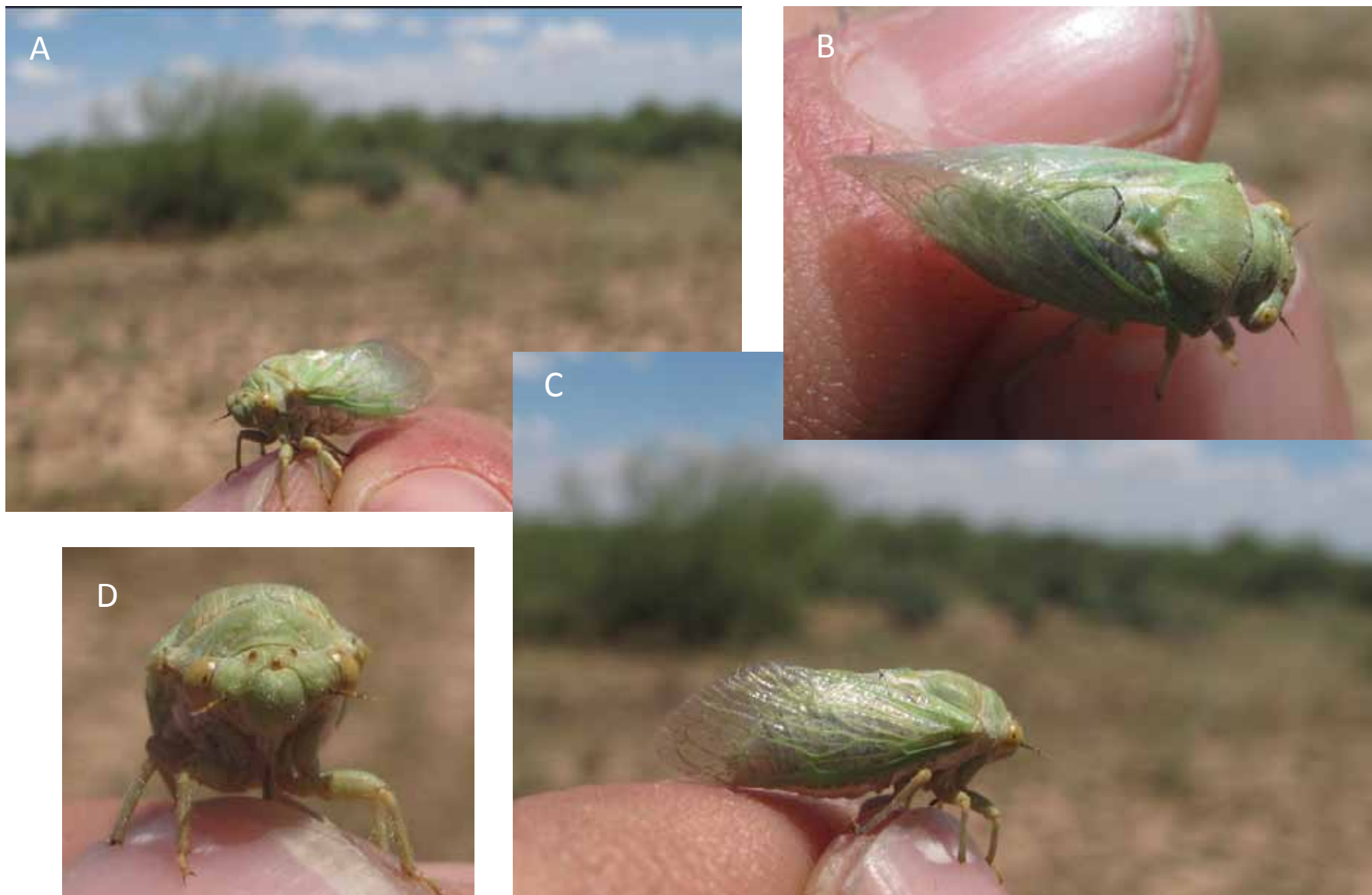


Figure S2. *Derotettix mendosensis* male (PL 618), Ruta 141, San Juan Province, Argentina. Clockwise from upper left. (A) side view, (B) dorsal, (C) side, (D) head. Photos PŁ. See Table 1, Fig. 2 for exact locality.



Figure S3. PL 755 & 756.
Derotettix mendosensis,
Río Negro Province, AR

(A) female, green morph, dorsal; (B) female, green, ventral, (C) male yellow morph, ventral, (D) male, yellow, dorsal, (E) male, yellow, side. Photos PŁ. See Table 1, Fig. 2 for exact locality.



Figure S4. PL767 *Derotettix mendosensis*, NW de Rincón de Los Sauces, Neuquén Province, AR. (A) male, side, (B) male, ventral, (C) male, dorsal. Photos PŁ. See Table 1, Fig. 2 for exact locality.



Figure S5. *Derotettix mendosensis* (PL954), Ruta de Pomona, Provincia Rio Negro, AR. (A) male, dorsal, (B) male, side, (C) male, ventral. Photos PŁ. See Table 1, Fig. 2 for exact locality.





Figure S6. *Derotettix mendosensis* site (18.AR.MZ.CLG), highway 34, east of Villa Tulumaya, Mendoza Province AR. Three views of *D. mendosensis* habitat with obvious human disturbance. Photos CS. See Table 1, Fig. 2 for exact locality.

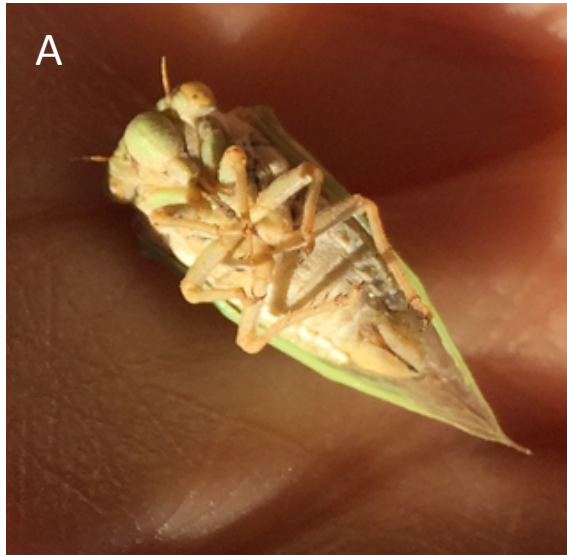


Figure S7. *Derotettix mendosensis*, east of Villa Tulumaya, Mendoza Province, AR. 18.AR.MZ.EVT. (A) female, ventral, (B) female, side, (C) male, side, (D) male, ventral. Photos CS. See Table 1, Fig. 2 for exact locality.





Figure S8. *Derotettix mendosensis* habitat, 18.AR.MZ.CLG, Calle Lugones, just off Provincial Road 96, Mendoza Province, AR. Three views of habitat with obvious human disturbance. Photos CS. See Table 1, Fig. 2 for exact locality.

Figure S9. RAxML phylogeny nuclear genes only, RNA + codon partitioned, (28S, 18S, EF1a, ARD1). Of 6,652 total characters, 1034 are parsimony informative; for the Cicadidae ingroup only, 714 are parsimony informative.

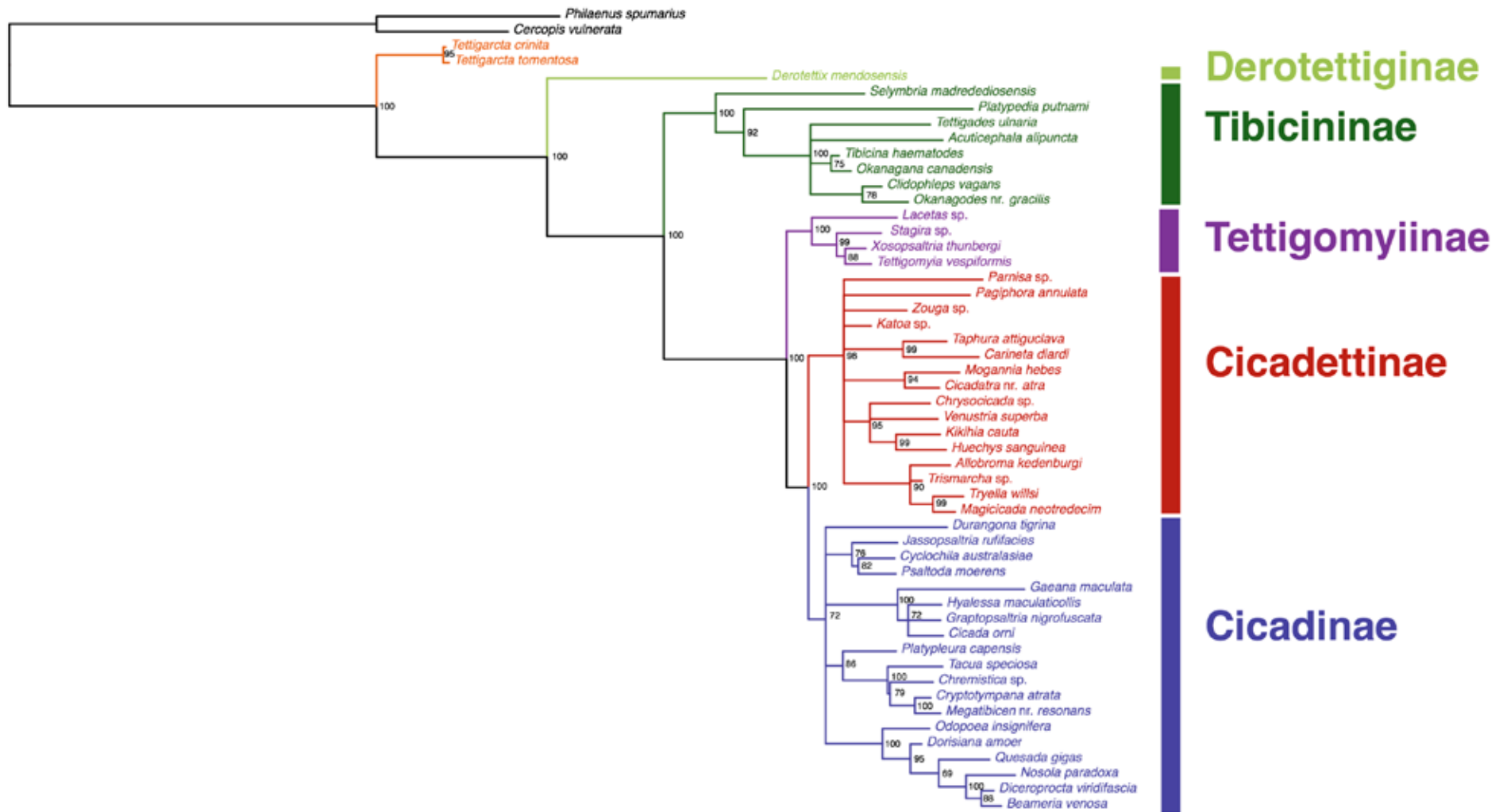
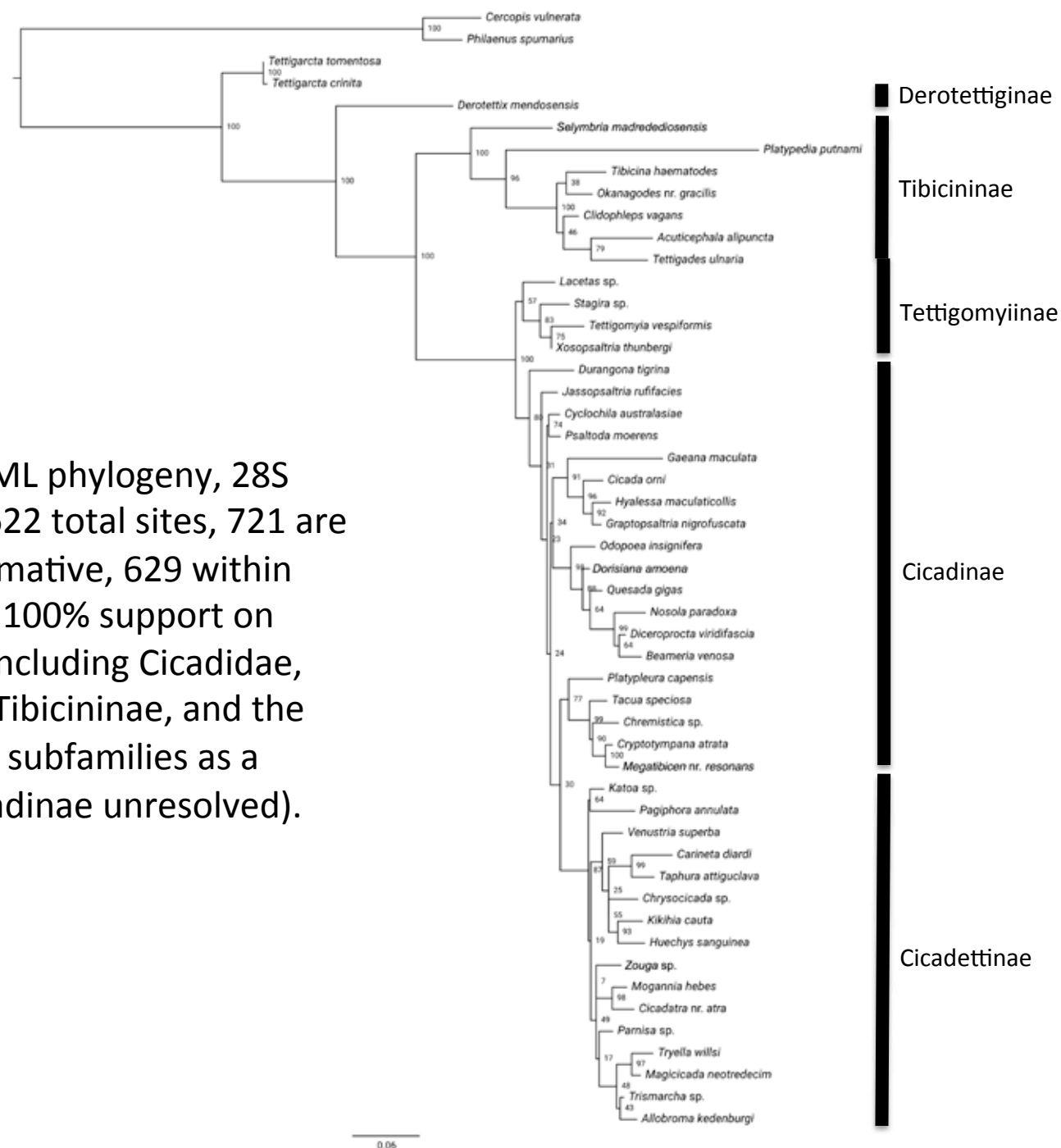
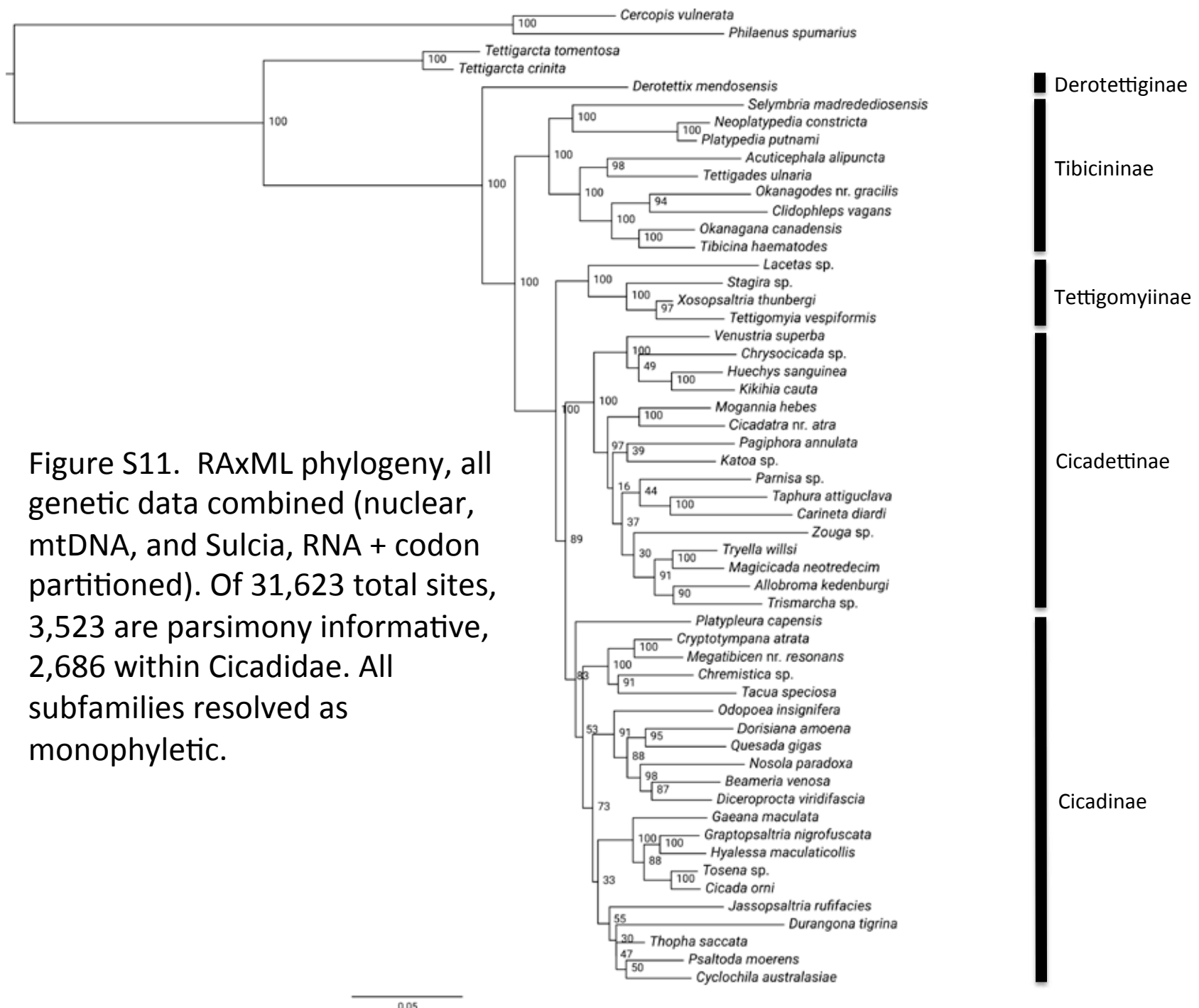


Figure S10. RAxML phylogeny, 28S gene only. Of 4622 total sites, 721 are parsimony informative, 629 within Cicadidae. Note 100% support on deepest nodes including Cicadidae, Derotettiginae, Tibicininae, and the three remaining subfamilies as a trichotomy (Cicadinae unresolved).





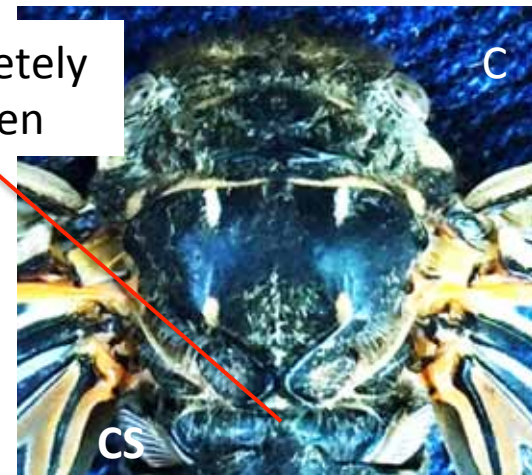
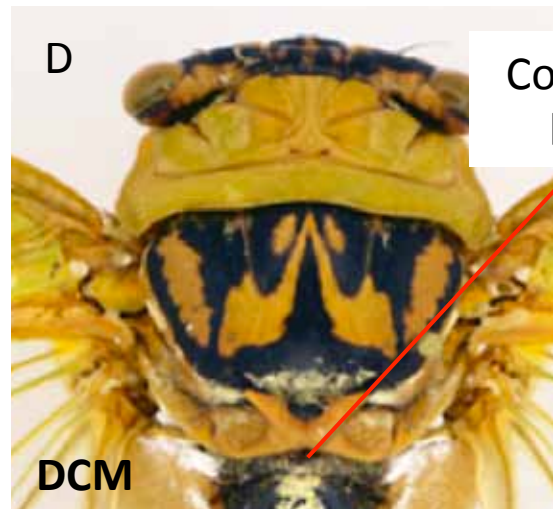
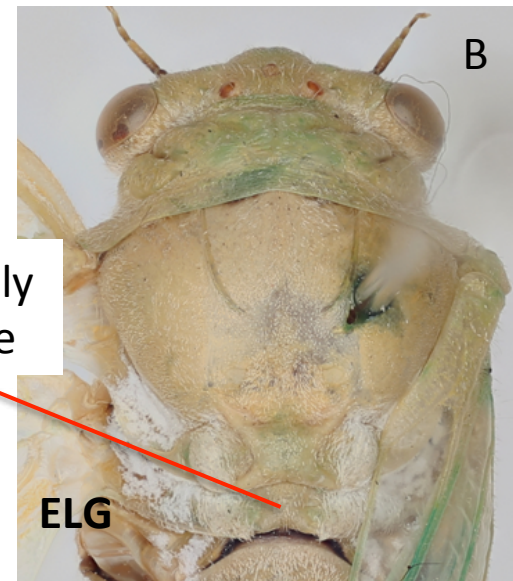
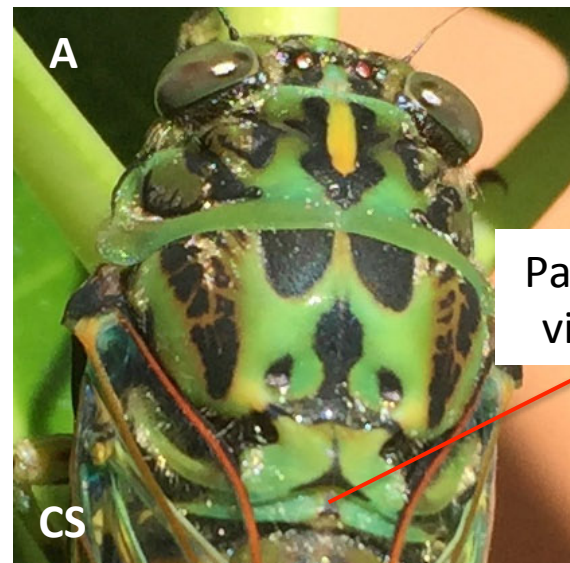
Supplementary Table 1. Character matrix of the 83 taxa used in the parsimony analysis. Missing data and character states not relevant to a taxon are scored as '?'.

	000000000	000000000	000000000	000000000	000000000	000000000	000000000	000000000	000000000	000000000	111111111	1111111
	000000000	111111111	222222222	333333333	444444444	555555555	666666666	777777777	888888888	999999999	000000000	1111111
	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	1234567
Cercopidae (outgroup)	??000???	??0?????	0????0??	0???????	0???????	0???????	0???????	0???????	0???????	00000000	00000000	0?00000
<i>Adelia borealis</i>	001000010	001000?00	110010000	010100110	0000???	0110???	102020110	210101100	0000???	1??111111	111111000	0?00000
<i>Aleeta curvica</i>	100000000	001000?00	010010000	110100040	0010???	0110???	113020120	1?30?0000	001111101	111111111	111111000	0000000
<i>Anapsaltoda pulchra</i>	200001100	010000?00	000010000	000100020	102203000	010???	0100240	1?10?0000	0000???	1??111111	111111000	0?00000
<i>Arenopsaltria fullo</i>	200000000	010000?00	000110000	000100020	100203000	00?????	0100240	1?10?0000	0000???	1??111111	111111000	0?00000
<i>Arunta perulata</i>	200001000	010000?00	000110000	000100060	10121?000	00?????	01000190	1?00?0000	000012101	111111111	111111000	0000000
<i>Atrapsalta encaustica</i>	001000000	001000?00	040010000	110100110	0000???	11111??	102020170	200110000	0000?2101	1?1111111	111111000	0?00000
<i>Birima castanea</i>	001000000	001000?00	040010000	010100110	0010???	0110???	102020360	1?0120010	100001101	111111111	111111000	0000000
<i>Burbunga albofasciata</i>	200000000	000000?00	000110000	000100000	000200000	00?????	01000240	1?40?0000	0000???	1?1111111	111111000	0?00000
<i>Burbunga gilmorei</i>	200000000	0?0000?00	000110000	000100000	000200000	00?????	0100240	1?40?0000	00001?101	1?1111111	111111000	0?00000
<i>Burbunga hillieri</i>	200000000	000000?00	000110000	000100000	000200000	00?????	01000240	1?40?0000	0000???	1?1111111	111111000	0?00000
<i>Chlorocysta vitripennis</i>	011000000	001031111	011?10001	010100031	0000???	010???	103020150	1?20?0000	000012101	1?1111111	111111000	0?00000
<i>Chrysocicada franceaustrale</i>	000000000	001000?00	010010000	010100040	0010???	0110???	113020120	1?30?0000	0000???	1??111111	111111000	0?00000
<i>Cicada orni</i>	100000000	010000?00	010110000	000100000	010200100	00?????	01000230	1?10?0000	00001?101	1?1111111	111111000	0?00000
<i>Cicadetta montana</i>	001000000	001000?00	030010000	110100110	00?0???	0110???	102020360	1?0120000	0000???	1??111111	111111000	0?00000
<i>Clinata nodicosta</i>	000000000	001000?02	040010000	010100180	0000?????	0110???	102020110	1?1011100	0000???	1??111111	111111000	0000000
<i>Cosmopsaltria doryca</i>	100000010	010000?00	010110000	001100050	110202100	00?????	2?1100270	1?00?0000	0000???	1??111111	111111000	0?00000
<i>Cryptotympana facialis</i>	200001000	010000?00	000010000	000100020	100203000	00?????	01000240	1?10?0010	0000???	1??111111	111111000	0?00000
<i>Cyclochila australasiae</i>	100002201	010000?00	000111000	000102020	?00200000	00?????	01000240	1?10?0000	000012101	111111111	111111000	0000000
<i>Cyclochila virens</i>	100002201	010000?00	000111000	000102020	?00200000	00?????	01000240	1?10?0000	000012101	111111111	111111000	0000000
<i>Cystopsaltria immaculata</i>	011000000	10105????	010?10110	010110011	0000???	010???	103020150	1?20?0000	0000???	1??111111	111111000	0?00000
<i>Cystosoma saundersii</i>	011000000	10105????	020?10110	010110011	0000???	010???	103020150	1?20?0000	000011101	111111111	111111000	0000000
<i>Derotettix mendosensis</i>	100000000	001070?00	000?00000	010000010	0000???	007???	0700?1400	0000?0110	0000?0001	1??111101	111111000	0?00000
<i>Diceropyga subapicalis</i>	100000010	000000?00	010110000	001100050	110202100	00?????	2?1100270	1?00?0010	0000???	1??111111	111111000	0?00000
<i>Diemeniana frenchi</i>	001000010	001000?00	010010000	010100010	0000???	0110???	102020110	210101100	000002101	111111111	111111000	0000000
<i>Dundubia vaginata</i>	100001010	010000?00	010110000	001100050	110202100	00?????	01000230	1?10?0000	0000???	1??111111	111111000	0?00000
<i>Froggattoides typicus</i>	000000000	001000?00	040010000	011100110	0000???	007???	102020170	210101100	0000???	1??111111	111111000	0?00000
<i>Galanga labeculata</i>	001000000	001000?00	040010000	010100110	0000???	0110???	102020110	210101100	0000?2101	1??111111	111111000	0?00000
<i>Glaucopsaltria viridis</i>	011000000	001041111	011?10001	010100031	0000???	010???	103020150	1?20?0000	0000???	1??111111	111111000	0?00000
<i>Gudanga boulayi</i>	000000000	001000?00	010010000	010100010	0000???	01110??	102020110	1?0101000	0000???	1??111111	111111000	0?00000
<i>Guineapsaltria flava</i>	011000000	001000?01	011010000	010100031	0000???	010???	103020150	1?20?0000	00000?101	1?1111111	111111000	0?00000
<i>Gymnotympana strepitans</i>	011000000	001000?01	011010000	011100071	0000???	010???	103020150	1?20?0000	0000???	1??111111	111111000	0?00000
<i>Gymnotympana varicolor</i>	011000000	001000?01	011010000	011100031	0000???	010???	103020150	1?20?0000	0000???	1??111111	111111000	0?00000
<i>Henicopsaltria eydouxii</i>	200000000	0?0000?00	000?10000	000100020	100203000	010???	01000240	1?10?0000	000011101	111111111	111111000	0000000
<i>Henicopsaltria rufivelum</i>	200000000	0?0000?00	000110000	000100020	100203000	010???	01000240	1?10?0000	0000???	111111111	111111000	0000000
<i>Illyria burkei</i>	200000000	010000?00	000110000	000100060	000202000	00?????	01000240	1?10?0000	0100?1101	11?111111	111111000	0000000
<i>Jassopsaltria rufifacies</i>	100010000	000000?00	000110000	000100000	000200000	00?????	01000240	1?10?0000	0000???	1??111111	111111000	0?00000
<i>Jassopsaltria sp. A</i>	100010000	000000?00	000110000	000100000	000200000	00?????	01000240	1?10?0000	0000???	1??111111	111111000	0?00000
<i>Kikihia subalpina</i>	001000000	001000?00	040010000	010100110	0000???	0110???	102020360	1?0120000	0000???	1??111111	111111000	0?00000
<i>Kobonga umbrimargo</i>	001000010	001000?00	010110000	010100110	0000???	0110???	102020110	210101100	0000???	1??111111	111111000	0?00000

Supplementary Table 1 (continued). Character matrix of the 83 taxa used in the parsimony analysis. Missing data and character states not relevant to a taxon are scored as '?'.

	000000000	000000000	000000000	000000000	000000000	000000000	000000000	000000000	000000000	000000000	111111111	1111111
	000000000	111111111	222222222	333333333	444444444	555555555	666666666	777777777	888888888	999999999	000000000	1111111
	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	123456789	1234567
<i>Lembeja vitticollis</i>	011000000	101000?00	010010110	0?0100010	0000???710	010?70111	103020110	1?00?0000	0000??7101	1??111111	111111000	0?00000
<i>Lyrastes plebejus</i>	200000000	010000?00	000?10000	000100020	100203000	00?????101	0?1000240	1?10?0010	0000??7101	1??111111	111111000	0?00000
<i>Macrotristria angularis</i>	200001000	010000?00	000110000	000100020	000202000	00?????131	0?1000240	1?10?0000	010011101	111111111	111111000	0000000
<i>Macrotristria intersecta</i>	200001000	010000?00	000110000	000100020	000202000	00?????101	0?1000240	1?10?0000	0100??7101	111111111	111111000	0000000
<i>Magiciada septendecim</i>	000000010	001000?00	010010000	0101000?0	0000???710	0113??201	113020120	1?30?0000	00011?7101	1?1111111	111111000	0?00000
<i>Marteena rubricincta</i>	001000010	001000?00	110010000	010100110	0000???701	0110??7101	102020110	210101100	0000??7101	1??111111	111111000	0?00000
<i>Mugadina marshalli</i>	000000000	001000?01	040010000	010100110	0000???701	0110??7101	112020180	200130000	0000??7101	1??111111	111111000	0000000
<i>Myopsalta atrata</i>	001000000	001000?00	040010000	010100110	0000???701	0110??7101	102020110	210101100	0000??7101	1??111111	111111000	0?00000
<i>Neopsaltoda crassa</i>	200001100	010000?00	010?10000	000100020	101203000	010?70100	0?1010240	1?10?0000	0000??7101	1??111111	111111000	0?00000
<i>Neopunia graminis</i>	001000000	001000?01	040010000	010100110	0000???701	0113??121	102020170	200110000	0000??7101	1??111111	111111000	0?00000
<i>Nanopsalta basalis</i>	001000000	001000?01	040010000	010100110	0000???701	0113??121	102020170	200110000	0000??7101	1??111111	111111000	0?00000
<i>Odopoea dilatata</i>	001100300	000100?00	020110000	001110000	000201000	00?????101	0?1000010	1?00?0000	0000??7101	1??111111	111111000	0?00000
<i>Owra insignis</i>	011000000	001021011	011?10100	0101000?1	0000???710	010?71111	103020150	1?20?0000	0000??7101	1??111111	111111000	0?00000
<i>Oxypleura calypso</i>	200000300	010000?00	000110000	000110020	000202000	00?????101	0?1000240	1?00?0000	0000??7101	1??111111	111111000	0?00000
<i>Palapsalta circumdata</i>	001000000	001000?00	040010000	110100110	0000???701	11111?7101	102020170	200110000	0000??7101	1?1111111	111111000	0?00000
<i>Palapsalta eyrei</i>	001000000	001000?01	040010000	010100110	0000???701	01111?7101	112020170	200110010	000002101	111111111	111111000	0000000
<i>Palapsalta</i> sp. M	001000000	001000?01	040010000	010100110	0000???701	01111?7101	112020170	200110010	00000?7101	111111111	111111000	0000000
<i>Pamisa designata</i>	01?000000	001010?01	010010000	010100011	0000???710	010?70111	103020?70	1?70?0000	0000??7101	1??111111	111111000	0?00000
<i>Parnkalla muelleri</i>	100000000	010000?00	010110000	000100000	000???700	00?????101	0?1000171	1?00?0000	00000?7101	1?1111111	111111000	0?00000
<i>Pauropsalta mneme</i>	001000010	001000?00	040010000	110100110	0000???701	01110?7101	102020170	200110000	000002101	1?1111111	111111000	0?00000
<i>Pictila occidentalis</i>	000000000	001020?00	010010000	010100040	0000???710	0110??7101	103020120	1?30?0000	0000??7101	1??111111	111111000	0?00000
<i>Platypoea putnami</i>	100000000	001000?00	000?00000	000000090	0001?????	00?????100	0?1001010	1?00?0001	0000??7101	1??111111	111111000	0?00000
<i>Platypoea stridula</i>	200002200	010000?00	000010000	000110020	?00202000	00?????101	0?7010240	1?00?0000	0000??7101	1??111111	111111000	0?00000
<i>Plautilla venedictoffae</i>	000100300	000100?00	030?10100	011110070	00?20?700	00?????101	0?1000010	1?00?0000	0000??7101	1??111111	111111000	0?00000
<i>Prasia faticina</i>	01?000000	101000?00	110010100	011100010	0000???710	010?70111	103020110	1?70?0000	0000??7101	1??111111	111111000	0?00000
<i>Psaltoda moerens</i>	200001100	010000?00	010?10000	000100020	102203000	010?70100	0?1010240	1?10?0000	000011101	111111111	111111000	0000000
<i>Punia minima</i>	001000000	001000?01	040010000	010100110	0000???701	01130?7101	102020170	200110000	0000??7101	1??111111	111111000	0?00000
<i>Tamasa tristigma</i>	100000000	010000?00	010?10000	000100000	000200000	00?????10?	0?1000171	1?00?0000	000002101	111111111	111111000	0000000
<i>Taphura misella</i>	000000000	001000?00	010010000	010100080	0000???710	010?71101	1130201?0	1?00?0000	0000??7101	1??111111	111111000	0?00000
<i>Taurella forresti</i>	001000000	001000?00	040010000	010100180	0000???711	01110?7101	1020201?0	200110000	0000??7101	1??111111	111111000	0?00000
<i>Terepsalta infans</i>	00?000000	001000?00	010010000	010100110	0000???701	0110??7101	102020110	210101100	0000??7101	1??111111	111111000	0?00000
<i>Tettigades ulnaria</i>	100000200	010000?00	000?00000	000000090	0001?????	00?????101	0?1001010	1?00?0001	0000??7001	1??111111	111111000	0?00000
<i>Tettigarcta crinita</i>	00000?000	0?0000???	0?0001000	000011?70	0000???700	00?????0?0	0?0030400	0?00?0000	?00000001	110000000	000000111	1111111
<i>Tettigarcta tomentosa</i>	00000?000	0?0000???	0?0001000	000011?70	0000???700	00?????0?0	0?0030400	0?00?0000	?00000001	110000000	000000111	1111111
<i>Tettigomyia vespiformis</i>	000000000	0010700?0	020010100	011100000	0000???700	00?????120	0?1001110	1?00?0?00	0000??7101	111111111	111111000	0000000
<i>Thaumastopsaltia globosa</i>	011000000	001030?10	010?10100	010110031	0000???710	010?71111	103020150	1?20?0000	0000??7101	1??111111	111111000	0?00000
<i>Thopha saccata</i>	200001100	010000?00	000110000	000100060	10121?000	00?????101	0?1000190	1?00?0000	0000??7101	1??111111	111111000	0?00000
<i>Tibicina haematodes</i>	100000000	001000?00	000?00000	000000090	0001?????	00?????100	0?1001010	1?00?0001	0000??7101	1??111111	111111000	0?00000
<i>Tryella ochra</i>	100000000	001000?00	010010000	110100040	0010???710	01120?221	113020120	1?30?0000	0011??7101	1??111111	111111000	0?00000
<i>Urabanana sericeivitta</i>	001000000	001000?01	040010000	010100110	0000???701	0110??7101	1?2020180	200130000	0000??7101	1??111111	111111000	0?00000
<i>Venustria superba</i>	011000000	001010?01	011010000	010100010	0000???710	00?????111	1030201?0	1?20?0000	0000??7101	1??111111	111111000	0?00000
<i>Yoyetta celis</i>	001000000	001000?00	040010000	010100110	0000???701	0110??7101	102020360	1?0120010	1000?2101	11?111111	111111000	0000000
<i>Zammara intricata</i>	001100300	000100?00	030110000	001110000	000201000	00?????201	0?1000010	1?00?0000	0000??7101	1??111111	111111000	0?00000

Figure S12. Illustration of the subfamily character “metanotum at dorsal midline” with states “partially visible” and “completely hidden”. Clockwise from upper left, (A) Cicadettinae, *Amphipsalta zelandica* photo, CS; (B) Derotettiginae, *Derotettix mendosensis*, photo ERLG; (C) Tibicininae, *Alarcta micromacula*, Photo CS; (D) Cicadinae, *Neotibicen pronotalis*, Photo David C. Marshall (DCM).





Figs S13. (A-C) *Derotettix mendosensis*, Macropod® photos. 18.AR.MZ.EVT (Table 1). Color faded by ethanol. (A) dorsal, (B) head, (C) lateral view. Photos by ERLG.



Figure S14. *Derotettix mendosensis* male holotype, La Plata. (A) dorsal, (B) ventral; Macropod® Photos KN.



Figure S15. *Derotettix mendosensis* female allotype, La Plata. (A) dorsal, (B) ventral; Macropod[®] Photos KN.



Figure S16. *Derotettix wagneri* (= *D. proseni*) male holotype, La Plata. (A) dorsal, (B) ventral; Macropod® Photos KN.



Figure S17. (A) *D. wagneri* (= *D. proseni*) male holotype, lateral view, La Plata; (B) *D. mendosensis* male holotype, lateral view, La Plata. Macropod® Photos KN.



Table S2. Characters for the four subfamilies of the family Cicadidae modified from Marshall et al. (2018). Autapomorphies are highlighted in gray. Note that the Tettigomyiinae is diagnosable only by a combination of attributes.

CHARACTER	SUBFAMILY					
	Cicadinae	Cicadettinae	Tettigomyiinae	Tibicininae	Derottiginae	Tettigarctidae
Metanotum	Entirely concealed at dorsal midline.	Partly visible at dorsal midline.	Exposed or concealed (in Lacetasini n. tribe) at dorsal midline.	Concealed at dorsal midline (except in <i>Platypedia</i> , <i>Tibicina</i>).	Partially visible at dorsal midline	Exposed
Forewing veins CuP and 1A	Fused in part.	Fused in part.	Fused in part.	Unfused (fused in <i>Platypedia</i> and <i>Neoplatypedia</i>).	Unfused; adjacent for two thirds of length but widely diverging in distal third.	Unfused, clearly separated for their length.
Hindwing veins RP and M	Fused at their bases.	Fused at their bases.	Fused at their bases.	Unfused at their bases.	Unfused at their bases.	Unfused at their bases.
Male opercula shape	Not strongly S-shaped and not with lateral margins deeply concave.	Not strongly S-shaped and not with lateral margins deeply concave.	Not strongly S-shaped and not with lateral margins deeply concave.	A distinctive S-shape with lateral margins deeply concave, and with distal margins not reaching distal margins of tympanal cavities.	Rounded, enclosing tympanal cavity but not meeting.	Absent
Male timbal covers	Present but lost in a few genera.	Lacking timbal covers or with a partial turned-back rim.	Lacking timbal covers or with a partial turned-back rim.	Lacking timbal covers or with a partial turned-back rim.	Lacking timbal covers or a turned-back rim.	Absent
Pygofer distal shoulder	Well developed, often the most distal part of pygofer.	Undeveloped.	Well developed, often the most distal part of pygofer.	Undeveloped.	Undeveloped	Undeveloped
Pygofer upper lobe	Absent or present.	Present.	Absent.	Usually absent (present in Selymbriini n. tribe).	Absent	Absent

Table S2. Continued.

CHARACTER	SUBFAMILY					
	Cicadinae	Cicadettinae	Tettigomyiinae	Tibicininae	Derottiginae	Tettigarctidae
Uncus	Of moderate length and retractable within pygofer.	Absent or very small and duck-bill shaped, and retractable within pygofer.	Long, directed distally, not retractable within pygofer.	Very long and not retractable within pygofer.	Of moderate length, directed distally, not retractable within pygofer.	Very long, directed distally and not retractable within pygofer.
Claspers	Absent, but present in some Dundubiini (if present, spined).	Usually large (small in Kaotini n. tribe), not spined.	Absent.	Absent.	Absent.	Absent
Ventrobasal pocket of aedeagus	Absent.	Absent.	Absent.	Present.	Present	Present
Aedeagal restraint	Before or below uncus.	By claspers.	Before or below uncus.	By tubular encapsulation below uncus.	By tubular encapsulation below uncus.	By an apical slit in uncus.
Apical part of theca	Without leaf-like lateral lobes.	Without leaf-like lateral lobes.	Without leaf-like lateral lobes.	With a pair of leaf-like lateral lobes.	Without leaf-like lateral lobes.	With scleritised flanges.
Basal plate	Basally undivided	Basally undivided	Basally undivided	Basally undivided	Reduced on its basal half to form a pair of long slender lateral arms attached to the theca by sinuation.	Reduced on more than its basal half to form a pair of long slender lateral arms attached to the theca by sinuation.

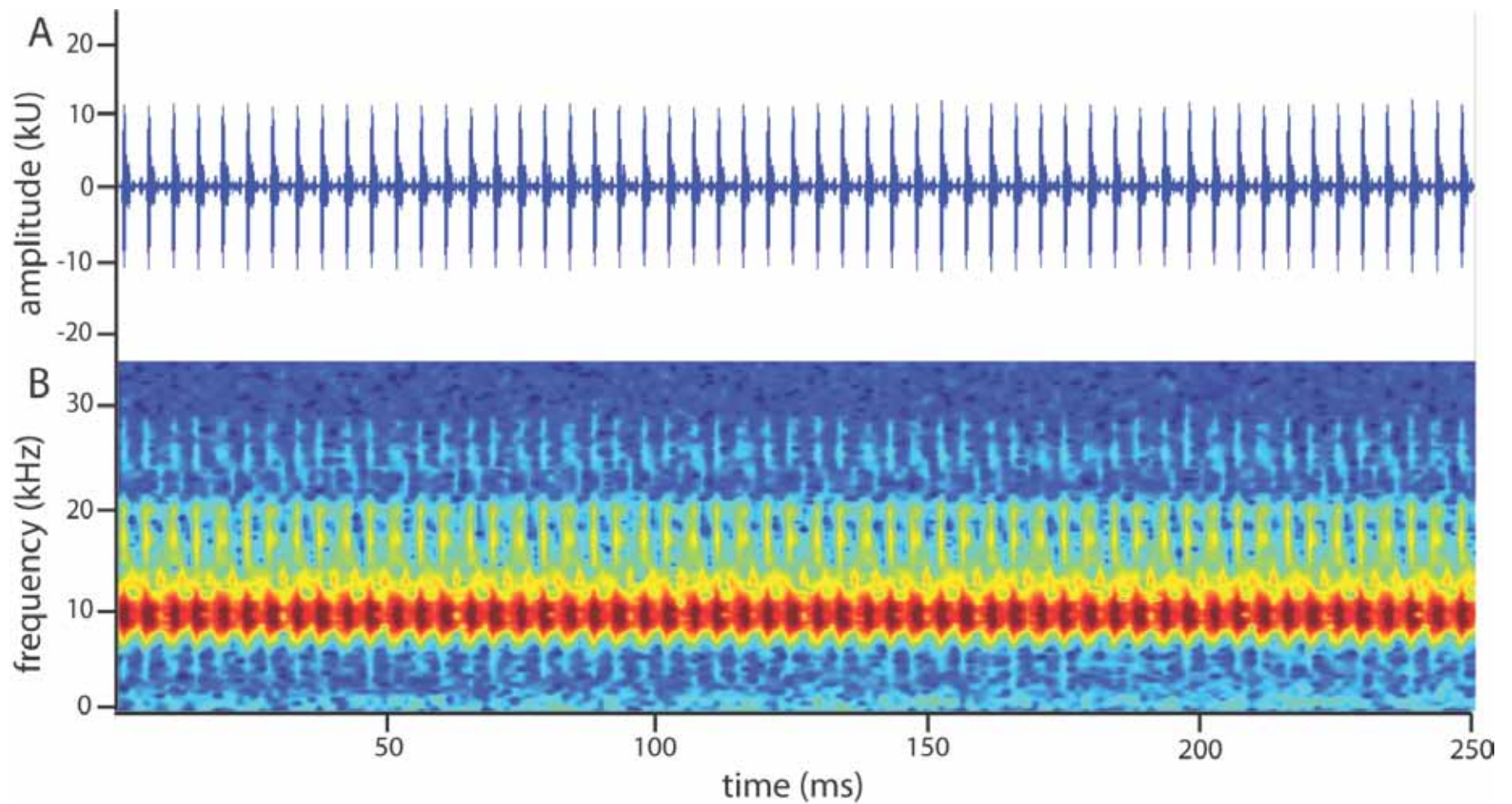


Figure S18. (A) *Derotettix mendosensis* song structure, recording 080110-05, 35.4 °C 18.AR.MZ.CLG. a. 0.5 s oscillogram (above), (B) spectrogram.

Fig. S19. Paleomap reconstructions redrawn/excerpted as permitted from Scotese, C. R., 2001. Atlas of Earth History, Volume 1, Paleogeography, PALEOMAP Project, Arlington, Texas, 52 pp. Note extensive South American inland sea incursions 80 Mya and again 20 Mya. See also Hernandez et al. 2005, Figure 2, Paranense Sea, 13-15 Mya.

