

300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling

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Abstract

Orthoptera is the most diverse order among the polyneopteran groups and includes familiar insects, such as grasshoppers, crickets, katydids, and their kin. Due to a long history of conflicting classification schemes based on different interpretations of morphological characters, the phylogenetic relationships within Orthoptera are poorly understood and its higher classification has remained unstable. In this study, we establish a robust phylogeny of Orthoptera including 36 of 40 families representing all 15 currently recognized superfamilies and based on complete mitochondrial genomes and four nuclear loci, in order to test previous phylogenetic hypotheses and to provide a framework for a natural classification and a reference for studying the pattern of divergence and diversification. We find strong support for monophyletic suborders (Ensifera and Caelifera) as well as major superfamilies. Our results corroborate most of the higher-level relationships previously proposed for Caelifera, but suggest some novel relationships for Ensifera. Using fossil calibrations, we provide divergence time estimates for major orthopteran lineages and show that the current diversity has been shaped by dynamic shifts of diversification rates at different geological times across different lineages. We also show that mitochondrial tRNA gene orders have been relatively stable throughout the evolutionary history of Orthoptera, but a major tRNA gene rearrangement occurred in the common ancestor of Tetrigoidea and Acridomorpha, thereby representing a robust molecular synapomorphy, which has persisted for 250 Myr.

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Introduction

With more than 25 700 extant species, Orthoptera is the most diverse order among the polyneopteran insect lineages (Grimaldi and Engel, 2005; Eades et al., 2014). The order includes familiar singing insects, such as crickets and katydids, as well as often devastating pests, such as grasshoppers and locusts (Gangwere

et al., 1997). Orthopteran insects have diversified into numerous lineages that occupy every conceivable terrestrial habitat outside the polar regions and play integral roles in the ecosystem (Uvarov, 1966; Kevan, 1982). Such diversity in form and function has attracted researchers who use these insects as model systems for studying anatomy, physiology, neurobiology, bioacoustics, chemical ecology, life-history traits, speciation research, and evolutionary ecology (Uvarov, 1966, 1977; Baccetti, 1987; Chapman and Joern, 1990; Gangwere et al., 1997; Pener and Simpson, 2009).

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The monophyly of Orthoptera is strongly supported by several morphological characters, such as the presence of the cryptopleuron and jumping hind legs among others (Kevan, 1982; Kristensen, 1991; Grimaldi and Engel, 2005), and the order is considered to be divided into two suborders: Ensifera (crickets, katydids, wetas, and their relatives) (Fig. 1) and Caelifera (grasshoppers, locusts, and their relatives) (Fig. 2). Despite the familiarity and the diversity of the group, the phylogenetic relationships within Orthoptera are poorly understood and its higher classification remains unstable due to a long history of conflicting taxonomic

hypotheses based on different character sets, such as fossil wing venation (Zeuner, 1942; Sharov, 1968; Gorochov, 1995a), internal organs (Slifer, 1939; Judd, 1947; Dirsh, 1957; Baccetti, 1987), external morphology (Blackith and Blackith, 1968; Vickery and Kevan, 1983), and the male phallic complex (Chopard, 1920; Ander, 1939; Roberts, 1941; Dirsh, 1973; Amédégato, 1974; Eades, 2000). Most of these hypotheses are pre-cladistic and lack formal analyses, and there are major disagreements among even those based on the same set of morphological characters. In 1975, the higher classification of Orthoptera reached its most chaotic



Fig. 1. Representatives of Ensifera. (a) Grylloidea: Gryllidae: *Brachytrupes membranaceus*; (b) Gryllotalpoidea: Gryllotalpidae: *Gryllotalpa africana*; (c) Schizodactyloidea: Schizodactylidae: *Comicus capensis*; (d) Stenopelmatoidea: Stenopelmatidae: *Sia* sp.; (e) Rhaphidophoroidea: Rhaphidophoridae: *Diestrammena asynamora*; (f) Hagloidea: Prophalangopsidae: *Cyphoderris monstrosa*; (g) Tettigonioidae: Tettigoniidae: *Pterochroza ocellata* (Photograph credit: Piotr Naskrecki).



Fig. 2. Representatives of Caelifera. (a) Tridactyloidea: Tridactylidae: *Xya* sp.; (b) Tetrigoidea: Tetrigidae: *Afrolarcus* sp.; (c) Proscopioidea: Proscopidae: *Tetanorhynchus* sp.; (d) Eumastacoidea: Episactidae: *Episactus tristani*; (e) Tanaoceroidea: Tanaoceridae: *Tanaocerus koebeleii*; (f) Pneumoroidea: Pneumoridae: *Bullacris* sp.; (g) Trigonopterygoidea: Trigonopterygidae: *Systella rafflesii*; (h) Pyrgomorphoidea: Pyrgomorphidae: *Zonocerus variegatus*; (i) Acridoidea: Acrididae: *Locustana pardalina* (Photograph credit: Piotr Naskrecki [a, b, f, h, i], Paul Lenhart [c], Robert A. Behrstock [d], Hartmut Wisch [e], Kurt Orion G [g]).

state when Dirsh proposed a superorder Orthopteroidea with ten new orders (Dirsh, 1975). Although contemporary orthopterists agree that Dirsh's treatment was extreme (Gurney, 1976; Kevan, 1976), the aftermath of taxonomic instability still lingers today and many new species are being described under conflicting classification schemes (Song, 2010).

Several researchers have proposed different higher classification schemes for Orthoptera and the major lineages based on modern cladistic methods using morphology and molecules (Fig. 3). Flook et al. (1999)

produced the first modern phylogeny of Orthoptera based on 31 ingroup taxa representing all major lineages and three ribosomal loci, and redefined some superfamily concepts. Due to small taxon and character sampling, however, some of the relationships were not fully resolved, but this work still stands as the most comprehensive work for the entire order until now. In recent years, a number of studies used complete mitochondrial genome (mtgenome) sequences to infer the phylogeny of Orthoptera (Fenn et al., 2008; Sheffield et al., 2010; Zhou et al., 2010; Zhang et al.,

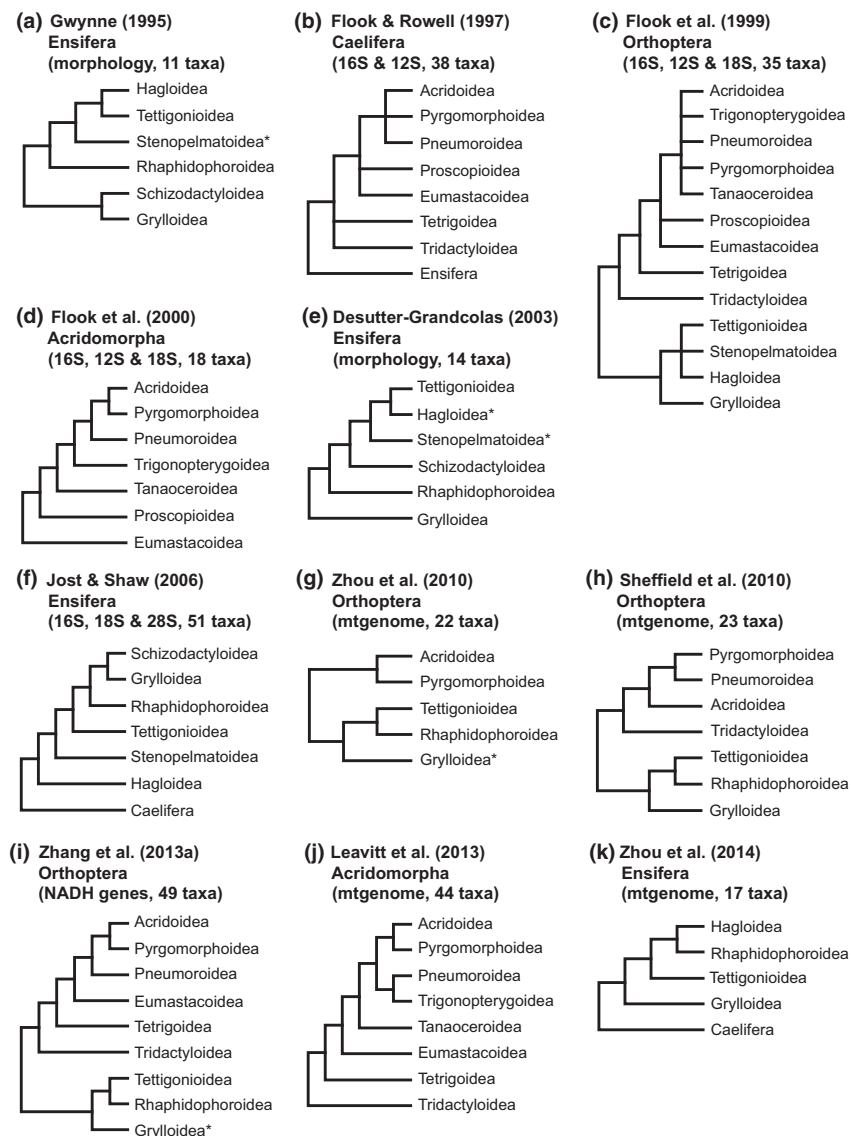


Fig. 3. Previous phylogenetic hypotheses of Orthoptera based on modern cladistic methods. Nodes from the original studies have been collapsed to show superfamily-level relationships. Asterisks represent superfamilies *sensu* Eades et al. (2014) that were not found to be monophyletic in the original studies.

2013a), but most of these had a relatively small taxon sampling often based only on available mtgenome data, not enough to seriously test previous classification schemes. At the level of suborders, Gwynne (1995) presented the first explicit cladistic analysis of Ensifera based on morphological characters coded from the taxonomic literature and used it to address the evolution of acoustic communication and mating behaviour in Ensifera. Nevertheless, this study also met with some sharp criticisms based on methodological grounds (Nickle and Naskrecki, 1997; Desutter-Grandcolas, 2003). Desutter-Grandcolas (2003) coded 85 morphological characters from 12 ensiferan terminals (three emblematic genera and nine families), which resulted in a well-resolved phylogeny, but this

taxon sampling did not allow generalizations to be made about ensiferan evolution because polymorphism was included in the data matrix. Jost and Shaw (2006) then reconstructed the phylogeny of Ensifera based on three ribosomal loci and 51 terminals, which resulted in a radically different phylogenetic relationship from previous hypotheses, but the dataset used in this work was later shown to be highly sensitive to different analytical methods (Legendre et al., 2010). Zhou et al. (2014) presented a mtgenome phylogeny of Ensifera based on a newly sequenced mtgenome of *Prophalangopsidae* (representing Hagloidea) and available ensiferan mtgenomes, but the study lacked several key taxa from basal ensiferans to make a significant impact. As for Caelifera, Flook and Rowell (1997) proposed the

Table 1
Taxonomic information and Genbank accession numbers for 258 taxa used in total evidence analysis

		Species	Voucher ID	18S	28S	H3	Wingless
ENSIFERA							
Grylloidea	Gryllidae	<i>Brachytrupes</i> sp.	OR426	KM853299	N/A	KM853569	KM853737
		<i>Meloimorpha japonica</i>	OR451	KM853308	KM853384	KM853561	KM853729
		<i>Eneroptera guyanensis</i>	OR434	KM853304	KM853388	KM853564	KM853732
		<i>Euscyrtus japonicus</i>	OR462	KM853311	KM853381	KM853558	N/A
		<i>Modicogryllus volivoli</i>	OR432	KM853302	KM853390	KM853566	KM853734
		<i>Sciobia finoti</i>	OR431	KM853301	KM853391	KM853567	KM853735
		<i>Acheta domesticus</i>	OR429	KM853300	KM853392	KM853568	KM853736
		<i>Gryllus assimilis</i>	OR016	KM853171	KM853519	KM853693	KM853856
		<i>Hapithus symphonos</i>	OR460	KM853310	KM853382	KM853559	KM853727
		<i>Nemobius sylvestris</i>	OR472	KM853315	KM853378	KM853554	KM853724
		<i>Argizala braziliensis</i>	OR468	KM853314	N/A	KM853555	N/A
		<i>Oecanthus niveus</i>	OR031	KM853175	KM853514	KM853689	KM853853
		<i>Paragryllus</i> sp.	OR458	KM853309	KM853383	KM853560	KM853728
		<i>Pentacentrus</i> sp.	OR463	KM853312	KM853380	KM853557	KM853726
		<i>Selvacla troxalis</i>	OR443	KM853306	KM853386	KM853563	KM853731
		<i>Phaloria</i> sp.	OR447	KM853307	KM853385	KM853562	KM853730
		<i>Sclerogryllus</i> sp.	OR433	KM853303	KM853389	KM853565	KM853733
		<i>Paroecanthus</i> sp.	OR440	KM853305	KM853387	N/A	N/A
		<i>Anaxipha praeopostera</i>	OR465	KM853313	KM853379	KM853556	KM853725
	Gryllotalpidae	<i>Gryllotalpa</i> sp.	OR050	KM853176	KM853513	KM853688	KM853852
		<i>Gryllotalpa</i> sp.	OR162	KM853183	KM853507	KM853682	KM853845
		<i>Scapteriscus</i> sp.	OR161	KM853182	N/A	N/A	N/A
	Mogoplistidae	<i>Hoplosphyrum</i> sp.	OR424	KM853298	N/A	KM853570	N/A
	Myrmecophilidae	<i>Myrmecophila manni</i>	OR022	KM853173	KM853517	KM853691	KM853855
Hagloidea	Prophalangopsidae	<i>Cyphoderris monstrosa</i>	OR021	KF570814	KF570943	KF571077	KF571212
		<i>Tarragoilus diuturnus</i> (mtgenome only)		N/A	N/A	N/A	N/A
Rhaphidophoroidea	Rhaphidophoridae	<i>Diestrammena unicolor</i>	OR418	KM853293	KM853396	KM853574	KM853742
		<i>Ceuthophilus utahensis</i>	OR017	KM853172	KM853518	KM853692	N/A
		<i>Gammarotettix genitalis</i>	OR029	N/A	KM853515	N/A	N/A
		<i>Troglophilus neglectus</i>	OR083	KF570820	KF570948	KF571092	KF571227
Schizodactyloidea	Schizodactylidae	<i>Comicus campestris</i>	OR416	KM853292	KM853397	KM853575	KM853743
		<i>Schizodactylus inexpectatus</i>	OR563	KM853344	N/A	KM853524	KM853696
Stenopelmatoidea	Anostomatidae	<i>Henicus</i>	OR420	KM853295	KF570949	KF571122	KM853740
		<i>brevimucronatus</i>					
		<i>Cnemotettix miniatus</i>	OR415	KM853291	KM853398	KM853576	KM853744
	Cooloolidae	<i>Cooloola ziljan</i>	OR412	KM853290	KM853399	KM853577	KM853745
	Gryllacrididae	<i>Hadrogyllacris</i> sp.	OR421	KM853296	KM853394	KM853572	KM853739
		<i>Prosopogryllacris japonica</i>	OR419	KM853294	KM853395	KM853573	KM853741
		<i>Camptonotus carolinensis</i>	OR024	KF570818	KF570941	KF571078	KF571213
	Stenopelmatidae	<i>Stenopelmatus fuscus</i>	OR014	KF570813	KF570940	KF571076	KM853857
Tettigonioidae	Tettigoniidae	<i>Pachysaga</i> sp.	OR484	KF570757	KF570897	KF571124	KF571258
		<i>Hemisaga</i> sp.	OR483	KF570758	KF570896	KF571123	KF571257
		<i>Deracantha onos</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Macroxiphus sumatranus</i>	OR381	KF570803	KF570930	KF571110	KF571245
		<i>Ruspolia</i> sp.	OR380	KF570793	KF570923	KF571109	KF571244
		<i>Odontolakis virescens</i>	OR379	KF570792	KF570932	KF571108	KF571243
		<i>Salomona</i> sp.	OR145	KF570791	KF570928	KF571100	KF571235
		<i>Copiphora rhinoceros</i>	OR142	KF570790	KF570918	KF571099	KF571234
		<i>Conocephalus dorsalis</i>	OR082	N/A	N/A	KF571091	KF571226
		<i>Enyaliopsis</i> sp.	OR177	KF570690	KF570871	KF571102	KF571237
		<i>Acanthoplus</i> sp.	OR176	KF570692	KF570873	KF571101	KF571236
		<i>Glenophis borneo</i>	OR638	KF570686	KF570903	KF571193	KF571327
		<i>Hexacentrus japonicus</i>	OR382	KF570685	N/A	KF571111	KF571246
		<i>Lipotactes maculatus</i>	OR634	KF570698	KF570876	KF571189	KF571323
		<i>Mortoniellus ovatus</i>	OR633	KF570697	KF570875	KF571188	KF571322

Table 1
(Continued)

		Species	Voucher ID	18S	28S	H3	Wingless
Tettigonioidea	Tettigoniidae	<i>Meiophis micropennis</i>	OR657	KF570812	KF570889	KF571458	KF571346
		<i>Arachnoscelis rehni</i>	OR582	KF570695	KF570900	KF571139	KF571273
		<i>Alloteratura</i> sp.	OR636	KF570703	KF570878	KF571191	KF571325
		<i>Kuzicus megaterminatus</i>	OR635	KF570701	KF570877	KF571190	KF571324
		<i>Xizicus fascipes</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Mecopoda elongata</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Eumecopoda</i> sp.	OR385	KF570771	KF570912	KF571113	KF571248
		<i>Zitsikama tessellata</i>	OR384	KF570756	KF570881	KF571112	KF571247
		<i>Phylloptera</i> sp.	OR388	KF570858	KM853413	KF571116	KF571251
		<i>Eurycophora</i> sp.	OR387	KF570743	KF570863	KF571115	KF571250
		<i>Dysonia</i> sp.	OR386	KF570722	KF570849	KF571114	KF571249
		<i>Trigonocorypha</i> sp.	OR378	KF570745	KF570844	KF571107	KF571242
		<i>Phaneroptera falcata</i>	OR076	KF570718	KF570864	KF571088	KF571223
		<i>Barbitistes serricauda</i>	OR069	KF570742	KF570859	KF571084	KF571219
		<i>Acrometopa macropoda</i>	OR043	KF570717	KF570853	KF571082	KF571217
		<i>Sinochlora longifissa</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Elimaea cheni</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Phasmodes</i> sp.	OR485	KF570817	KF570944	KF571125	KF571259
		<i>Phyllophora</i> sp.	OR132	KF570816	KF570911	KF571096	KF571231
		<i>Sasima</i> sp.	OR131	KF570770	KF570910	KF571095	KF571230
		<i>Phrictaetypus viridis</i>	OR393	KF570772	KF570909	KF571121	KF571256
		<i>Pantecphylus</i> sp.	OR392	KF570707	KF570823	KF571120	KF571255
		<i>Teleutias</i> sp.	OR391	KF570815	KF570829	KF571119	KF571254
		<i>Idiarthron</i> sp.	OR389	KF570712	KF570827	KF571117	KF571252
		<i>Panoploscelus</i> sp.	OR377	KF570713	KF570826	KF571106	KF571241
		<i>Typophyllum</i> sp.	OR196	KF570693	KF570946	KF571103	KF571238
		<i>Cymatomera</i> sp.	OR139	KF570779	KF570885	KF571098	KF571233
		<i>Zabalius ophthalmicus</i>	OR138	KF570884	KF570975	KF571369	KM853848
		<i>Clonia</i> sp.	OR201	KF570699	KF570880	KF571105	KF571240
		<i>Peringueyella</i> sp.	OR199	KF570810	KF570905	KF571104	KF571239
		<i>Requena</i> sp.	OR553	KF570696	KF570901	KF571133	KF571267
		<i>Pholidoptera</i> <i>griseoptera</i>	OR079	KF570767	KF570893	KF571089	KF571224
		<i>Tettigonia cantans</i>	OR075	KF570765	N/A	KF571087	KF571222
		<i>Anabrus simplex</i>	OR034	KF570763	KF570890	KF571081	KF571216
		<i>Gampsocleis gratiosa</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Tympanophora</i> sp.	OR486	KF570777	KF570947	KF571126	KF571260
		<i>Kawanaphila</i> sp.	OR487	KF570700	KF570882	KF571127	KF571261
CAELIFERA	Acridoidea						
	Acrididae	<i>Truxalis</i> sp.	OR510	KM853325	KM853367	KM853543	KM853714
		<i>Hyalopteryx rufipennis</i>	OR240	KM853210	KM853480	KM853655	KM853819
		<i>Calephorus</i> <i>compressiocornis</i>	OR192	KM853192	KM853498	KM853673	KM853836
		<i>Acrida willemsei</i>	OR059	KM853177	KM853512	KM853687	KM853851
		<i>Phlaeoba albonema</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Paracaloptenus</i> <i>caloptenoides</i>	OR194	KM853194	KM853496	KM853671	KM853834
		<i>Calliptamus barbarus</i>	OR193	KM853193	KM853497	KM853672	KM853835
		<i>Kinangopa jeanelli</i>	OR574	KM853345	KM853348	KM853523	KM853695
		<i>Aresceutica morogorica</i>	OR506	KM853322	KM853371	KM853547	KM853717
		<i>Pezocatantops</i> sp.	OR505	KM853321	KM853372	KM853548	KM853718
		<i>Urnisiella rubropunctata</i>	OR499	KM853320	KM853373	KM853549	KM853719
		<i>Stenocatantops</i> <i>vitripennis</i>	OR498	KM853319	KM853374	KM853550	KM853720
		<i>Rusurplia tristis</i>	OR497	KM853318	KM853375	KM853551	KM853721

Table 1
(Continued)

		Species	Voucher ID	18S	28S	H3	Wingless
Acridoidea	Acrididae	<i>Retuspia validicornis</i>	OR496	KM853317	KM853376	KM853552	KM853722
		<i>Porraxia</i> sp.	OR494	KM853316	KM853377	KM853553	KM853723
		<i>Catantops</i> sp.	OR237	KM853209	KM853481	KM853656	KM853820
		<i>Macrolopholia</i> sp.	OR235	KM853208	KM853482	KM853657	KM853821
		<i>Xenocatantops</i> <i>brachycerus</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Traulia szetschuanensis</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Cyphacris</i> sp.	OR334	KM853251	KM853439	KM853615	KM853782
		<i>Copiocera</i> sp.	OR333	KM853250	KM853440	KM853616	KM853783
		<i>Eucoptacra</i> sp.	OR509	KM853324	KM853368	KM853544	KM853715
		<i>Parepistaurus deses</i>	OR508	KM853323	KM853369	KM853545	KM853716
		<i>Rhadinacris</i> <i>schistocercoides</i>	OR547	KM853341	KM853351	KM853527	KM853699
		<i>Nomadacris</i> <i>septemfasciata</i>	OR545	KM853340	KM853352	KM853528	KM853700
		<i>Ornithacris</i> sp.	OR544	KM853339	KM853353	KM853529	KM853701
		<i>Schistocerca</i> sp.	OR185	KM853186	KM853504	KM853679	KM853842
		<i>Anacridium</i> sp.	OR184	KM853185	KM853505	KM853680	KM853843
		<i>Cyrtacanthacris tatarica</i>	OR181	KM853184	KM853506	KM853681	KM853844
		<i>Chondracris rosea</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Euryphymus</i> sp.	OR314	KM853243	KM853447	KM853623	KM853790
		<i>Rhachitopsis</i> sp.	OR312	KM853242	KM853448	KM853624	KM853791
		<i>Calliptamulus</i> sp.	OR311	KM853241	KM853449	KM853625	KM853792
		<i>Heteracris</i> sp.	OR310	KM853240	KM853450	KM853626	KM853793
		<i>Eyprepocnemis plorans</i>	OR309	KM853239	KM853451	KM853627	KM853794
		<i>Cataloipus</i> sp.	OR218	KM853201	KM853489	KM853664	KM853828
		<i>Shirakiacris shirakii</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Aulocara ellioti</i>	OR521	KM853329	KM853363	KM853539	KM853710
		<i>Mermiria intertexta</i>	OR520	KM853328	KM853364	KM853540	KM853711
		<i>Rhammatocerus</i> <i>schistocercoides</i>	OR346	KM853258	KM853432	KM853608	KM853776
		<i>Prorocorypha snowi</i>	OR228	KM853207	KM853483	KM853658	KM853822
		<i>Syrbula montezuma</i>	OR227	KM853206	KM853484	KM853659	KM853823
		<i>Dichromorpha viridis</i>	OR226	KM853205	KM853485	KM853660	KM853824
		<i>Gomphocerus sibiricus</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Arcyptera coreana</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Kassongia vittata</i>	OR507	N/A	KM853370	KM853546	N/A
		<i>Leptacris</i> sp.	OR304	KM853238	KM853452	KM853628	KM853795
		<i>Pristocorypha</i> sp.	OR303	KM853237	KM853453	KM853629	KM853796
		<i>Loryma</i> sp.	OR302	KM853236	KM853454	KM853630	KM853797
		<i>Stenacris</i> sp.	OR342	KM853255	KM853435	KM853611	KM853778
		<i>Tetrataenia</i> sp.	OR338	KM853254	KM853436	KM853612	KM853779
		<i>Leptysma marginicollis</i>	OR214	KM853199	KM853491	KM853666	KM853830
		<i>Marellia remipes</i>	OR344	KM853256	KM853434	KM853610	N/A
		<i>Ognevia longipennis</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Hesperotettix viridis</i>	OR517	KM853327	KM853365	KM853541	KM853712
		<i>Bradynotes obesa</i>	OR515	KM853326	KM853366	KM853542	KM853713
		<i>Prumna halrasana</i>	OR395	KM853277	KM853412	KM853589	KM853758
		<i>Anapodisma miramae</i>	OR356	KM853265	KM853425	KM853601	KM853769
		<i>Jivarus ronderosi</i>	OR328	KM853249	KM853441	KM853617	KM853784
		<i>Dichroplini</i>	OR325	KM853248	KM853442	KM853618	KM853785
		<i>Melanoplus bivittatus</i>	OR245	KM853211	KM853479	KM853654	KM853818
		<i>Ceracris kiangsue</i> (mtgenome only)		N/A	N/A	N/A	N/A

Table 1
(Continued)

		Species	Voucher ID	18S	28S	H3	Wingless
Acridoidea	Acrididae	<i>Gastrimargus marmoratus</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Tomonotus ferruginosus</i>	OR523	KM853331	KM853361	KM853537	KM853708
		<i>Psinidia fenestralis</i>	OR522	KM853330	KM853362	KM853538	KM853709
		<i>Heteropternis</i> sp.	OR225	KM853204	KM853486	KM853661	KM853825
		<i>Locusta migratoria</i>	OR191	KM853191	KM853499	KM853674	KM853837
		<i>Acrotylus patruelis</i>	OR190	KM853190	KM853500	KM853675	KM853838
		<i>Aiolopus simulatrix</i>	OR188	KM853189	KM853501	KM853676	KM853839
		<i>Xanthippus</i> sp.	OR187	KM853188	KM853502	KM853677	KM853840
		<i>Trimerotropis</i> sp.	OR186	KM853187	KM853503	KM853678	KM853841
		<i>Locheuma brunneri</i>	OR366	KM853268	KM853422	KM853598	KM853766
		<i>Ommatolampis quadrimaculata</i>	OR364	KM853267	KM853423	KM853599	KM853767
		<i>Aspidophyma americana</i>	OR361	KM853266	KM853424	KM853600	KM853768
		<i>Vileria</i> sp.	OR336	KM853252	KM853438	KM853614	KM853781
		<i>Syntomacrella</i> sp.	OR323	KM853247	KM853443	KM853619	KM853786
		<i>Abracris</i> sp.	OR222	KM853202	KM853488	KM853663	KM853827
		<i>Kosciuscola tristis</i>	OR396	KM853278	KM853411	KM853588	KM853757
		<i>Oxya hyla</i>	OR315	KM853244	KM853446	KM853622	KM853789
		<i>Paulinia acuminata</i>	OR345	KM853257	KM853433	KM853609	KM853777
		<i>Poecilocloeus napoana</i>	OR368	KM853270	KM853420	KM853596	N/A
		<i>Coscineuta</i> sp.	OR249	KM853212	KM853478	KM853653	KM853817
		<i>Galidacris variabilis</i>	OR371	KM853271	KM853419	KM853595	KM853764
		<i>Paropaon</i> sp.	OR337	KM853253	KM853437	KM853613	KM853780
		<i>Spathosternum</i> sp.	OR224	KM853203	KM853487	KM853662	KM853826
		<i>Petamella prosternalis</i>	OR560	KM853343	KM853349	KM853525	KM853697
		<i>Tristria discoidalis</i>	OR543	KM853338	KM853354	KM853530	KM853702
	Lentulidae	<i>Rhainopomona magnificum</i>	OR539	KM853336	KM853356	KM853532	N/A
		<i>Usambilla olivacea</i>	OR538	KM853335	KM853357	KM853533	KM853704
		<i>Betiscoides</i> sp.	OR296	KM853235	KM853455	KM853631	KM853798
		<i>Lentula callani</i>	OR295	KM853234	KM853456	KM853632	KM853799
	Lithidiidae	<i>Lithidiopsis carinatus</i>	OR316	KM853245	KM853445	KM853621	KM853788
	Ommexechidae	<i>Graea horrida</i>	OR579	N/A	N/A	KM853522	N/A
		<i>Ommexecha brunneri</i>	OR367	KM853269	KM853421	KM853597	KM853765
	Pamphagidae	<i>Hoplolopha</i> sp.	OR288	KM853231	KM853459	KM853635	KM853802
		<i>Porthetis carinata</i>	OR216	KM853200	KM853490	KM853665	KM853829
		<i>Prionotropis hystrix</i>	OR151	KM853180	KM853509	KM853684	KM853847
		<i>Pseudotmethis rubimarginis</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Filchnerella helanshanensis</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Asiomethis zacharjini</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Thrinchus schrenkii</i> (mtgenome only)		N/A	N/A	N/A	N/A
	Pamphagodidae	<i>Hemicharilaus monomorphus</i>	OR540	KM853337	KM853355	KM853531	KM853703
	Pyrgacrididae	<i>Pyrgacris descampsi</i>	OR317	KM853246	KM853444	KM853620	KM853787
	Romaleidae	<i>Megacheilacris graminicola</i>	OR355	KM853264	KM853426	KM853602	KM853770
		<i>Aphanolampis aberrans</i>	OR354	KM853263	KM853427	KM853603	KM853771
		<i>Pseudonautia</i> sp.	OR353	KM853262	KM853428	KM853604	KM853772
		<i>Lagarolampis amazonica</i>	OR352	KM853261	KM853429	KM853605	KM853773
		<i>Hyleacris rubrogranulata</i>	OR350	KM853260	KM853430	KM853606	KM853774
		<i>Aeolacris octomaculata</i>	OR376	KM853276	KM853414	KM853590	KM853759
		<i>Agriacris magnifica</i>	OR375	KM853275	KM853415	KM853591	KM853760
		<i>Maculiparia rotundata</i>	OR374	KM853274	KM853416	KM853592	KM853761

Table 1
(Continued)

		Species	Voucher ID	18S	28S	H3	Wingless
Acridoidea	Romaleidae	<i>Acridophaea</i> sp.	OR373	KM853273	KM853417	KM853593	KM853762
		<i>Pareusychius defurcus</i>	OR372	KM853272	KM853418	KM853594	KM853763
		<i>Costalimacris</i> sp.	OR347	KM853259	KM853431	KM853607	KM853775
		<i>Diponthus argentinus</i>	OR267	KM853222	KM853468	KM853643	KM853810
		<i>Xyleus modestus</i>	OR265	KM853221	KM853469	KM853644	KM853811
		<i>Titanacris albipes</i>	OR085	KM853178	KM853511	KM853686	KM853850
	Tristiridae	<i>Tropidostethus angusticollis</i>	OR203	KM853196	KM853494	KM853669	KM853833
		<i>Atacamacris diminuta</i>	OR202	KM853195	KM853495	KM853670	N/A
		<i>Bufonacris claraziana</i>	OR207	KM853198	KM853492	KM853667	KM853831
		<i>Tristira magellanica</i>	OR204	KM853197	KM853493	KM853668	KM853832
		<i>Elasmoderus lutescens</i>	OR532	KM853334	KM853358	KM853534	KM853705
		<i>Erianthus versicolor</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Chorotypus fenestratus</i>	OR422	KM853297	KM853393	KM853571	KM853738
		<i>Xenerianthus affinis</i>	OR397	KM853279	KM853410	KM853587	KM853756
Eumastacoidea	Chorotypidae	<i>Pielomastax zhengi</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Eumastax salazari</i>	OR406	KM853285	KM853404	KM853581	KM853750
		<i>Morsea</i> sp.	OR401	KM853282	KM853407	KM853584	KM853753
	Episactidae	<i>Paramastax nigra</i>	OR407	KM853286	KM853403	KM853580	KM853749
		<i>Pseudomastax</i> sp.	OR408	KM853287	KM853402	KM853579	KM853748
		<i>Biroella</i> sp.	OR403	KM853283	KM853406	KM853583	KM853752
	Morabidae	<i>Warramunga</i> sp.	OR404	KM853284	KM853405	KM853582	KM853751
		<i>Thericlesiella</i> sp.	OR399	KM853281	KM853408	KM853585	KM853754
		<i>Pseudothericles compressifrons</i>	OR398	KM853280	KM853409	KM853586	KM853755
	Pneumoridae	<i>Physemacris variolosa</i>	OR293	KM853233	KM853457	KM853633	KM853800
	Proscopiidae	<i>Proscopia</i> sp.	OR411	KM853289	KM853400	KM853578	KM853746
		<i>Apioscelis</i> sp.	OR409	KM853288	KM853401	N/A	KM853747
Pyrgomorphaidea	Pyrgomorphidae	<i>Colemania sphenarioides</i>	OR286	KM853230	KM853460	KM853636	KM853803
		Orthacridinae	OR280	KM853226	KM853464	KM853639	N/A
		<i>Monistria discrepans</i>	OR527	KM853332	KM853360	KM853536	KM853707
		<i>Chrotogonus</i> sp.	OR284	KM853229	KM853461	KM853637	KM853804
		<i>Atractomorpha</i> sp.	OR282	KM853228	KM853462	N/A	KM853805
		<i>Algete brunneri</i>	OR281	KM853227	KM853463	KM853638	KM853806
		<i>Desmoptera</i> sp.	OR278	KM853225	KM853465	KM853640	KM853807
		<i>Pyrgomorpha granulata</i>	OR277	KM853224	KM853466	KM853641	KM853808
		<i>Phymateus morbillosus</i>	OR273	KM853223	KM853467	KM853642	KM853809
		<i>Mekongiella xizangensis</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Mekongiana xiangchengensis</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Tanaocerus koebelei</i>	OR559	KM853342	KM853350	KM853526	KM853698
	Tetrigoidea	Batrachideinae	OR261	KM853219	KM853471	KM853646	N/A
		<i>Systolederus spicupennis</i>	OR251	KM853214	KM853476	KM853651	N/A
		<i>Mazarredia convexa</i>	OR250	KM853213	KM853477	KM853652	KM853816
		<i>Thoradonta nodulosa</i>	OR253	KM853216	KM853474	KM853649	N/A
		<i>Scelimena melli</i>	OR252	KM853215	KM853475	KM853650	KM853815
		<i>Alulatettix yunnanensis</i> (mtgenome only)		N/A	N/A	N/A	N/A
		<i>Euparatettix nigrilibis</i>	OR259	KM853218	KM853472	KM853647	KM853813
		<i>Tetrix japonica</i>	OR257	KM853217	KM853473	KM853648	KM853814
		<i>Trachytettix bufo</i>	OR263	KM853220	KM853470	KM853645	KM853812
		<i>Cylindraustralia</i> sp.	OR027	KM853174	KM853516	KM853690	KM853854
Tridactyloidea	Cylindrachetidae	<i>Mirhipipteryx andensis</i>	OR530	KM853333	KM853359	KM853535	KM853706
	Ripterygidae	<i>Ellipes minutus</i>	OR153	KM853181	KM853508	KM853683	KM853846
Trigonopterygoidea	Trigonopterygidae	<i>Trigonopteryx hopei</i>	OR290	KM853232	KM853458	KM853634	KM853801
	Xyronotidae	<i>Xyronotus aztecus</i>	OR1175	KM853179	KM853510	KM853685	KM853849

Table 1
(Continued)

Species			Voucher ID	18S	28S	H3	Wingless
OUTGROUP							
Grylloblattodea	Grylloblattidae	<i>Grylloblatta sculleni</i>		DQ457301	KM853520	KM853694	N/A
Mantodea	Mantidae	<i>Tamolanica tamolana</i>		EF383483	EF383645	GU064764	FJ802940
Mantophasmatodea	Mantophasmatidae	<i>Sclerophasma paretisense</i>		DQ457302	DQ457337	AY521712	N/A
Phasmatodea	Timematidae	<i>Timema californicum</i>		KM853346	KM853347	KM853521	N/A

Taxonomic classification used in this table follows the Orthoptera Species File. Voucher ID corresponds to Brigham Young University Insect Genomic Collection (BYU-IGC) ID numbers. Specific collecting information and other additional information is provided in Table S1.

first phylogeny of the suborder based on 32 ingroup taxa representing all major lineages and two mitochondrial ribosomal genes, but this dataset was not sufficient enough to resolve the basal relationships. Flook et al. (2000) studied the phylogenetic relationships among the lower caeliferans based on a slightly more expanded taxon sampling and redefined some superfamily concepts. Leavitt et al. (2013) conducted a thorough analysis of mtgenome data across major caeliferan superfamilies, but their taxon sampling was more biased toward Acridoidea than other lineages. All of these previous studies were lacking either in taxon or character sampling, and resulted in conflicting higher classification schemes. Therefore, a well-resolved higher-level phylogeny of Orthoptera is desperately needed.

The main objective of this research is to fill the void in the study of Orthoptera by establishing a robust phylogeny of Orthoptera based on comprehensive taxon and character sampling to provide a framework for natural classification and a reference for studying interesting evolutionary patterns within the order. We present a large-scale molecular phylogeny of Orthoptera based on 254 ingroup taxa and complete mtgenome data and nuclear genes. Using our phylogeny, we test previous phylogenetic hypotheses and propose a new classification scheme for the group. Using strongly corroborated fossil evidence, we present divergent time estimates for major orthopteran lineages and also explore whether different lineages have undergone different tempos of diversification throughout 300 Myr of evolution. Furthermore, we explore the evolution of mitochondrial gene rearrangements across the order based on complete mtgenome sequences.

Materials and methods

Taxon and character sampling

In this study, we generally used the classification scheme adopted by the Orthoptera Species File (Eades

et al., 2014) in order to test it with our phylogenetic analysis. One exception was in the case of Tettigonioidae, in which we recognized only a single family Tettigoniidae, as no alternative hypothesis based on well-corroborated monophyletic groups have been proposed. We sampled a total of 254 taxa covering the phylogenetic diversity within Orthoptera, which represents the most comprehensive taxon sampling for this group to date (Table 1). Specifically, we included extant representatives from all 15 currently recognized orthopteran superfamilies (six ensiferan and nine caeliferan) representing all 12 ensiferan families and 24 out of 28 caeliferan families (Eades et al., 2014). We were not able to obtain DNA-grade samples for four families, which were Dericorythidae, Euschmidtidae, Lathiceridae, and Mastacideidae. For outgroup taxa, we included four polyneopteran species, *Grylloblatta sculleni* (Grylloblattodea), *Tamolanica tamolana* (Mantodea), *Timema californicum* (Phasmatodea), and *Sclerophasma paretisense* (Mantophasmatodea). For 69 terminals, which represented key taxa for understanding higher-level relationships, we included complete mtgenome data, ten of which were newly sequenced for this study. The remaining mtgenomes were either previously generated by us (Fenn et al., 2007, 2008; Sheffield et al., 2010; Leavitt et al., 2013) or obtained from GenBank (Table 2). For all taxa, we generated complete 18S and 28S ribosomal RNA sequences and two conserved nuclear protein-coding genes, histone 3 and wingless, all of which have been frequently used for higher-level insect phylogenetics (Flook et al., 1999; Whiting, 2002; Whiting et al., 2003; Svenson and Whiting, 2004; Jost and Shaw, 2006). For the 26 taxa for which we obtained mtgenome sequences from GenBank, we were not able to generate the other four genes due to an obvious lack of access to specimens. The DNA-grade tissue samples used for this study were either collected by the authors or provided by collaborators. They were preserved in 100% ethanol and vouchered to the −80 °C cryofacility in the Insect Genomic Collection at Brigham Young University (BYU-IGC). We followed standard protocols for DNA extraction, polymer chain reaction (PCR),

sequencing, and primer walking for mtgenome sequencing, which we described in detail elsewhere (Sheffield et al., 2010; Leavitt et al., 2013; Mugleston et al., 2013). All newly generated mtgenomes were first uploaded as raw fasta files to MITOS (Bernt et al., 2013) to identify open reading frames (ORFs) and tRNAs. The initial MITOS annotation was used as a guideline to delimit gene boundaries and start and stop codons of each protein-coding gene were manually identified in Sequencher 4.8 (GeneCode), following the recommendation by Cameron (2014a). The annotations for the 10 newly sequenced mtgenomes are presented in Table S1. The DNA sequence data generated for this study are deposited to GenBank with accession numbers: KM657331–KM657340 (mtgenomes) and KM853171–KM853857 (nuclear genes).

Phylogenetic analyses

We employed several alignment strategies for different loci in our dataset. For both mitochondrial and nuclear protein-coding genes, we aligned based on the conservation of reading frames by first translating into amino acids in MEGA 5 (Tamura et al., 2011), aligning individually in MUSCLE (Edgar, 2004) using default parameters, and back-translating to nucleotides. The tRNA sequences were individually aligned in MUSCLE using default parameters. Mitochondrial ribosomal RNA (16S and 12S) genes were first aligned using E-INS-i strategy in MAFFT (Katoh et al., 2005). Then, we used predicted secondary structures of 16S and 12S of *Locusta migratoria* in RNAfold Web-Server (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>) as input constraint files to perform secondary structure-based alignment in RNAsalsa (Stocsits et al., 2009). For nuclear ribosomal RNA (18S and 28S) genes, we first aligned using E-INS-i strategy in MAFFT and used GBLOCKS 0.91b (Castresana, 2000), while allowing gap positions within the final blocks. All these individual alignments were concatenated into a single matrix in MacClade 4 (Maddison and Maddison, 2005), with the data divided into a total of 71 data blocks (15 protein-coding genes divided into individual codon positions, 22 tRNAs, and two mitochondrial and two nuclear ribosomal genes). We then used PartitionFinder (Lanfear et al., 2012) using the “greedy” algorithm (heuristic search) with branch lengths estimated as “unlinked” to search for the best-fit scheme as well as to estimate the model of nucleotide evolution for each partition.

We performed two separate phylogenetic analyses differing in taxon and character sampling strategies. The first analysis was based on the complete mtgenome data (16 758 aligned bp) from 69 taxa to establish deep relationships within Orthoptera, which was also used for a divergence time estimate analysis. The

second analysis was based on the total evidence dataset (21 025 aligned bp and 258 taxa), which represented a taxon-rich analysis, but had heterogeneously missing data. For 189 of 258 taxa, we did not have mtgenome data, and for 26 taxa, we did not have nuclear gene data. The rationale behind this second analysis was that the mtgenome data would establish robust “backbone” relationships, while the taxa without mtgenome data would still be able to form accurate phylogenetic relationships based on four nuclear genes.

We used parsimony and maximum likelihood (ML) inference methods to reconstruct the phylogeny of Orthoptera. For the parsimony analyses, we used TNT (Goloboff et al., 2003) using New Technology Search options with gaps treated as missing. We first reset RAM to 1000 Mbytes, held 1 000 000 trees, and set *Grylloblatta* as a root prior to tree search. The minimum length was searched ten times using ratchet (Nixon, 1999), sectorial search, drift, and tree fusing (Goloboff, 1999) under default parameters and by setting random seed as 0 and the initial driven search level at 15. Bootstrap support values were calculated to assess nodal support. For the ML analyses, we used the best-fit partitioning scheme recommended by PartitionFinder with the GTRGAMMA model applied to each partition and analysed using RAxML 7.2.8 (Stamatakis et al., 2008) on XSEDE (Extreme Science and Engineering Discovery Environment, <https://www.xse.de.org>) through CIPRES Science Gateway (Miller et al., 2011). Nodal support was evaluated using 5000 replications of rapid bootstrapping implemented in RAxML. The resulting trees were visualized using Fig-Tree (Rambaut, 2006–2009) and iTOL (Letunic and Bork, 2011). Our aligned datasets and the resulting trees were deposited to Dryad (doi: 10.5061/dryad.96r8b).

Topology test

In order to statistically test the previous phylogenetic hypotheses against our results, we performed the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) and the approximately unbiased (AU) test (Shimodaira, 2002). We ran two rounds of the topological tests, one for the mtgenome analysis and another for the total evidence data. For the mtgenome phylogeny, we first obtained the best likelihood score from the ML analysis. Then, we ran three separate ML analyses using topological constraints that matched (i) the caeliferan superfamily-level relationships proposed by Flook et al. (1999, 2000), and the ensiferan relationships proposed by (ii) Desutter-Grandcolas (2003), and (iii) Jost and Shaw (2006). When making these constraints, unconstrained relationships were left as polytomy. Using the best trees

Table 2

Taxonomic information and Genbank accession numbers for taxa used in mtgenome analysis

			Species	Genbank	Reference
ENSIFERA					
Grylloidea	Gryllidae		<i>Teleogryllus emma</i>	NC_011823	Ye et al. (2008)
	Gryllotalpidae		<i>Gryllotalpa pluvalis</i>	NC_011302	Fenn et al. (2008)
	Myrmecophilidae		<i>Myrmecophilus manni</i>	NC_011301	Fenn et al. (2008)
Hagloidea	Prophalangopsidae		<i>Cyphoderris monstrosa</i>	KM657332	This study
			<i>Tarragoilus diuturnus</i>	NC_021397	Zhou et al. (2014)
Rhaphidophoroidea	Rhaphidophoridae		<i>Troglophilus neglectus</i>	NC_011306	Fenn et al. (2008)
Schizodactyloidea	Schizodactylidae		<i>Comicus campestris</i>	KM657337	This study
Stenopelmatoidea	Anostomatidae		<i>Henicus brevimucronatus</i>	KM657338	This study
	Gryllacrididae		<i>Camptonotus carolinensis</i>	KM657333	This study
Tettigonioidea	Stenopelmatidae		<i>Stenopelmatus fuscus</i>	KM657331	This study
	Tettigoniidae		<i>Deracantha onos</i>	NC_011813	Zhou et al. (2009)
			<i>Ruspolia dubia</i>	NC_009876	Zhou et al. (2007)
			<i>Conocephalus maculatus</i>	NC_016696	Z. J. Zhou, Y. Huang, and F. M. Shi (unpubl.)
			<i>Xizicus fascipes</i>	NC_018765	Yang et al. (2012)
			<i>Mecopoda elongata</i>	NC_021380	Zhou et al. (2013)
			<i>Elimaea cheni</i>	NC_014289	Zhou et al. (2010)
			<i>Sinochlora longifissa</i>	NC_021424	Liu et al. (2013)
			<i>Anabrus simplex</i>	NC_009967	Fenn et al. (2007)
			<i>Gampsocleis gratiosa</i>	NC_011200	Zhou et al. (2008)
CAELIFERA					
Acridoidea	Acrididae		<i>Acrida willemsei</i>	NC_011303	Fenn et al. (2008)
			<i>Phlaeoba albonema</i>	NC_011827	Shi et al. (2008)
			<i>Calliptamus italicus</i>	NC_011305	Fenn et al. (2008)
			<i>Traulia sztschuanensis</i>	NC_013826	Y. Huang and C.-Y. Zhang (unpubl.)
			<i>Xenocatantops brachycerus</i>	NC_021609	Y. Liu and Y. Huang (unpubl.)
			<i>Schistocerca gregaria</i>	NC_013240	Erler et al. (2010)
			<i>Chondracris rosea</i>	NC_019993	G. F. Jiang and W. B. Qiang (unpubl.)
			<i>Shirakiacris shirakii</i>	NC_021610	Y. Liu and Y. Huang (unpubl.)
			<i>Arcyptera coreana</i>	NC_013805	Y. Huang and N. Liu (unpubl.)
			<i>Gomphocerus sibiricus</i>	NC_015478	Yin et al. (2011)
			<i>Prumna arctica</i>	NC_013835	Sun et al. (2010)
			<i>Ognevia longipennis</i>	NC_013701	Y. Huang and C.-Y. Zhang (unpubl.)
			<i>Locusta migratoria</i>	NC_001712	Flook et al. (1995)
			<i>Ceracris kiangsu</i>	NC_019994	G. F. Jiang, J. D. Ma, and M. Han (unpubl.)
			<i>Gastrimargus marmoratus</i>	NC_011114	Ma et al. (2009)
Acridoidea	Lentulidae		<i>Oxya chinensis</i>	NC_010219	Zhang and Huang (2008)
			<i>Lentula callani</i>	NC_020774	Leavitt et al. (2013)
			<i>Lithidiopsis carinatus</i>	NC_020775	Leavitt et al. (2013)
			<i>Ommexecha virens</i>	NC_020778	Leavitt et al. (2013)
	Pamphagidae		<i>Thrinchus schrenkii</i>	NC_014610	Zhang et al. (2011)
			<i>Prionotropis hystrix</i>	JX913764	Leavitt et al. (2013)
			<i>Pseudotmethis rubimarginis</i>	NC_020330	Zhang et al. (2013b)
			<i>Asiometis zacharjini</i>	NC_020328	Zhang et al. (2013b)
			<i>Filchnerella helanshanensis</i>	NC_020329	Zhang et al. (2013b)
			<i>Hemicharilaus monomorphus</i>	JX913773	Leavitt et al. (2013)
	Pyrgacridae		<i>Pyrgacris descampsi</i>	NC_020776	Leavitt et al. (2013)
			<i>Xyleus modestus</i>	NC_014490	Sheffield et al. (2010)
	Romaleidae		<i>Tristira magellana</i>	NC_020773	Leavitt et al. (2013)
			<i>Chorotypus fenestratus</i>	KM657339	This study
Eumastacoidea	Chorotypidae		<i>Erianthus versicolor</i>	NC_020045	S. Z. Wei and Y. Huang (unpubl.)
			<i>Pielomastax zhengi</i>	NC_016182	Yang and Huang (2011)
Pneumoroidea	Episactidae		<i>Paramastax nigra</i>	JX913772	Leavitt et al. (2013)
	Eumastacidae		<i>Pseudothericles compressifrons</i>	KM657335	This study
	Thericleidae		<i>Physemacris variolosa</i>	NC_014491	Sheffield et al. (2010)

Table 2
(Continued)

		Species	Genbank	Reference
Proscopioidea	Proscopiidae	<i>Proscopia</i> sp.	KM657336	This study
Pyrgomorphaidea	Pyrgomorphidae	<i>Atractomorpha sinensis</i>	NC_011824	Ding et al. (2007)
		<i>Mekongiella xizangensis</i>	NC_014451	Zhao et al. (2010)
		<i>Mekongiana xiangchengensis</i>	NC_014450	Zhao et al. (2010)
Tanaoceroidea	Tanaoceridae	<i>Tanaocerus koebeli</i>	NC_020777	Leavitt et al. (2013)
Tetrigoidea	Tetrigidae	<i>Tetrix japonica</i>	NC_018543	Xiao et al. (2012a)
		<i>Alulatettix yunnanensis</i>	NC_018542	Xiao et al. (2012b)
		<i>Trachytettix bufo</i>	JX913766	Leavitt et al. (2013)
		<i>Cylindraustralia</i> sp.	KM657334	This study
Tridactyloidea	Ripterygidae	<i>Mirhipipteryx andensis</i>	KM657340	This study
	Tridactylidae	<i>Ellipes minuta</i>	NC_014488	Sheffield et al. (2010)
	Trigonopterygidae	<i>Trigonopteryx hopei</i>	JX913767	Leavitt et al. (2013)
OUTGROUP				
Grylloblattodea	Grylloblattidae	<i>Grylloblatta sculleni</i>	DQ241796	Cameron et al. (2006)
Mantodea	Mantidae	<i>Tamolanica tamolana</i>	NC_007702	Cameron et al. (2006)
Mantophasmatodea	Mantophasmatidae	<i>Sclerophasma paretisense</i>	NC_007701	Cameron et al. (2006)
Phasmatodea	Timematidae	<i>Timema californicum</i>	DQ241799	Cameron et al. (2006)

Taxonomic classification used in this table follows the Orthoptera Species File.

from these analyses, we calculated per-site log-likelihood score for each tree in RAXML using the $-f$ g option, which we used as input data to obtain P -values for the SH and the AU tests in CONSEL (Shimodaira and Hasegawa, 2001). For the total evidence phylogeny, we ran four separate ML analyses using the topological constraints that matched the three above-mentioned studies as well as the mtgenome phylogeny, and performed the tests using the same procedure.

Divergence time estimate analysis

In order to estimate timing and rates of divergence across major orthopteran lineages using abundant fossil records, we performed a divergence time estimate analysis using BEAST v.1.8 (Drummond et al., 2012). For this analysis, we used the 69-taxa dataset based on the complete mtgenome data using the partitioning scheme and the models of nucleotide evolution recommended by PartitionFinder. We created an xml file in BEAUti (Drummond et al., 2012), specifying the starting tree, fossil priors, monophyly constraints, and parameters for molecular clock models. We used Yule process as a tree prior and exponential distribution as a distribution prior for fossil calibration points, which is shown to be suitable for modelling fossil calibrations (Ho, 2007). The ML phylogram generated from RAXML was transformed into an ultrametric chronogram using non-parametric rate smoothing (NPRS) in TreeEdit (Rambaut and Charleston, 2001), and the base of the ingroup was re-scaled to the minimum age of the earliest definitive fossil Orthoptera (299 MYA) to scale the entire chronogram. This rescaled chronogram was utilized as a user-specified starting tree for the BEAST

analyses. We placed monophyly constraints on nine nodes on the phylogeny based on the previous studies (Flook et al., 1999; Leavitt et al., 2013) as well as the ML analysis, and we selected the corresponding nine fossil calibration points from the literature (Brongniart, 1885; Scudder, 1885; Piton, 1940; Sharov, 1968; Riek, 1976; Bethoux et al., 2002; Heads and Leuzinger, 2011) and applied age constraints as priors (Table 3). To assess convergence across independent runs, we conducted two separate analyses each for 100 million generations, sampling every 1000 generations. We inspected the results using Tracer (Rambaut and Drummond, 2003–2009) and discarded 25% of each run as burn-in, and combined the trees using LogCombiner (Rambaut and Drummond, 2002–2013a). A maximum clade credibility tree was summarized in TreeAnnotator (Rambaut and Drummond, 2002–2013b), and visualized in FigTree.

Diversification analysis

We used the program MEDUSA (Alfaro et al., 2009) in order to test whether certain lineages have given rise to clades with unusual species richness. We first reduced the total evidence ML phylogeny into a family-level tree by collapsing the nodes so that each terminal represented a single monophyletic family. We converted this reduced tree into a chronogram using the results from the divergence time estimate analysis. As for the species richness, we obtained the number of valid extant species in each family from the Orthoptera Species File (Eades et al., 2014) and the Mantodea Species File (Otte et al., 2014). We fitted a piecewise birth–death model to the chronogram and allowed the

Table 3

Fossil calibration constraints used in the divergence time estimate analysis

			Median age [MYA] (minimum–maximum)	Notes	Reference
ORTHOPTERA					
Oedischioidea	Oedischidae	<i>Oedischia williamsoni</i>	301.45 (299–303.9)	Oldest definitive Orthoptera	Brongniart (1885)
ENSIFERA					
Grylloidea	Raphoglidae	<i>Raphogla rubra</i>	255.7 (251–260.4)	Oldest definitive Ensifera	Bethoux et al. (2002)
	Protogryllidae	<i>Protogryllus</i>	231.5 (228–235)	Oldest definitive Grylloidea (undescribed)	Heads and Leuzinger (2011)
Tettigonioidea	Tettigoniidae	<i>Tettigonella chazei</i>	28.5 (23.1–33.9)	Oldest definitive Tettigoniidae	Piton (1940)
CAELIFERA					
Locustopsoidae	Eolocustopsidae	<i>Eolocustopsis primitiva</i>	255.7 (251–260.4)	Oldest definitive Caelifera and Acridomorpha	Riek (1976)
Tridactyloidea	Mongoloxyninae	<i>Monodactylus curtippensis</i>	131.15 (129.4–132.9)	Oldest definitive Tridactyloidea	Sharov (1968)
Tetrigioidea	Tetrigidae	<i>Prototetrix reductus</i>	131.15 (129.4–132.9)	Oldest definitive Tetrigidae	Sharov (1968)
Eumastacoidea	Eumastacidae	<i>Archaeomastax jurassicus</i>	154.25 (145–163.5)	Oldest definitive Eumastacoidea and Eumastacidae	Sharov (1968)
Acridoidea	Acrididae	<i>Tyrbula russelli</i>	35.95 (33.9–38)	Oldest definitive Acridoidea and Acrididae	Scudder (1885)

breakpoints to either be at the nodes or the stems. We used the sample size corrected Akaike information criterion (AICc) to assess the models and the net diversification rate (r) and relative extinction (ϵ) were also estimated for each model.

Results

For the parsimony analysis of the complete mtgenome data (Fig. 4a), we found a single most parsimonious tree ($L = 130\,483$; $CI = 0.199$; $RI = 0.365$). We failed to recover monophyly of Orthoptera as well as Stenopelmatoidea, Eumastacoidea, and Acridoidea, although we recovered monophyletic suborders. Nodal supports were generally poor across all backbone nodes. For the ML analysis of the same data (Fig. 4b), we recovered robust monophyly for Orthoptera as well as each suborder and all of the superfamilies except Stenopelmatoidea. The parsimony analysis of the total evidence data yielded three most parsimonious trees ($L = 148\,589$; $CI = 0.204$; $RI = 0.414$). A strict consensus tree (not shown) recovered monophyly of Orthoptera, Ensifera, and Caelifera, as well as Schizodactyloidea, Grylloidea, Hagloidea, Rhaphidophoroidea, Tettigonioidea, Tridactyloidea, Tetrigioidea, Proscopioidea, and Eumastacoidea. However, it did not find Stenopelmatoidea, Pyrgomorpha, and Acridoidea as monophyletic, and had wildly different placements for Proscopioidea, Trigonopterygoidea, and Tanaoceroidea from previous phylogenetic studies of Caelifera (Flook and Rowell, 1997; Flook et al., 1999, 2000; Leavitt et al., 2013). The ML analysis of

the total evidence data recovered monophyly of Orthoptera, both suborders, and all 15 superfamilies (Fig. 5). Because mtgenome data and nuclear rRNA genes of Orthoptera have been known to exhibit base compositional heterogeneity and variable substitution rates (Legendre et al., 2010; Sheffield et al., 2010; Song et al., 2010; Leavitt et al., 2013), the poor performance of the parsimony analyses was to be expected. Thus, the further comments on phylogenetic relationships are based on the ML analyses.

Within Ensifera, we recovered monophyly for five of six superfamilies in the mtgenome analysis, and all six superfamilies in the total evidence analysis. In the mtgenome analysis, Gryllacrididae did not form a clade with other members of Stenopelmatoidea, but in the total evidence analysis, Gryllacrididae, Stenopelmatidae, Anostomatidae, and Cooloolidae formed a monophyletic group. Schizodactyloidea was found to be sister to Grylloidea in the mtgenome analysis, but it was placed basally to the non-grylloid ensiferans in the total evidence analysis. Within Grylloidea, two clades were consistently found, one consisting of Gryllidae, and the other consisting of Gryllotalpidae, Mogoplistidae, and Myrmecophilidae. In both analyses, Rhaphidophoroidea, Stenopelmatoidea, and Hagloidea formed a monophyletic group, which in turn was sister to Tettigonioidea. Within Caelifera, we recovered monophyly for all nine superfamilies with the following phylogenetic relationships: (Tridactyloidea (Tetrigioidea ((Proscopioidea + Eumastacoidea) (Tanaoceroidea ((Pneumoroidea + Trigonopterygoidea) (Pyrgomorpha + Acridoidea)))))). Within Acridoidea, Pyrgacrididae was the most basal lineage, followed by

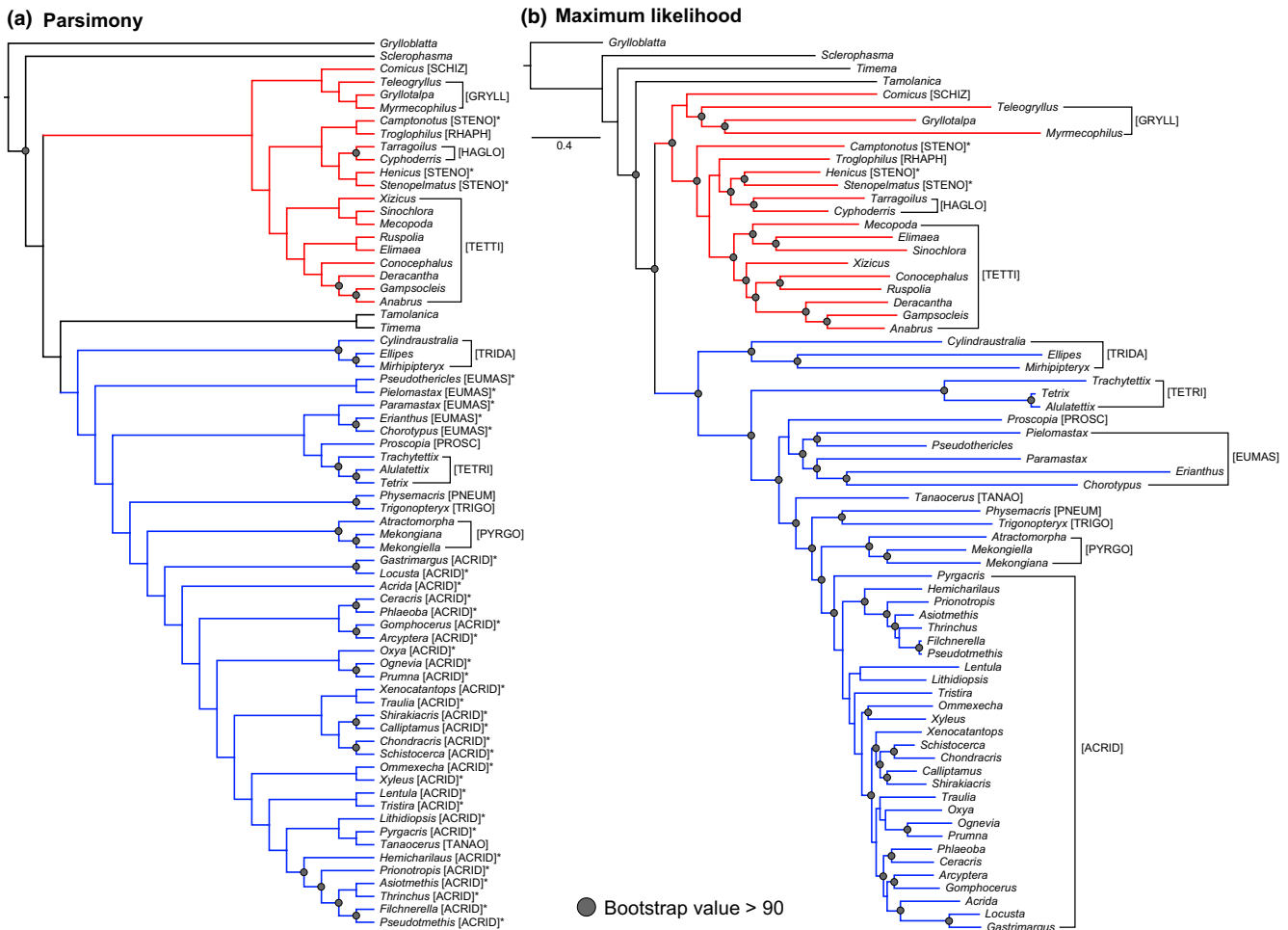


Fig. 4. Phylogenetic analyses based on complete mtgenome data. (a) The single most parsimonious tree from the parsimony analysis; (b) the most likelihood phylogram from the ML analysis. Branches are colour coded in red to indicate Ensifera and blue to indicate Caelifera. In the brackets are abbreviations of superfamily names. [SCHIZ]: Schizodactyloidea; [GRYLL]: Grylloidea; [STENO]: Stenopelmatoidea; [RHAPH]: Rhaphidophoroidea; [HAGLO]: Hagloidea; [TETTI]: Tettigonioidae; [TRIDA]: Tridactyloidea; [PROSC]: Proscopioidae; [EUMAS]: Eumastacoidae; [TANAO]: Tanaoceroidea; [PNEUM]: Pneumoroidea; [TRIGO]: Trigonopterygoidea; [PYRGO]: Pyrgomorphoidea; [ACRID]: Acridoidea. Asterisk denotes paraphyletic groups. Superfamily names used in this figure follow the currently recognized 15 superfamilies according to the Orthoptera Species File (Eades et al., 2014).

Pamphagodidae + Pamphagidae, Lentulidae + Lithidiidae, and Tristiridae. Ommexechidae showed a close relationship with Romaleidae in both analyses, but Romaleidae did not form a monophyletic group in the total evidence analysis. The monophyly of Acrididae was supported in the mtgenome analysis, but was not supported in the total evidence analysis due to paraphyly with Romaleidae.

When we compared our topology with the previous phylogenetic hypotheses using the SH and the AU test (Table 4), we found that the caeliferan relationship was not statistically different from that of Flook et al. (1999, 2000), but the ensiferan relationship was significantly different from both that of Desutter-Grandcolas (2003) and Jost and Shaw (2006). This pattern was found in both the mtgenome and the total evidence

analyses. The topology deduced from the mtgenome data did not statistically differ from the total evidence topology.

The divergence time-estimate analysis based on nine fossil calibration points suggested that Orthoptera originated in the Carboniferous and the two suborders diverged in the Permian (Fig. 6). Within Ensifera, Grylloidea was the first lineage to diversify, starting in the Early Triassic and continuing throughout the Mesozoic. Other ensiferans diversified mostly in the Mesozoic and most of the major lineages within Tettigonioidae diversified in the Cretaceous. Caeliferan lineages showed different patterns of diversification and diverged into two groups that gave rise to two infraorders, Tridactylidea (Tridactyloidea) and Acrididea (the remaining eight superfamilies) in the

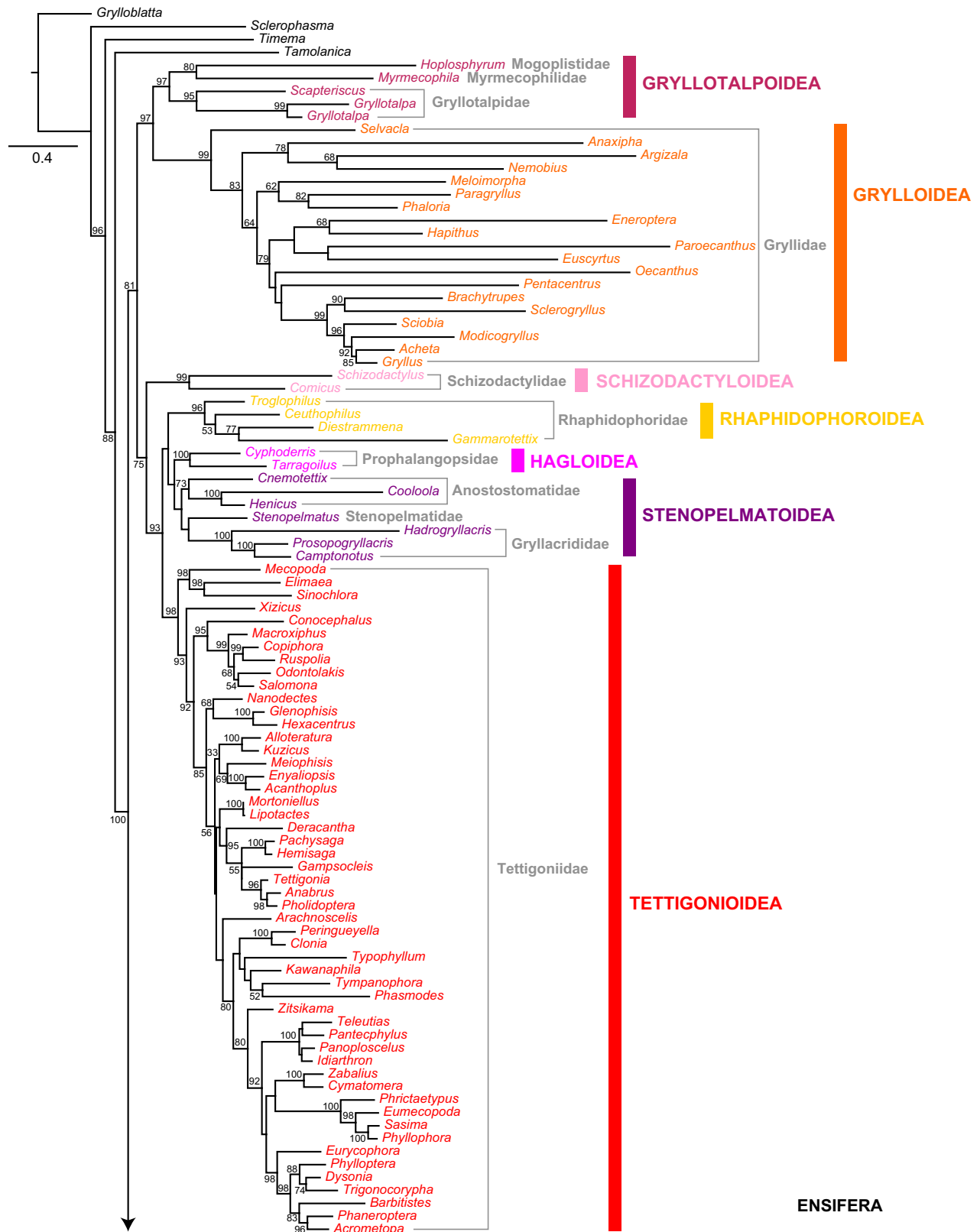


Fig. 5. Total evidence phylogeny of Orthoptera inferred from ML analysis. Numbers above the nodes are bootstrap support values. Asterisk denotes paraphyletic groups. Superfamily names used in this figure follow the proposed classification scheme in the present study. Thus, Gryllotalpoidea is indicated here as a distinct superfamily, separate from Grylloidea.

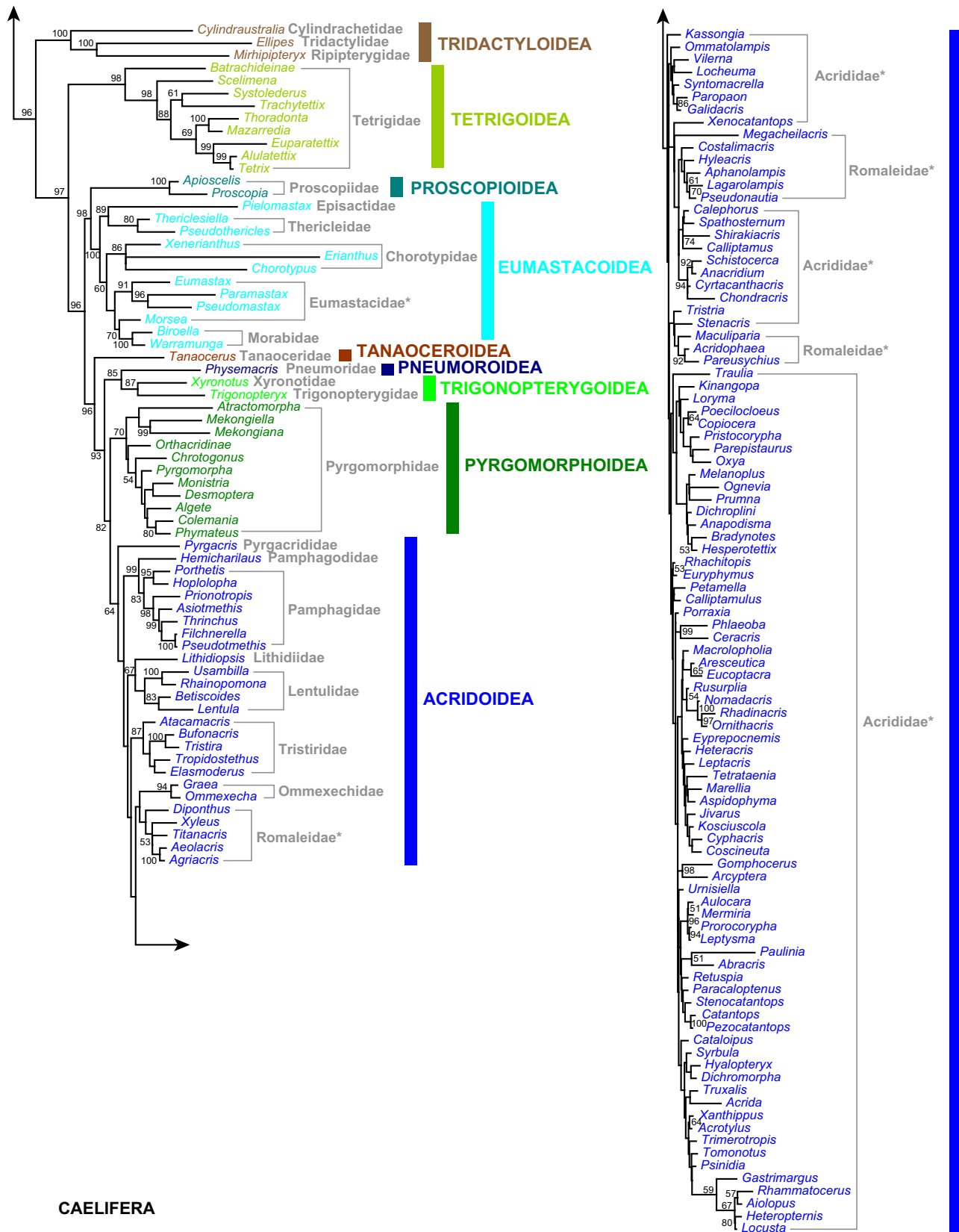


Fig. 5. (continued)

Table 4

Results of the Shimodaira–Hasegawa (SH) test and the approximately unbiased (AU) test against previous hypotheses

Alternative hypothesis	Ln-likelihood score	SH test (<i>P</i> -value)	AU test (<i>P</i> -value)	Significantly different?
Mtgenome best tree	–491397.738	–	–	–
Caelifera relationship <i>sensu</i> Flook et al. (1999, 2000)	–491492.498	1.000	1.000	No
Ensifera relationship <i>sensu</i> Desutter-Grandcolas (2003)	–491668.521	0.000	0.000	Yes
Ensifera relationship <i>sensu</i> Jost and Shaw (2006)	–491771.721	0.000	0.000	Yes
Total evidence best tree	–588308.136	–	–	–
Mtgenome relationship (this study)	–588420.665	0.796	0.544	No
Caelifera relationship <i>sensu</i> Flook et al. (1999, 2000)	–588429.772	0.731	0.456	No
Ensifera relationship <i>sensu</i> Desutter-Grandcolas (2003)	–588908.165	0.000	0.000	Yes
Ensifera relationship <i>sensu</i> Jost and Shaw (2006)	–588812.682	0.000	0.000	Yes

late Palaeozoic. In the Late Triassic, Acrididea diverged into two lineages, Tetrigoidea and Acridomorpha, a monophyletic group characterized by grasshopper-like morphology that includes seven superfamilies (Dirsh, 1975; Song, 2010). Eumastacoidea and Proscopidoidea flourished in the Mesozoic and modern grasshoppers of the superfamily Acridoidea were the most recently diverged group, which diversified in the mid- to the Late Cretaceous. The major lineages of Acrididae seemed to have radiated in the Cenozoic.

Our MEDUSA analysis showed that the background tempo of diversification across Orthoptera was characterized by a relatively low net rate ($r = 0.027$ lineages per Myr) and a moderate turnover rate ($\epsilon = 0.596$). Using the number of validly described species as a proxy for clade-level diversity, we found that there were three episodes where the tempo of diversification significantly increased in Orthoptera (Fig. 7). The most significant change occurred in the clade consisting of Acrididae, Romaleidae, and Ommexechidae, which appeared to have gone through a major adaptive radiation with no major extinctions in the Cenozoic (node 1 in Fig. 7). The clade containing Tettigoniidae, Rhaphidophoridae, Prothalangopsiidae, Anostostomatidae, Gryllacrididae, and Stenopelmaticidae also went through an increased rate of diversification (node 2 in Fig. 7). Pamphagidae independently went through an increased rate of diversification (node 3 in Fig. 7). The third most diverse lineage in Orthoptera is Gryllidae, but our analysis did not find it to show any major shift in tempo of diversification, which suggests that the clade has continued to diversify throughout the evolutionary history of Orthoptera.

Finally, the gene arrangement in the mtgenome across Orthoptera was examined and we found that most ensiferan lineages retained the ancestral gene

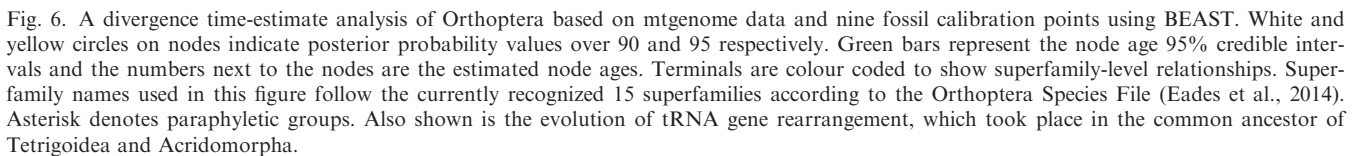
arrangement, except two reported cases of gene rearrangements in Gryllidae and Tettigoniidae (Ye et al., 2008; Liu et al., 2013). We found an additional exception in a prothalangopsid *Cyphoderris monstrosa*, which had tRNA-Ala and tRNA-Arg reversed from the ancestral arrangement (Fig. 8). Within Caelifera, all three families of Tridactyloidea retained the ancestral condition, while the remaining caeliferan superfamilies had the tRNA genes between cytochrome *c* oxidase subunit II (COII) and ATP synthase protein 8 (ATP8) rearranged so that tRNA-Asp was positioned before tRNA-Lys, which was reversed from the ancestral insect arrangement in which tRNA-Lys precedes tRNA-Asp. When this tRNA rearrangement was mapped onto the mtgenome phylogeny, we inferred that the rearrangement evolved in the common ancestor of Acrididea in the Late Permian or the Early Triassic (Fig. 6).

Discussion

Towards a phylogeny-based natural classification scheme for Orthoptera

This work represents the most comprehensive phylogenetic analysis of Orthoptera to date (Fig. 9) and presents an excellent opportunity to test previous hypotheses about the phylogenetic relationships among the major lineages within the order. Below, we comment on the higher-level relationships, which we can confidently resolve using the current data and we propose a new phylogeny-based natural classification scheme for Orthoptera (Table 5).

The phylogeny of Ensifera has been contentious over the years and numerous hypotheses have been proposed based on different character systems (Ander, 1939; Zeuner, 1939; Judd, 1947; Blackith and Blackith,



sisting of Gryllotalpidae (mole crickets), Mogoplistidae (scaly crickets), and Myrmecophilidae (ant-loving crickets). The close relationship between Gryllidae and Gryllotalpidae has always been supported by previous studies (Ander, 1939; Zeuner, 1939; Judd, 1947; Sharov, 1968; Vickery, 1977), but their relationship with respect to the other two families has not been resolved because they have sometimes been included as subfamilies of Gryllidae (Rehn and Hebard, 1912). Our phylogeny strongly suggests that Mogoplistidae and Myrmecophilidae form a clade, which is in turn sister to Gryllotalpidae. The clade formed by these three families is quite divergent from Gryllidae. Furthermore, many lineages within Gryllidae are also very divergent from each other, reflecting the ancient age of

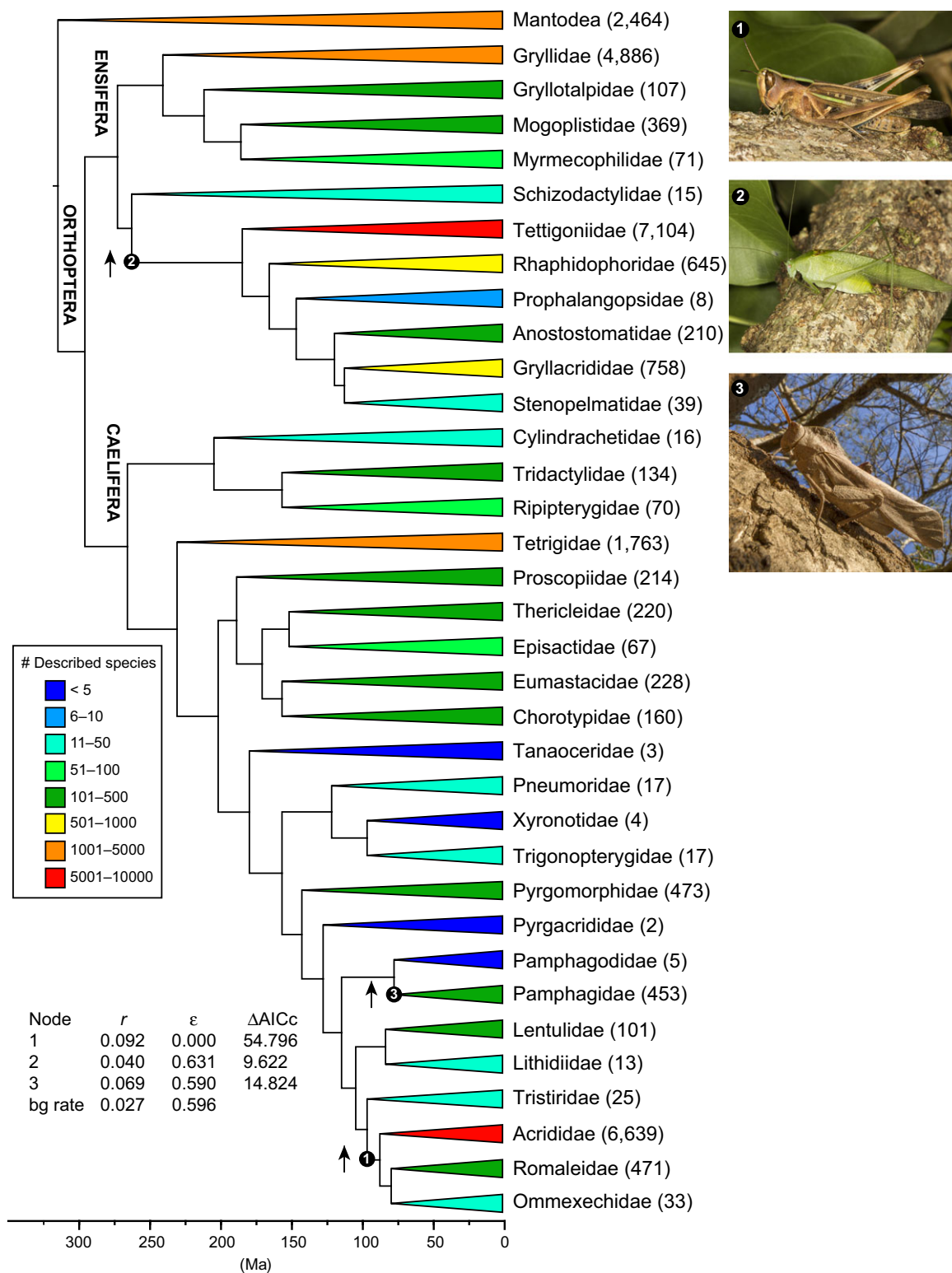


Fig. 7. Diversification patterns of major lineages of Orthoptera as deduced from MEDUSA analysis. Each terminal represents a monophyletic family and the number in parenthesis next to the family name indicates the number of validly described species within the family. Terminals are colour coded to show the species diversity. The MEDUSA analysis identified three episodes where diversification rate (r) and extinction rate (ϵ) significantly changed, which are indicated by black circles on nodes. The up arrows indicate an increase in diversification rates. The images on the right show the representatives of the clades that experienced the shifts in diversification rate. (Photograph credit: Hojun Song [1, 2], Piotr Naskrecki [3])

(a) Insect ancestor, Ensifera and Tridactyloidea

Ile (I)	Gln (Q)	Met (M)	nad2	Trp (W)	Cys (C)	Tyr (Y)	cox1	Leu (L)	cox2	Lys (K)	Asp (D)	atp8	atp6	cox3	Gly (G)	nad3	Ala (A)	Arg (R)	Asn (N)	Ser (S)	Glu (E)	Phe (F)	nad5	His (H)	nad4	nad4l	Thr (T)	Pro (P)	nad6	cytb	Ser (S)	nad1	Leu (L)	16S	Val (V)	12S	CR
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(b) Acrididea (Tetrigioidea and Acridomorpha)

Ile (I)	Gln (Q)	Met (M)	nad2	Trp (W)	Cys (C)	Tyr (Y)	cox1	Leu (L)	cox2	Asp (D)	Lys (K)	atp8	atp6	cox3	Gly (G)	nad3	Ala (A)	Arg (R)	Asn (N)	Ser (S)	Glu (E)	Phe (F)	nad5	His (H)	nad4	nad4l	Thr (T)	Pro (P)	nad6	cytb	Ser (S)	nad1	Leu (L)	16S	Val (V)	12S	CR
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(c) *Teleogryllus emma* & *T. commodus*

Ile (I)	Gln (Q)	Met (M)	nad2	Trp (W)	Cys (C)	Tyr (Y)	cox1	Leu (L)	cox2	Lys (K)	Asp (D)	atp8	atp6	cox3	Gly (G)	nad3	Ala (A)	Arg (R)	Glu (E)	Ser (S)	Asn (N)	Phe (F)	nad5	His (H)	nad4	nad4l	Thr (T)	Pro (P)	nad6	cytb	Ser (S)	nad1	Leu (L)	16S	Val (V)	12S	CR
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(d) *Sinochlora longifissa* & *S. retrolateralis*

Ile (I)	Met (M)	nad2	CR	Gln (Q)	Trp (W)	Cys (C)	Tyr (Y)	cox1	Leu (L)	cox2	Lys (K)	Asp (D)	atp8	atp6	cox3	Gly (G)	nad3	Ala (A)	Arg (R)	Asn (N)	Ser (S)	Glu (E)	Phe (F)	nad5	His (H)	nad4	nad4l	Thr (T)	Pro (P)	nad6	cytb	Ser (S)	nad1	Leu (L)	16S	Val (V)	12S
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(e) *Cyphoderris monstrosa*

Ile (I)	Gln (Q)	Met (M)	nad2	Trp (W)	Cys (C)	Tyr (Y)	cox1	Leu (L)	cox2	Lys (K)	Asp (D)	atp8	atp6	cox3	Gly (G)	nad3	Arg (R)	Ala (A)	Asn (N)	Ser (S)	Glu (E)	Phe (F)	nad5	His (H)	nad4	nad4l	Thr (T)	Pro (P)	nad6	cytb	Ser (S)	nad1	Leu (L)	16S	Val (V)	12S	CR
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Fig. 8. Mitochondrial genome structures and known gene arrangements within Orthoptera. (a) Ancestral insect mtgenome structure, which is found in most ensiferans and Tridactyloidea; (b) tRNA rearrangement found in Acrididea, which is a molecular synapomorphy for the group; (c) Unique tRNA rearrangement found in *Teleogryllus* (Ye et al., 2008; Wolff et al., 2012); (d) Unique gene rearrangement found in *Sinochlora* (Liu et al., 2013); (e) Unique tRNA rearrangement found in *Cyphoderris monstrosa*.

the group, and it may be reasonable to elevate some of the gryllid subfamilies to the family level, given more thorough analyses with more taxon sampling in the future. In recognition of the two deeply divergent clades within Gryllidea, we propose recognition of two superfamilies within the infraorder, Grylloidea, which includes Gryllidae and Gryllotalpoidea, which includes the remaining three families. As our taxon sampling for Gryllidea is relatively weak compared with other groups included in this study, additional sampling may be necessary to test this hypothesis, but an independent analysis using a larger taxon sampling finds a similar relationship (Chintauan-Marquier et al., in press), lending further support for this taxonomic change.

Within Tettigoniidea, we find a basal position of Schizodactyloidea, which comprises a small relict family Schizodactylidae (sand crickets), which is a novel hypothesis. The phylogenetic position of Schizodactyloidea within Ensifera has not been clear (Hedges and Leuzinger, 2011). Jost and Shaw (2006), Legendre et al. (2010), as well as our mtgenome analysis, found it to be sister to Gryllidea, while other morphology-based studies placed them near Tettigoniidea (Ander, 1939; Gorochov, 1995a; Desutter-Grandcolas, 2003). Hedges and Leuzinger (2011) supported a sister relationship between Schizodactyloidea and Gryllidea, which was first proposed by Gwynne (1995), but it

was not based on a formal cladistic analysis. Upon close examination of our data, we find that the branch lengths of the members of Gryllidea are exceptionally long and it is possible that our mtgenome analysis may have been affected by long-branch attraction (Felsenstein, 1978; Bergsten, 2005), meaning that the resulting topology may be an analytical artefact, rather than an accurate relationship. The larger taxon and character sampling of the total evidence analysis seems to have overcome this issue.

We then recover a sister relationship between Tettigoniidea and the remaining three monophyletic superfamilies (Hagloidea, Rhaphidophoroidea, and Stenopelmatoidea). Many of the earlier taxonomists have considered Tettigoniidea and Hagloidea to be closely related because these are the only two superfamilies outside Gryllidea that emit sounds with a tegminal design and hear with tibial tympanal structures (Ander, 1939; Zeuner, 1939; Ragge, 1955; Gwynne, 1995; Desutter-Grandcolas, 2003). However, it is important to recognize that many ensiferans within Tettigoniidea can also produce femoro-abdominal stridulation, which can be perceived by either membranous tympana or mechanoreceptors (Desutter-Grandcolas, 2003). Tettigoniidea includes the most diverse and speciose orthopteran family, Tettigoniidae (katydids), which has the stridulatory file on the left tegmen (Gwynne, 2001), and Hagloidea, which includes only a

Table 5

A phylogeny-based natural classification scheme proposed in this study

Suborder	Infraorder	Superfamily group	Superfamily	Included families (alphabetical)	Number of described species (extant)
ENSIFERA					
	Gryllidea		Grylloidea*	Gryllidae	4886
			Gryllotalpoidea [†]	Gryllotalpidae, Mogoplistidae, Myrmecophilidae	547
	Tettigoniidea		Schizodactyloidea	Schizodactylidae	15
			Tettigoniioidea	Tettigoniidae [‡]	7104
			Rhaphidophoroidea	Rhaphidophoridae	645
			Stenopelmatoidea	Anostomatidae [§] , Gryllacrididae, Stenopelmatidae	913
			Hagloidea	Prophalangopsidae	8
CAELIFERA					
	Tridactylidea		Tridactyloidea	Cylindrachetidae, Ripipterygidae, Tridactylidae	205
	Acrididea		Tetrigoidea	Tetrigidae	1763
		Acridomorpha	Proscopioidae	Proscopiidae	214
			Eumastacoidea	Chorotypidae, Episactidae, Eumastacidae, Euschmidtidae [¶] , Mastacideidae [¶] , Morabidae, Thericleidae	1008
			Tanaoceroidea	Tanaoceridae	3
			Pneumoroidea	Pneumoridae	17
			Trigonopterygoidea	Trigonopterygidae, Xyronotidae	21
			Pyrgomorpha	Pyrgomorphidae	473
			Acridoidea	Acrididae, Derycorythidae [¶] , Lathiceridae [¶] , Lentulidae, Lithidiidae, Ommexechidae, Pamphagidae, Pamphagodidae, Pyrgacrididae, Romaleidae, Tristiridae	7976

*Grylloidea used to be the sole superfamily within Gryllidea. Now we recognize it as containing a single family Gryllidae. Upon further studies in the future, Gryllidae may be divided into more families.

[†]Here, we recognize the superfamily Gryllotalpoidea as a distinct lineage from Grylloidea to include Gryllotalpidae, Mogoplistidae, and Myrmecophilidae based on our phylogenetic analyses.

[‡]In this study, we recognize a single family Tettigoniidae, which includes Phaneropteridae *sensu* Heller et al. (2014). The katydid classification is clearly in a state of flux and a major revision of the classification will be required with more data in the future.

[§]We consider Cooloolidae as an aberrant member of Anostomatidae and do not recognize it as a valid family.

[¶]These four families are not included in this study.

single extant family, Prophalangopsidae (ambidextrous crickets), and numerous extinct families, all of which have stridulatory files on both tegmina (Spooner, 1973). Jost and Shaw (2006) proposed a more radical scheme, which placed Hagloidea (represented by *Cyphoderris* only) as the most basal lineage within Ensifera and suggested that acoustic communication is the ancestral condition for Ensifera and has been lost multiple times. Recently, Zhou et al. (2014) suggested a sister relationship between Hagloidea and Rhaphidophoroidea based on mtgenome data, but the study lacked robust taxon sampling because it included only one species per superfamily and did not include the members of Stenopelmatoidea. Our study finds a novel relationship and does not find support for the sister relationship between Tettigoniioidea and Hagloidea, or the sister relationship between Hagloidea and Rhaphidophoroidea, or the basal placement of Hagloidea. Instead, we find the clade (Rhaphidophoroidea (Hagloidea + Stenopelmatoidea)), which is, in turn, sister to Tettigoniidae. The acoustic communication is certainly a very complex syndrome that can be achieved from diverse ways of producing sound and equally

diverse ways of perceiving sound (Desutter-Grandcolas, 2003) and it is not well justified to treat it as a simple binary character to test whether it is ancestral or derived. A more appropriate way is to conduct a detailed anatomical study to carefully form homology statements of various components of sound production and hearing to optimize on to the phylogeny. Thus, a study with larger taxon sampling and detailed morphological analyses is necessary to fully understand the evolution of acoustic communication in these insects.

Recently, Heller et al. (2014) elevated the status for the tettigoniid subfamily Phaneropterinae to full family Phaneropteridae, which includes four plant-feeding subfamilies, Phaneropterinae, Pseudophyllinae, Mecopodinae, and Phyllophorinae, based on a recent molecular study by Mugleston et al. (2013) and a previous hypothesis by Gorochoy (1995b). This taxonomic change implies that Phaneropteridae as a whole is a monophyletic group, but Mugleston et al. (2013) found that one of the pseudophylline tribes, Pterochrozini, did not group with other pseudophyllines, but was placed at the base of katydid phylog-

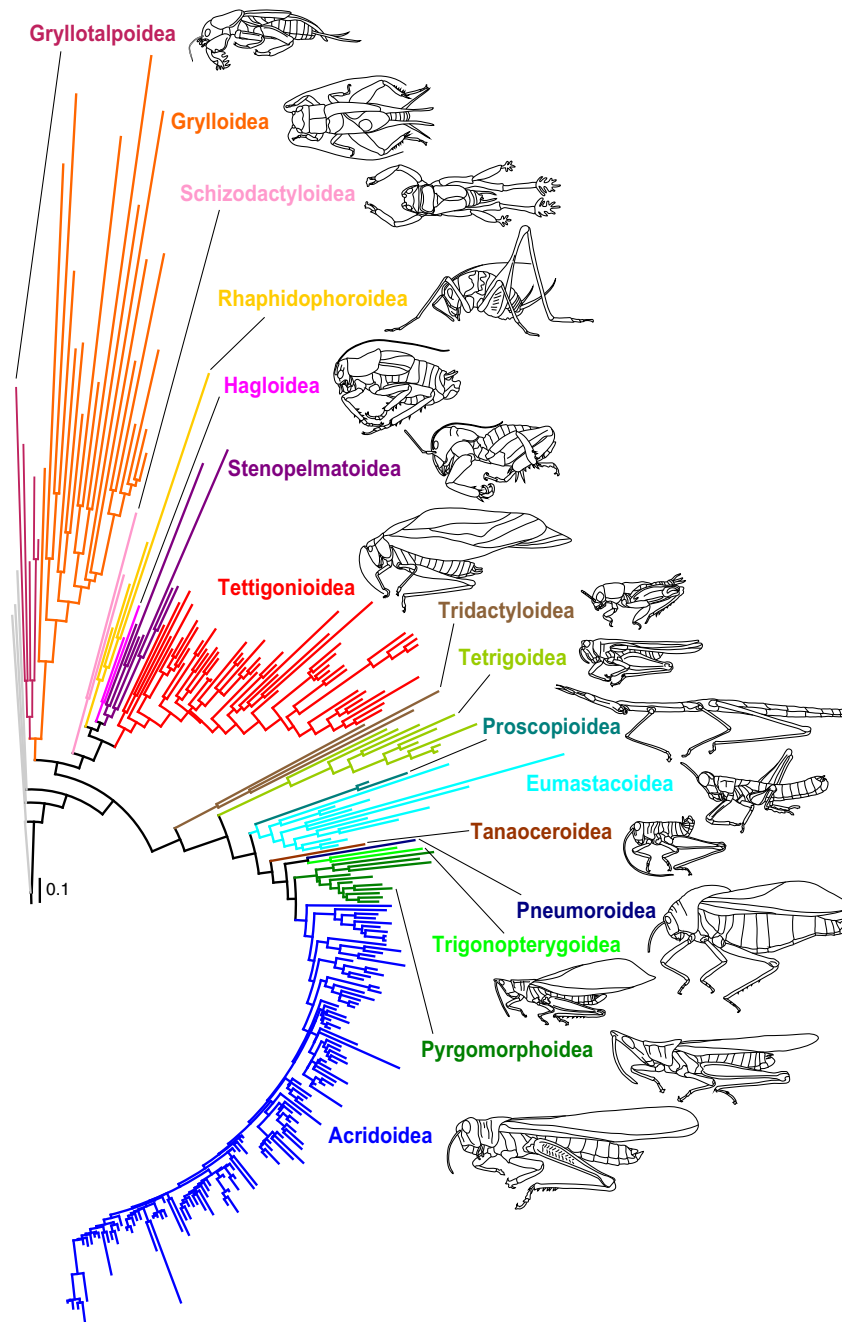


Fig. 9. A summary phylogeny of Orthoptera based on ML analysis of total evidence data. For readability, terminals are not shown, but branches are colour coded to show their superfamily identity. Superfamily names used in this figure follow the proposed classification scheme in the present study.

eny, thereby rendering the concept of Phaneropteridae paraphyletic. Our study is built upon the data generated by Mugleston et al. (2013), and we also do not find strong support for the family Phaneropteridae. To make matters worse, Gorochoy (2012) elevated several tribes within Pseudophyllinae to subfamilies and created a “subfamily group” Pseudophyllidae, but this hypothesis has not been fully tested. The katydid classification is

clearly in a state of flux and a major revision of the classification will be required once a definitive phylogeny of Tettigoniidae becomes available. Thus, in this study, we recognize a single family, Tettigoniidae, that has always been found monophyletic.

Our study proposes a novel phylogenetic position for Rhaphidophoroidea, which includes a single cosmopolitan family Rhaphidophoridae (camel crickets).

Gorochov (2001) considered Rhaphidophoridae as a member of Stenopelmatoidea, but our study shows that it is divergent from Stenopelmatoidea. Likewise, a sister relationship between Stenopelmatoidea and Hagloidea is also novel. Although our analysis based on the mtgenome finds only paraphyletic Stenopelmatoidea, the total evidence analysis (Fig. 5) based on more taxon sampling strongly recovers the monophyly of the superfamily consisting of Anostomatidae (wetas and king crickets), Stenopelmidae (Jerusalem crickets), and Gryllacrididae (raspy crickets and leaf-rolling crickets). Anostomatidae is found to be paraphyletic with respect to Cooloolidae (cooloola monsters), but evidence suggests that Cooloolidae are actually aberrant Anostomatidae, and Gorochov (2001) also expressed this view. However, more taxon sampling is required to fully resolve the relationships among Rhaphidophoroidea, Stenopelmatoidea, and Hagloidea, and allow the definition of monophyletic units of evolution for extant taxa and fossils.

We now have a very clear understanding of the phylogeny of Caelifera and the relationships among superfamilies. Like Ensifera, Caelifera is mainly divided into two infraorders, Tridactylidea *sensu* Kevan (1982), which comprises a single superfamily, Tridactyloidea, and Acrididea *sensu* Kevan (1982), which includes all the other caeliferan superfamilies (Fig. 5). Previous molecular phylogenetic hypotheses of Caelifera (Flook and Rowell, 1997; Flook et al., 1999; Leavitt et al., 2013; Zhang et al., 2013a,b) consistently recovered the basal position of Tridactyloidea *sensu* Dirsh (1975), followed by Tetrigoidea *sensu* Dirsh (1975), and our study also confirms this hypothesis. Although some earlier studies focusing on the fossil evidence proposed a sister relationship between Tridactyloidea and Tetrigoidea (Ragge, 1955; Sharov, 1968), other morphologists thought that Tetrigoidea was more closely related to other caeliferan superfamilies (Dirsh, 1975; Kevan, 1982; Vickery, 1997) and our study concurs with the latter. Tridactyloidea is the earliest branch within Caelifera, currently consisting of three families, Tridactylidae (pygmy mole crickets), the Neotropical relative Ripipterygidae, and the subterranean Cydrachetidae (sandgropers). Although Vickery (1997) considered Cydrachetidae to form its own superfamily, Cydrachetidae, Flook and Rowell (1997) included it within Tridactyloidea and our findings support this position. The infraorder Acrididea consists of Tetrigoidea and Acridomorpha, the former comprised of a single cosmopolitan family, Tetrigidae (pygmy grasshoppers and grouse locusts), which is characterized by the extension of the pronotum over the entire dorsal surface of the abdomen (Kevan, 1982). The latter is a group of seven superfamilies collectively known as Acridomorpha for their grasshopper-like morphology and herbivorous feeding habit (Dirsh, 1975; Flook and Rowell, 1997;

Song, 2010). The higher-level classification within Acridomorpha has been largely informed by male phallic structures (Dirsh, 1973; Amédégato, 1974; Eades, 2000; Song, 2010), but individual taxonomists have interpreted these morphological characters differently from each other, resulting in several conflicting schemes (Song, 2010). Molecular data have been very useful in resolving these conflicts (Flook and Rowell, 1997; Flook et al., 1999, 2000; Leavitt et al., 2013). Recently, Song and Mariño-Pérez (2013) compared the phylogenetic signal in male phallic structures with that of molecular data, and showed that many traditionally used characters are highly homoplasious, while some phallic structures do have a strong signal. The present study is largely congruent with the previous molecular studies (Flook and Rowell, 1997; Flook et al., 1999, 2000; Leavitt et al., 2013) and clarifies some of the conflicts from the earlier taxonomic work.

In this study, we find that Acridomorpha is divided into two groups: Proscopioidea *sensu* Descamps (1973b) + Eumastacoidea *sensu* Descamps (1973b) and the clade consisting of the remaining five superfamilies. Proscopioidea comprises a single family Proscopiidae (jumping sticks). Although this phasmid-looking grasshopper family has sometimes been considered a member of Eumastacoidea (Dirsh, 1961, 1975), we consider it here as a separate superfamily based on its unique apomorphies (Descamps, 1973a,b) as well as its robust basal position relative to other eumastacoids. Our study clarifies the ambiguous position of Proscopioidea as found by Matt et al. (2008). Eumastacoidea includes seven families (Eades et al., 2014) that are commonly referred to as monkey grasshoppers. Of the remaining five superfamilies within Acridomorpha, the earliest diverging lineage is Tanaoceroidea *sensu* Kevan (1982), which includes a small relict family Tanaoceridae, known only from three species endemic to the southwestern USA, and characterized by extremely long antennae and a rudimentary male phallic complex (Rehn, 1948; Dirsh, 1955; Grant and Rentz, 1967).

Then the lineage is divided into two clades, one consisting of Pneumoroidea *sensu* Flook et al. (2000) and Trigonopterygoidea *sensu* Flook et al. (2000) and the other consisting of Pyrgomorphaidea *sensu* Flook et al. (1999) and Acridoidea *sensu* Flook et al. (2000). Pneumoroidea contains one family, Pneumoridae (bladder grasshoppers, flying gooseberries), and 17 described species mostly found in South Africa, and is known for its femoro-abdominal stridulatory mechanism (Dirsh, 1965). Trigonopterygoidea consists of two rather divergent families, Trigonopterygidae and Xyronotidae. The former is endemic to Southeast Asia, contains 17 species, and is characterized by reversed male genitalia and foliaceous tegmina (Dirsh, 1952). The latter contains four species endemic to central Mexico and can be characterized by rudimentary

male genitalia and a stridulatory ridge on the third abdominal tergite (Dirsh and Mason, 1979). Flook et al. (2000) recovered these two families as a monophyletic group and our study corroborates their findings. However, the sister relationship between Pneumoroidea and Trigonopterygoidea is novel. Dirsh and Mason (1979) considered Tanaoceridae, Pneumoridae, and Xyronotidae to form a monophyletic lineage because of the apparently shared femoro-abdominal stridulatory mechanism, but Flook et al. (2000) thoroughly showed that this morphological trait must have evolved multiple times within the basal caeliferans and our findings bolster this idea.

Pyrgomorphae includes a single family, Pyrgomorphidae (gaudy grasshoppers), that contains about 470 species distributed globally, with most of its diversity found in the Old World, and is characterized by the presence of a groove in the fastigium (Kevan and Akbar, 1964). Members of this family are often large, strikingly coloured, and known to feed on toxic plants for their defense (Rowell, 1967; Chapman et al., 1986; Whitman, 1991). Dirsh (1975) placed the family Pamphagidae within Pyrgomorphae based on the similarity of male phallic structures, but our study clearly shows that Pyrgomorphidae alone forms a distinct sister lineage to Acridoidea, and Pamphagidae is firmly included within Acridoidea, which corroborates the earlier findings by Flook and Rowell (1997) and Flook et al. (1999).

Finally, Acridoidea is the largest superfamily within Acridomorpha, and currently includes 11 recognized families and more than 7900 described species, which are defined by the morphology of the male phallic complex and the lack of a basioccipital slit, among other characters (Roberts, 1941; Chopard, 1949; Dirsh, 1973; Amédégnato, 1974; Kevan, 1982; Eades, 2000). Many species in this group can be recognized as typical and familiar grasshoppers. Within Acridoidea, we find that Pyrgacrididae, which is endemic to Réunion Island in the Indian Ocean (Hugel, 2005), is the earliest diverging lineage, representing a transitional form between Pyrgomorphae and Acridoidea (Eades, 2000). Leavitt et al. (2013) found Pamphagidae + Pamphagodidae to be the basal lineage within Acridoidea, but their nodal support for the backbone relationships was poor. The present study is based on large taxon and character sampling and our nodal support is much stronger than that of Leavitt et al. (2013).

Divergence and diversification patterns of major orthopteran lineages

Orthoptera has evolved over 300 Myr (Sharov, 1968; Gorochoy, 1995a; Storozhenko, 1997; Grimaldi and Engel, 2005) and its current diversity has been shaped

by dynamic shifts of diversification rates at different geological times across different lineages within the order. Some clades are very diverse and show a cosmopolitan distribution, while others are represented by only a small number of species and have a very limited geographical distribution (Kevan, 1982). Moreover, different clades show characteristic morphological, ecological, and behavioral traits, which appear to be phylogenetically conserved within each lineage (Kevan, 1982). Using the divergence time estimates (Fig. 6) and the MEDUSA analysis (Fig. 7), we have revealed some interesting patterns of diversification, and here we comment on the evolution of the three most diverse and cosmopolitan orthopteran clades, crickets (Grylloidea), katydids (Tettigoniidae), and grasshoppers (Acrididae), to highlight the complexity of orthopteran evolution.

Crickets represent one of the most ancient lineages within Orthoptera and we estimate that they diverged from other groups probably in the Triassic. They form the third most diverse clade with more than 4800 known species (Eades, 2000) and our MEDUSA analysis shows that they did not experience any major shifts in diversification rates, implying that the lineage has continued to diversify since the origin of the clade (Fig. 7). However, this result needs to be interpreted cautiously because it is possible that different patterns might emerge if the whole diversity of Grylloidea indeed consists of multiple valid families (Chopard, 1949). Modern crickets are mostly nocturnal, brown or black in colour, and omnivorous scavengers (Alexander, 1968), and these ecological characters could have originated in the early Mesozoic. What sets the crickets apart from other ancient insects of similar ecological habits is their ability to communicate acoustically (Greenfield, 1997), even if the loss of acoustic communication is frequent in crickets, contrary to tettigoniids. Cricket songs are mainly used in the context of intraspecific sexual behaviour (Otte, 1992; Greenfield, 1997), which suggests that acoustic crickets are under sexual selection. Many closely related species can be diagnosed by their male calling songs. Sexually selected characters tend to evolve rapidly (Lande, 1981; West-Eberhard, 1983) and, in fact, sexual selection on male calling songs has been postulated as a likely reason for the extremely rapid speciation in a Hawaiian genus, *Laupala* (Mendelson and Shaw, 2005). At the same time, these songs can attract predators and parasitoids, which can provide strong selective pressure for the crickets to evolve silence rapidly (Pascoal et al., 2014), or modify their signals into less conspicuous ones, such as the ultrasounds used by Eneopterinae (Robillard et al., 2007). Therefore, it is conceivable that both sexual selection on songs and natural selection may have played an important role for cricket diversification over the past 200 Myr, which may have contributed to the current diversity.

Katydidids represent the most successful lineage within Orthoptera in terms of species diversity (Eades, 2000). They are also very diverse in terms of ecological traits, with their diets ranging from herbivory to carnivory, and they can be active during both the day and night (Gwynne, 2001). Like crickets, katydidids communicate acoustically, with their songs ranging from audible sound to ultrasound (Greenfield, 1997; Gwynne, 2001; Montealegre-Z, 2009), with very rare loss of acoustic communication, and sexual selection has likely played an important role in their diversification. Although definitive fossils of Tettigoniidae are only known from the Cenozoic (Piton, 1940; Sharov, 1968; Gorochoy, 1995b; Storozhenko, 1997), our divergence time-estimate analysis suggests that the family probably originated in the Late Jurassic and diversified into major lineages in the Cretaceous (Grimaldi and Engel, 2005). Furthermore, the MEDUSA analysis suggests that there was an increase in diversification rate in the lineage leading to this clade (Fig. 7). This period coincides with the diversification of angiosperms (Soltis et al., 2005) and complex palaeoclimatic patterns (Bender, 2013). Many modern katydidids have tegmina resembling angiosperm leaves, which function as a defense mechanism against visual predators via crypsis or mimesis (Nickle and Castner, 1995), and a recent molecular phylogeny of Tettigoniidae (Mugleston et al., 2013) showed that leaf-like wings have evolved multiple times within the family. Among the herbivorous katydidids, the diet habits vary across florivory, granivory, and folivory (Gwynne, 2001), which suggests that the availability of diverse angiosperms could have promoted the diversification of katydidids. Although katydidids appear to show a cosmopolitan distribution, only three groups, Conocephalinae, Phaneropterinae, and Pseudophyllinae, are truly cosmopolitan, while the other lineages have more restricted distributions (Kevan, 1982; Eades et al., 2014). This suggests that the numerous vicariance events that followed the break-up first of Pangaea, and later of Gondwana and Laurasia, may have played an important role in the divergence of major katydid lineages. This biogeographical pattern also seems to explain the distribution of crickets.

Grasshoppers represent the most recently diverged lineage within Orthoptera. Fossil acridids are frustratingly scarce and most of the known fossils are from the mid- to late Cenozoic (Sharov, 1968; Storozhenko, 1997; Grimaldi and Engel, 2005). We estimate that the ancestral Acrididae probably originated in the Late Cretaceous, but it is likely that the major diversification events happened after the Cretaceous–Paleogene (K–Pg) boundary, well into the Cenozoic. The MEDUSA analysis identifies the lineage leading up to Acrididae to have undergone a significant increase in diversification rate with little or no extinction (Fig. 7).

During the Cenozoic, global climate generally became temperate (Bender, 2013), and grasses evolved and became dominant (Strömberg, 2011). Grasshoppers are exclusively herbivores and many lineages have adapted to graminivory (Uvarov, 1977). They are among the dominant herbivores of grasslands and rangelands, known to consume nearly 10% of plant biomass in such habitats (Gangwere et al., 1997). The most speciose acridid subfamily is Gomphocerinae (Eades et al., 2014), which has its mouthparts adapted for graminivory (Isley, 1944). Thus, it is conceivable that the diversification of grasses, and particularly the spread of open grasslands (Dixon et al., 2014), could have played an important role in the diversification of grasshoppers. By the Cenozoic, the continents had approached their present-day configuration and large oceanic barriers existed between them. Many grasshopper lineages are known to be strong fliers and colonizers, and an extreme example of their flight can be illustrated from locust swarms that can migrate long distances, often across continents (Pener and Simpson, 2009). This suggests a possibility that at least some of the current cosmopolitan distribution of Acrididae could have been due to dispersal events. Our total evidence phylogeny (Figs 5 and 9) shows that the branch lengths for taxa belonging to Acrididae are extremely short, suggesting that only few changes have accumulated in the nuclear genes we have used amongst grasshoppers. From another angle, this pattern highlights a possibility that Acrididae may have undergone an explosive adaptive radiation, powered by the evolution of a new niche space (grasslands) and frequent founder events after the colonization of new habitats.

Mitochondrial genome evolution

A typical metazoan mtgenome consists of 37 genes (13 protein-coding, two ribosomal RNAs and 22 transfer RNAs) and a variable size of AT-rich control region (Clary and Wolstenhome, 1985; Boore, 1999), and has been shown to accurately resolve phylogenetic relationships over broad evolutionary periods across many insect groups (Cameron, 2014b). In this study, we show that the mtgenome data are very useful in resolving the phylogeny of Orthoptera. We also demonstrate that not only the sequence data of the mtgenome, but also its gene arrangements, contain important phylogenetic information. Flook et al. (1995) sequenced the first orthopteran mtgenome from the migratory locust, *Locusta migratoria*, and noticed that two tRNA genes (tRNA-Lys and tRNA-Asp) between COII and ATP8 were reversed from the ancestral insect arrangement. Subsequently, this tRNA rearrangement has been reported from other caeliferans, but not in any of the ensiferans (Flook and Rowell, 1995; Fenn et al., 2008; Sheffield et al., 2010).

Leavitt et al. (2013) recently showed that Tridactylidae was the only caeliferan that did not have the rearrangement, thus retaining the ancestral condition. In this study, we show that all of the ensiferan superfamilies as well as all three families (Tridactylidae, Ripipterygidae, and Cyldrachetidae) of the basal caeliferan superfamily Tridactyloidea retain the ancestral gene arrangement (Fig. 8).

There are three exceptions, however. Ye et al. (2008) showed that the tRNA gene cluster tRNA-Asn–tRNA-Ser(AGN)–tRNA-Glu was inversely located in the minor strand in the cricket *Teleogryllus emma*, and Wolff et al. (2012) also showed that the congeneric *T. commodus* had the same gene rearrangement. It is not clear if other gryllids have this rearrangement. Additionally, Liu et al. (2013) found a novel gene order, 12S rRNA–tRNA-Ile–tRNA-Met–nad2–control region–tRNA-Gln–tRNA-Trp, in two katydid species in the genus *Sinochlora*, which was uniquely different from the ancestral order of 12S rRNA–control region–tRNA-Ile–tRNA-Gln–tRNA-Met–nad2–tRNA-Trp. Interestingly, none of the other Tettigoniidae mtgenomes shows this rearrangement, which suggests that this is an isolated incidence. In the present study, we also find that *Cyphoderris monstrosa* has tRNA-Arg–tRNA-Ala, instead of the ancestral tRNA-Ala–tRNA-Arg, but this appears to be an isolated case because another prophalangopsid, *Tarragoilus diuturnus*, is not known to have any rearrangement (Zhou et al., 2014).

The only consistent tRNA gene rearrangement across the phylogeny is the one between COII and ATP8 and when this gene arrangement is mapped onto the phylogeny (Fig. 6), we find that it evolved in the common ancestor of Acrididea (Tetrigioidea + Acridomorpha). This suggests that this rearrangement has persisted for nearly 250 Myr since this lineage diverged from Tridactylidae. Thus, this gene rearrangement can be considered a clear molecular synapomorphy for Acrididea. Mitochondrial gene rearrangements have been frequently reported in Paraneoptera and Holometabola (Cameron, 2014b), but so far Orthoptera is the only polyneopteran lineage that shows this pattern and it appears to have happened infrequently. This pattern implies that such gene rearrangements must have been a rare event among polyneopteran insects.

Future directions and challenges

The present study represents a major milestone for orthopteran systematics and provides a framework for a phylogeny-based natural classification system for Orthoptera (Table 5). While this study has clarified many long-standing issues, it has also identified several areas of research that need further investigations. The first area is the relationship among basal ensiferans. Given the diversity of Rhaphido-

phoroidea and Stenopelmatoidea, our taxon sampling in these groups is too sparse and these are also among the least studied groups within Orthoptera. Similarly, more sampling for Gryllidea is required to formally test the validity of Gryllotalpoidea and the relationships among the families, and check the definition of monophyletic families within Grylloidea. The second area is to thoroughly re-evaluate the classification of Tettigoniidae, specifically to address the recent taxonomic issue regarding the status of Phaneropteridae. The third area is the phylogeny of Tetrigioidea, which is one of the more ancient lineages and the second most speciose superfamily within Caelifera. Tetrigioidea has a cosmopolitan distribution and shows exceptional diversity in pronotum morphology. Nevertheless, a phylogeny of Tetrigioidea is completely unknown at this time. The last area is the phylogeny of Acrididae and Romaleidae. Our current data are unable to resolve the majority of the relationships within these families, mainly because the markers that we have used are too conserved and because grasshoppers appear to have undergone explosive adaptive radiation. Despite the economical and ecological importance of the grasshoppers, we currently do not know the phylogenetic relationships among the major lineages. Therefore, there needs to be a collective effort to resolve these issues.

With advances in sequencing technologies (Shendure and Ji, 2008), a new approach to phylogenetic studies utilizing whole genome and transcriptome data, also known as phylogenomics, is becoming increasingly popular (Faircloth et al., 2012; Lemmon et al., 2012). For Orthoptera, however, phylogenomic studies currently remain a challenge because the order is known to have the largest nuclear genome size among insects (Hanrahan and Johnston, 2011) ranging from 1.52 to 16.56 Gb (Gregory, 2014). So far, the migratory locust, *Locusta migratoria*, is the only orthopteran to be sequenced and its genome is 6.5 Gb in size (Wang et al., 2014). Unfortunately, this genome is not fully annotated and there is currently no reference genome for any orthopteran, which makes gene annotation for any shotgun sequencing or RNA-seq very difficult. Yet, we remain hopeful that these challenges will be soon overcome with rapidly evolving sequencing technologies and bioinformatics, and we look forward to having a much more thorough understanding about the evolution of this fascinating group of insects.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Annotation of newly generated mitochondrial genomes in this study.