

Out of Africa? A dated molecular phylogeny of the cicada tribe Platycleurini Schmidt (Hemiptera: Cicadidae), with a focus on African genera and the genus *Platycleura* Amyot & Audinet-Serville

Short title: Phylogeny of Platycleurini

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Abstract

The Platycleurini is a large group of charismatic cicadas distributed from the Cape of South Africa, through tropical Africa, Madagascar, India, and eastern Asia to Japan, with generic diversity concentrated in equatorial and southern Africa. This distribution suggests the possibility of a Gondwanan origin and dispersal to eastern Asia from Africa or India. We used a four-gene (three mitochondrial) molecular dataset, fossil calibrations, and molecular clock information to explore the phylogenetic relationships of the platycleurine cicadas and the timing and geography of their diversification. The earliest splits in the tribe were found to separate forest genera in Madagascar and equatorial Africa from the main radiation, and all of the Asian/Indian species sampled formed a younger clade nested well within the African taxa. The tribe appears to have diversified during the Cenozoic, beginning ca. 50-32 Ma, with most extant African lineages originating in the Miocene or later, well after the breakup of the Gondwanan landmass. Biogeographical analysis suggests an African origin for the tribe and a single dispersal event founding the Asian platycleurines, although additional taxon sampling and genetic data will be needed to confirm this pattern because key nodes in the tree are still weakly supported. Two Platycleurini genera from Madagascar (*Pycna* Amyot & Audinet-Serville, *Yanga* Distant) are found to have originated by late Miocene dispersal of a single lineage from Africa. The genus *Platycleura* is recovered as polyphyletic, with *P. signifera* Walker from South Africa and many Asian/Indian species apparently requiring assignment to different genera, and a new *Platycleura* concept is proposed with the synonymisation of *Azanicada* Villet **syn.n.** The genera *Orapa* Distant and *Hamza* Distant, currently listed within separate tribes but suspected of platycleurine affinity, are nested deeply within the Platycleurini radiation. The tribe Orapini **syn.n.** is here synonymized while the tribe Hamzini is pending a decision of the ICZN to preserve nomenclatorial stability.

Key words: historical biogeography; divergence-time analysis; systematics; Cicadidae

Introduction

Many studies of taxa distributed in both Africa and Asia generally consider two biogeographical scenarios: the commonly cited “Out of Africa” hypothesis (post-Gondwanan, African origin followed by dispersal into Asia) and the less commonly cited “Out of India” hypothesis (Gondwanan origin with dispersal into Asia mediated by rafting on proto-India). “Out of Africa” dispersal is often followed by diversification in Asia and subsequent “Out of Asia” dispersal back into Africa (Folinsbee & Brooks, 2007; Kodandaramaiah & Wahlberg, 2007). It is postulated that the connection of Africa and the Arabian peninsula to Eurasia from the mid-Oligocene (30–25 Ma; Jolivet & Faccenna, 2000; Willis & McElwain, 2002; Bosworth *et al.*, 2005; Gheerbrant & Rage, 2006) facilitated the “geo-dispersal” (*sensu* Lieberman & Eldridge, 1996) of many vertebrate and invertebrate taxa (Mey, 2005; Bossuyt *et al.*, 2006; Folinsbee & Brooks, 2007; Kodandaramaiah & Wahlberg, 2007; Schaefer & Renner, 2008; Aduse-Poku *et al.*, 2009; Kelly *et al.*, 2009; Rasmussen & Cameron, 2010). Furthermore, the connection of Africa to Eurasia resulted in the closing of the Tethys Sea in the mid-Miocene (20–10 Ma), reorganizing oceanic currents (Hessami *et al.*, 2001). This oceanic reorganization is thought to have caused a drastic drop in global temperatures and the aridification of northern Africa and the Arabian Peninsula, resulting in disjunct distribution patterns involving Africa and Asia (Zachos *et al.*, 2001; Douady *et al.*, 2003; Teske *et al.*, 2007; Aduse-Poku *et al.*, 2009). The “Out of India” hypothesis is suggested as an explanation for the Asian distribution of otherwise Gondwanan lineages (e.g., Karanth, 2006; Klaus *et al.*, 2010). It is generally accepted that India and Madagascar began to move away from Gondwanan Africa in the late Jurassic / early Cretaceous (~ 160 Ma), and that Madagascar split from India in the mid- to late Cretaceous (~ 90 Ma; Karanth, 2006; Yoder & Nowak, 2006). This was followed by India colliding with Asia in the Eocene (50–35 Ma; Karanth, 2006; Aitchison *et al.*, 2007; Ali & Aitchison, 2008), facilitating the transfer of lineages of Gondwanan origin into Asia (Karanth, 2006). Although Madagascar has been separated from Africa for 160 million years, its proximity to the East African coast is thought to have allowed faunal interchange (Yoder & Nowak, 2006; Kainulainen *et al.*, 2017; Bourguignon *et al.*, 2018). Similarly, the proximity of India to the East African coast (Briggs, 2003) is thought to have facilitated faunal interchange before its eventual collision with Eurasia (Briggs, 2003).

The platyleurine cicadas, which occur in Africa, Madagascar, India, and Asia, offer a model taxon for exploring the significance of these hypotheses in explaining the diversity of modern animal groups. Cicadas (Hemiptera: Cicadidae) are an enigmatic group of insects with a global distribution, widely known for their loud songs used in mate attraction. The family currently consists of 53 tribes in four subfamilies: Cicadettinae, Cicadinae, Tettigomyiinae and Tibicininae, the latter *sensu* “Tettigadinae” of Moulds (2005) (Marshall *et al.*, 2018a). The tribe Platyleurini (Cicadinae) currently consists of 31 genera (Sanborn, 2013; Marshall *et al.*, 2018a) and about 264 species and subspecies distributed in Africa and Asia/India, with an African centre of diversity (Distant, 1906; Metcalf, 1963; Duffels & van der Laan, 1985; Sanborn, 2013) (Table S1). The concentration of platyleurine genera in sub-Saharan Africa, with additional genera extending to southern India, suggests the possibility of a Gondwanan origin, with subsequent dispersal into Asia and, eventually, Japan.

The most widespread (Distant, 1882) and speciose Platypleurini genus, *Platypleura* Amyot & Audinet-Serville, was most recently revised by Boulard (1972), who confined his attention to the African taxa. Morphological conservatism and convergence within *Platypleura* has resulted in very few characters with which to infer species' relationships, resulting in the need for additional data sources (Villet *et al.*, 2004). The first molecular phylogeny involving the genus (Villet *et al.*, 2004) suggested that the South African *Platypleura* cicadas might have an East African origin with dispersal and speciation in a southward and westward direction. The ancestral plant association of *Platypleura* was hypothesised to be with *Acacia* Miller (now *Vachellia* Wight & Walker-Arnott and *Senegalia* Rafinesque) species, with speciation sporadically associated with host plant shifts (Villet *et al.*, 2004). However, these inferences were based on limited taxon sampling (12 of 55 taxa) and only a ~500 bp portion of one mitochondrial gene. In addition the platypleurine genera *Oxypleura* Amyot & Audinet-Serville and *Pycna* Amyot & Audinet-Serville include both African and Asian species. The definition of *Oxypleura* as a platypleurine cicada with clear wings dates to Distant (1906), and while Boulard (1972) redefined the genus he did not consider the Asian species. Similarly, *Pycna* was originally defined by its narrow head (Amyot & Audinet-Serville, 1843) and has never been redefined.

The aim of this study was to investigate the biogeographical history of the tribe Platypleurini through molecular phylogenetic and divergence-time analyses. This enabled several hypotheses to be tested: (1) The tribe Platypleurini is of Gondwanan origin; (2) Asian platypleurine taxa resulted from dispersal from Africa that post-dates the meeting of Africa and Eurasia in the mid-Oligocene (“Out of Africa”; cf. Kodandaramaiah & Wahlberg, 2007); (3) Platypleurine genera containing both Asian and African representatives (*Platypleura*, *Pycna*, *Oxypleura*) do not represent monophyletic assemblages; (4) Forest-associated platypleurine lineages predate the aridification of Africa; (5) Madagascan platypleurine taxa (genera *Yanga* Distant and *Pycna*) are a result of recent dispersal from Africa, and not Gondwanan vicariance (cf. Yoder & Nowak, 2006); (6) The African members of *Platypleura* originated in north-eastern Africa, with a southward radiation (Villet *et al.*, 2004).

Methods

Sampling and laboratory protocols

A total of 24 of the 31 currently recognized genera in the Platypleurini were included among 84 sequenced cicadas; type species were used to represent genera where possible (Table 1). Forty-five species (out of an estimated 70) were sampled for the large genus *Platypleura*, including seven from Asia/India. In addition, five genera of platypleurine cicadas occur within Asia: *Suisha* Kato, *Oxypleura*, *Pycna*, *Kalabita* Moulton and *Hainanosemia* Kato, samples of which were available for the first four. The genera *Chremistica* Stål, *Cryptotympana* Stål (tribe Cryptotympanini), *Orapa* Distant (Orapini), *Hamza* Distant (Hamzini), *Hyalessa* China (Sonatini), and *Meimuna* Distant (Dundubiini) and *Cicada* Linnaeus (Cicadini) were included in the analyses to test the monophyly of the Platypleurini. The cryptotympanine genera were designated as outgroups following the relationships in Marshall *et al.* (2018a). Nicknames in quotation marks given for undescribed taxa are temporary and not intended for formal taxonomic nomenclature (Art. 8.2, ICZN 1999).

Table 1. Cicada specimens sequenced for this study, with taxonomic data, voucher numbers, collection localities, and GenBank accession information shown for each of four genes. Asterisked taxa are type species of their respective genera.

Species	Collection Locality	Voucher #	16S	COI	COII	EF1a
<u>Tribe Platypleurini</u>						
* <i>Afzeliada afzelii</i> (Stål)	unknown, Gabon	MHV0468	FJ168845	GU344060	GU344016	GU343836
* <i>Albanycada albiger</i> a (Walker)	Kieskamma River Pass, South Africa	MHV0334	GU344078	GU344055	GU344006	GU343825
<i>Albanycada</i> sp. 1	near Willowmore, South Africa	MHV0423	FJ168837	FJ169041	GU344010	GU343829
* <i>Azanicada zuluensis</i> (Villet)	Fish River Mouth, South Africa	MHV0236	GU344077	GU344053	GU344000	-
* <i>Brevisiana brevis</i> (Walker)	Mpumalanga, South Africa	MHV0650	FJ168914	FJ169115	GU344024	GU343843
* <i>Canualna liberiana</i> (Distant)	Annobon Island, Equatorial Guinea	MHV1553	GU344094	-	-	GU343869
* <i>Capcicada absimilis</i> (Distant)	Bainskloof Pass, South Africa	MHV0304	FJ168810	FJ169014	GU344002	GU343822
* <i>Hamza ciliaris</i> (Linnaeus)	Talilabu, Indonesia	MHV1562	GU344098	GU344073	GU344050	-
* <i>Ioba leopardina</i> (Distant)	Mazabuka, Zambia	MHV0643	FJ168912	FJ169114	-	GU343841
<i>Karscheliana parva</i> Boulard	Mpala Ranch, Kenya	MHV0686	GU344083	GU344062	GU344027	GU343846
* <i>Koma bombifrons</i> (Karsch)	Mazabuka, Zambia	MHV0644	FJ168913	FJ169081	GU344023	GU343842
* <i>Kongota punctigera</i> (Walker)	Ilfracombe, South Africa	MHV0343	FJ168825	FJ169030	GU344008	GU343827
* <i>Munza laticlav</i> ia (Stål)	Upington, South Africa	MHV0248	FJ168801	FJ169005	GU344001	GU343821
* <i>Orapa numa</i> (Distant)	Ngangao Forest, Taita Hills, Kenya	MHV0674	FJ168916	FJ169117	GU343941	GU343845
<i>Oxypleura calypso</i> Kirby	Christmas Island, Australia	MHV0448	-	GU344058	-	GU343831
<i>Oxypleura quadraticollis</i> (Butler)	Malilangwe GR, Zimbabwe	MHV0162	FJ168788	FJ168994	GU343993	-
<i>Platypleura arabica</i> Myers	Wadi Bih dam, United Arab Emirates	MHV1202	GU344086	GU344064	GU344041	-
<i>Platypleura</i> sp. 'breedeflumensis'	near Prince Albert, South Africa	MHV0602	FJ168898	FJ169101	GU344020	FJ215654
<i>Platypleura brunea</i> Villet	Cathcart, South Africa	MHV0825	FJ168923	FJ169127	GU343888	GU343852
<i>Platypleura capensis</i> (Linnaeus)	Cannon Rocks, South Africa	MHV0021	EF134521	FJ168988	GU343990	FJ215646
<i>Platypleura</i> sp. 'catenata' isolate CM	near Swellendam, South Africa	MHV0317	EF134579	GU344054	GU344004	-
<i>Platypleura</i> sp. 'catenata' isolate EM	Uniondale, South Africa	MHV0152	EF134560	FJ168993	GU343992	GU343815
<i>Platypleura</i> sp. 'catenata' isolate WM	near Worcester, South Africa	MHV0312	EF134578	FJ169015	GU344003	GU343823
<i>Platypleura</i> cf. <i>girardi</i> Boulard	unknown, Togo	MHV0459	FJ168841	FJ169045	GU344013	-
<i>Platypleura</i> cf. <i>girardi</i> Boulard	Comoe, Ivory Coast	MHV0720	-	FJ169120	GU344028	GU343847
<i>Platypleura chalybaea</i> Villet	Zuurberg Pass, South Africa	MHV1022	FJ168966	FJ169169	GU343912	GU343862
<i>Platypleura divisa</i> (Germar)	Whittlesea, South Africa	MHV0515	FJ168853	FJ169055	GU344017	GU343837
<i>Platypleura</i> sp.	nr Grasskop, South Africa	MHV0992	FJ168958	FJ169161	GU344037	GU343861
<i>Platypleura</i> sp. 'gamtoosflumensis'	nr Loerie, South Africa	MHV1018	FJ168962	FJ169165	GU344038	FJ215657

<i>Platypleura</i> sp. 'gariepflumensis'	Pella, South Africa	MHV0183	FJ168791	FJ168996	GU343994	FJ215647
<i>Platypleura gowdeyi</i> Distant	Mpala Ranch, Kenya	MHV0187	FJ168792	FJ168997	GU343995	GU343816
<i>Platypleura haglundi</i> Stål	Weenen Nature Res., South Africa	MHV0590	FJ168890	FJ169093	GU344018	GU343838
<i>Platypleura hilpa</i> Walker	Ban Sen Island, Vietnam	MHV0219	FJ168796	FJ169000	GU343996	GU343817
<i>Platypleura hirta</i> Karsch	Renosterpoort, South Africa	MHV0880	FJ168937	FJ169141	GU344032	GU343854
<i>Platypleura hirtipennis</i> (Germar)	Port Elizabeth, South Africa	MHV1020	FJ168964	FJ169167	GU344039	FJ215659
<i>Platypleura instabilis</i> Boulard	unknown, Gabon	MHV0465	FJ168844	FJ169048	GU344015	GU343835
<i>Platypleura</i> sp. 'intercapedinis'	Alexandria Forest, South Africa	MHV0912	FJ168946	FJ169150	GU343891	GU343857
<i>Platypleura kaempferi</i> (Fabricius)	Miuami, Ashikara City, Japan	MHV0461	FJ168842	FJ169046	GU344014	GU343834
<i>Platypleura kaempferi</i> (Fabricius)	Zhongjiang, China	MHV1479	GU344093	GU344070	GU344047	GU343868
<i>Platypleura</i> sp. 'karoensis'	Ghwarriepoort River, South Africa	MHV0323	EF134552	FJ169020	GU344005	GU343824
<i>Platypleura longirostris</i> Ashton	Mbololo Forest, Kenya	MHV1169	FJ168981	FJ169184	GU343915	GU343863
<i>Platypleura maytenophila</i> Villet	nr Jozini, South Africa	MHV0989	FJ168957	FJ169160	GU344036	GU343860
<i>Platypleura mijburghi</i> Villet	Pongolapoort Pass, South Africa	MHV0964	FJ168953	GU343741	GU343907	-
<i>Platypleura mira</i> Distant	Mae Ho, Thailand	MHV0669	-	-	GU344026	-
<i>Platypleura murchisoni</i> Distant	nr Makhado, South Africa	MHV0960	FJ168952	FJ169156	GU343906	GU343858
<i>Platypleura nobilis</i> (Germar)	Toungka Forest, Thailand	MHV0668	FJ168915	FJ169116	GU344025	GU343844
<i>Platypleura octoguttata</i> (Fabricius)	Nagar Parker, Pakistan	MHV1559	GU344096	-	GU344049	-
<i>Platypleura</i> sp. 'olifantsflumensis'	Vanrhynsdorp, South Africa	MHV0867	FJ168936	FJ169140	GU344031	GU343853
<i>Platypleura pinheyi</i> Boulard	Braganza South, Mozambique	MHV1289	GU344088	GU344066	GU344043	-
<i>Platypleura</i> sp. 10	Perekezi Forest, Malawi	MHV1410	GU344090	GU344068	GU344045	GU343866
<i>Platypleura plumosa</i> (Germar)	Klipplaat, South Africa	MHV1042	FJ168974	FJ169177	GU344040	-
<i>Platypleura signifera</i> Walker	nr Groenriviermond, South Africa	MHV0888	FJ168939	FJ169143	GU344034	GU343856
<i>Platypleura</i> sp. C	Mindoro, Philippines	MHV1561	GU344097	GU344072	-	-
<i>Platypleura</i> sp. 8	Collingham Ridge, South Africa	MHV0338	FJ168822	FJ169027	GU344007	GU343826
<i>Platypleura</i> sp. 10B	Van Rhynsdorp Pass, South Africa	MHV0010	EF134551	FJ168984	GU343989	GU343812
<i>Platypleura</i> sp. 10A	nr Montague, South Africa	MHV0600	FJ168896	FJ169099	GU344019	-
<i>Platypleura</i> sp. 14	nr Maclear, South Africa	MHV0988	FJ168956	FJ169159	GU344035	GU343859
<i>Platypleura</i> sp. 15	Wakkerstroom, South Africa	MHV0881	FJ168938	FJ169142	GU344033	GU343855
<i>Platypleura</i> sp. 17	Swan Mine, Dem. Rep. Congo	MHV1295	GU344089	GU344067	GU344044	GU343865
<i>Platypleura</i> sp. 18	Kakamega, Kenya	MHV0767	-	FJ169124	GU344030	GU343850
<i>Platypleura</i> sp. 19	Mughese Forest reserve, Malawi	MHV0757	FJ168921	FJ169123	-	GU343849
* <i>Platypleura stridula</i> (Linnaeus)	Citrusdal, South Africa	MHV0001	EF134568	FJ168982	GU343988	-
<i>Platypleura takasagona</i> Matsumura	unknown, Taiwan	MHV0551	-	KR674166	MK226183	MK226184

<i>Platypleura techowi</i> Schumacher	Kimberley, South Africa	MHV0232	FJ168797	FJ169001	GU343998	GU343819
<i>Platypleura wahlbergi</i> Stål	Beacon Bay, South Africa	MHV0365	EF134550	GU344056	GU344009	GU343828
<i>Pycna coelestia</i> Distant	Fa-Mu-Dui, Tibet	MHV1568	GU344099	-	-	-
<i>Pycna repanda</i> (Linnaeus)	Mari, Pakistan	MHV1558	GU344095	GU344071	GU344048	-
<i>Pycna semiclara</i> (Germar)	Grahamstown, South Africa	MHV0022	FJ168784	FJ168989	GU343932	GU343813
* <i>Pycna strix</i> Amyot & Audinet-Serville	Beparasy	MHV1579	-	MK226182	-	MK226185
<i>Sadaka radiata</i> (Karsch)	Gola National Forest, Liberia	MHV0396	GU344079	-	-	-
* <i>Severiana severini</i> (Distant)	Ongongo camp site, Namibia	MHV0035	FJ168785	FJ168990	GU343991	GU343814
<i>Soudaniella marshalli</i> (Distant)	Mazabuka, Zambia	MHV0642	FJ168911	FJ169113	GU344022	GU343840
<i>Strumosella limpida</i> (Karsch)	unknown, Ghana	MHV0457	GU344081	GU344059	GU344012	GU343833
<i>Suishia coreana</i> (Matsumura)	Ilsanseogu, South Korea	MHV0224	GU344075	-	-	-
<i>Ugada grandicollis</i> (Germar)	near Takoradi, Ghana	MHV0455	-	-	-	GU343832
<i>Yanga guttulate</i> (Signoret)	near Antsohihy, Madagascar	MHV0634	GU344082	GU344061	GU344021	GU343839
<u>Tribe Cicadini</u>						
* <i>Cicada orni</i> Linnaeus	Bouches-du-Rhône, France	07.FR.BD.CLP.06	-	GQ527099	EU401955	GQ527139
<u>Tribe Cryptotympanini</u>						
<i>Auritibicen bihamatus</i> (Motschulsky)	Iwaizumi, Japan	MHV0444	GU344080	GU344057	GU344011	GU343830
<i>Chremistica</i> sp.	Huai Kaeo, Thailand	MHV0771	GU344085	KR674240	-	GU343851
<i>Cryptotympana atrata</i> (Fabricius)	Zhongjiang, China	MHV1476	GU344091	GU344069	GU344046	-
<i>Cryptotympana mandarina</i> Distant	Ban Sen Island, Vietnam	MHV0220	GU344074	GU344051	GU343997	GU343818
<i>Cryptotympana takasagona</i> Kato	Kueitan Hot Spring, Taiwan	MHV0233	GU344076	GU344052	GU343999	GU343820
<u>Tribe Dundubiini</u>						
<i>Meimuna opalifera</i> (Walker)	Anyang, South Korea	MHV1237	GU344087	GU344065	GU344042	GU343864
<u>Tribe Sonatini</u>						
<i>Hyalessa maculaticollis</i> (Motschulsky)	Zhongjiang, China	MHV1477, 08.CN.AH.TIA.24	GU344092	MK226181	-	GU343867

Cicadas were collected and preserved in 95 % ethanol. Total genomic DNA was extracted from the wing or tymbal muscle tissue following the Chelex[®] 100 protocol (Walsh *et al.*, 1991). Small pieces of tissue (c. 2 mm³) were homogenised in 300 µl 5 % Chelex[®] 100 solution and incubated in a heating block at 105 °C for 15 minutes, vortexing the samples every five minutes. Following the incubation period, the supernatant was removed for subsequent use in PCR amplifications.

Portions of three mitochondrial genes and one nuclear gene were sequenced from each sample. The mitochondrial genes used were the small subunit ribosomal 16S RNA (16S) and the cytochrome oxidase subunits I (COI) and II (COII). The nuclear data derived from a portion of elongation factor 1 alpha (EF-1α) encoding exons 3, 4 and 5 and the two associated introns. Primers used for amplification and sequencing reactions were 16S: 16Sar with 16Sbr (Palumbi *et al.*, 1991); COI: COI-F2 (Price *et al.*, 2010) with C1-N-2568 (Brady *et al.*, 2000); COII: TL2-J-3033 with TK-N-3786 (Simon *et al.*, 1994); and EF-1α: EF1-F650-mod (Arensburger *et al.*, 2004) with EF1-N-1419 (Sueur *et al.*, 2007).

Polymerase chain reaction amplifications were performed in 25–50 µl reactions using the following protocol: initial denaturation at 95 °C for 5 minutes; followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 48 °C (16S, COI), 51 °C (COII) or 58 °C (EF-1α) for 45 seconds and extension at 72 °C for two minutes; followed by a final extension at 72 °C for five minutes. PCR products were confirmed by electrophoresis of 5 µl PCR product stained with ethidium bromide and 5 µl tracking dye in a 1 % agarose gel and visualized using a UV trans-illuminator.

PCR products were purified using the Invitex Invisorb MSB[®] Spin PCRapace purification kit (Invitex, Germany) and sequenced in both directions. The sequencing reactions were carried out using the ABI Big Dye Sequencing kit v.3.1, according to the manufacturer's instructions. Sequence trace files were generated using an ABI 3100 genetic analyzer at Rhodes University. Trace files were checked and edited using GeneStudio Pro v.1.0 (GeneStudio, Inc.). The sequence data were imported into MEGA v.4 (Tamura *et al.*, 2007) and aligned using the Clustal W algorithm (Thompson *et al.*, 1994). The alignments were then checked manually.

Phylogenetic analysis

Analyses were run on the CIPRES Science Gateway v3.3 (www.phylo.org; Miller *et al.*, 2010), the Computational Biology Core, Institute for Systems Genomics, University of Connecticut and on a mid-2015 model MacBook Pro.

Appropriate data partitions and models of sequence evolution for the Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were selected using PartitionFinder v2.1.1 (Guindon *et al.*, 2010; Lanfear *et al.*, 2016), the Bayesian Information Criterion, and the "greedy" search algorithm (Lanfear *et al.*, 2012). Branch lengths were set to "linked", and models were selected from the "MrBayes" available set. Eleven potential data subsets included 16S, the EF-1a intron region, and the COI, COII, and EF-1α 1st, 2nd, and 3rd codon positions. The final partition and the substitution models selected are shown in Table 2.

Table 2. Data characteristics showing final data partition and substitution models.

Partition	No. Sites	No. Variable	No. Pars Info	% Pars Info	Model
COI-1, COII-1	524	208	162	30.9	GTR+I+G
COI-2	313	45	21	6.7	GTR+I+G
COI-3, COII-3	523	505	479	91.6	GTR+G
COII-2, 16S	561	196	136	24.2	GTR+I+G
EF1 α -1, EF1 α -2	278	16	5	1.8	K80+I
EF-3	139	89	54	38.8	HKY+G
EF1 α intron	288	214	149	51.2	GTR+G
TOTAL	2626	1273	1006	38.3	

Partitioned Bayesian phylogenetic analysis was conducted using MrBayes v.3.2.3 and v3.2.4 (Huelsenbeck & Ronquist, 2001). This analysis consisted of paired runs each using random starting trees and four chains, sampling every 40,000 generations. Partition rate multipliers were unlinked to incorporate among-partition rate variation (Marshall *et al.*, 2006). An initial branch length prior was set using the *brlenspr* = *Unconstrained : Exponential (100.0)* command, because the taxon sample contained many congeneric species and long branch length priors can cause problems in such cases (Brown *et al.*, 2010; Marshall, 2010). Parameters other than branch lengths and topology were unlinked across partitions. Stationarity was assessed primarily by the average standard deviation of split frequencies, with the run set to terminate when this value dropped below 0.005 (with *burninfrac* set to 0.25). Stationarity was further confirmed by ensuring that the potential scale reduction factor values were 1.001 or less for all parameters, and that the effective sample sizes (checked in Tracer v. 1.5; Rambaut & Drummond, 2007) were above 1000 for all model parameters.

Partitioned ML analysis was undertaken using Garli v2.01 (Zwickl, 2006). Parameters for the evolutionary algorithm were set as in the default version of Garli except that *genthreshfortopoterm* was changed to 40,000 to increase the thoroughness of the search. *Subsetspecificrates* was set to 1 and *linkmodels* was set to 0 for a partitioned analysis. Ten heuristic search replicates were conducted for the initial ML search and for each of 200 bootstrap replicates.

To ascertain whether the current taxonomic understanding of *Platypleura* accurately reflected the most likely topology, separate partitioned likelihood analyses were performed in IQ-Tree v1.6.9 (Nguyen *et al.*, 2015; Chernomor *et al.*, 2016) first under a topologically unconstrained model and then with constraint trees enforced corresponding to the monophyly of all sampled members of *Platypleura* and the monophyly of the sampled African members of *Platypleura*. To remove the confounding effect of *Azanicada zuluensis* (Villet) being nested within the African *Platypleura* as indicated by the preliminary analyses, the two constraint analyses were repeated including *A. zuluensis* within the genus *Platypleura*. The significance of the differences in likelihood scores of the most likely topology with no constraints and the four most likely constrained topologies was assessed using the Approximately-Unbiased (AU) test (Shimodaira, 2002), as implemented in IQ-Tree. The AU

test overcomes bias that can be introduced when the commonly applied SH test (Shimodaira & Hasegawa, 1999; Goldman *et al.*, 2000) is used to compare a very small subset of trees, as when comparing a best-fitting unconstrained tree to a few constrained alternatives (Shimodaira, 2002). For this test, the number of bootstrap replicates was set to 10^4 and model parameters were separately estimated for each replicate.

Molecular dating

The age of each node in the Platyleurini tree and its corresponding 95 % confidence interval were estimated using an uncorrelated lognormal (UCLN) relaxed clock in BEAST v.2.5.0 (Drummond & Rambaut, 2007; Bouckaert *et al.*, 2014). The analysis used a birth-death speciation prior and a random starting tree. Each of the two analyses were run to 100 million generations, and the burn-in was estimated after examination of the samples in Tracer v1.5, such that the effective sample sizes for most substitution model parameters exceeded 100 (Rambaut & Drummond, 2007) and all rate-related parameters exceeded 1000. Maximum clade compatibility trees were generated from the posterior sample in TreeAnnotator v.1.7.5 and viewed using FigTree v.1.4.2. Ingroup monophyly constraints were used to define the Cryptotympanini clade as the most distant outgroup, followed by the *Meimuna+Cicada* clade, following the Bayesian and ML results (see below).

Although no fossils are known from tribe Platyleurini, some have been described from Cryptotympanini, Cicadini, Dundubiini and Sonatini, for which taxa were available in the molecular analyses (Moulds, 2018). Of these, two were chosen to time-calibrate the BEAST analyses. *Cryptotympana miocenica* Zhang & Zhang (age range 16.0–11.6 Ma) was used to constrain the minimum age (lower bound) of a uniform prior age for the most recent common ancestor (MRCA) of *Auritibicen* Lee and *Cryptotympana* to > 11.6 Ma (the taxa formed a monophyletic clade in the MrBayes tree, see Results). Following the same reasoning, *Meimuna protopalifera* Fujiyama (age 23.6–16 Ma) was used to constrain the most recent common ancestor of *Meimuna* + *Cicada* to > 16 Ma.

To further inform the maximum ages of clades in the divergence time analysis, a literature-based empirical molecular clock for the COI data partition was used as explained in Marshall *et al.* (2016). Clock-calibrated divergence time estimation can perform poorly when genetic distances are high for the calibrated locus, apparently due to saturation and poor phylogenetic signal for branch lengths, a problem observed at the tribe level in cicadas (Marshall *et al.*, 2016), and we used two methods proposed in that paper to reduce these artifacts: (1) the use of empirical rather than estimated base frequencies in the substitution model for the clock-calibrated locus, and (2) scaled clock priors for the other gene partitions based on partition-specific relative rates taken from maximum-likelihood analysis. The COI locus was modeled as a single data subset to accommodate the calibration; the remaining data partitions were modeled as in the Bayesian and ML analyses. Relative partition rates, under the composite BEAST substitution/partition model, were estimated in Garli. In addition, we omitted from the data matrix the two mtDNA genes for those taxa that fell outside the largest monophyletic group containing samples from the genus *Platyleura* in the phylogenetic analysis results. This allowed the COI rate information to constrain the age of the most recent common ancestor of the large *Platyleura* sensu lato group while reducing distortion of tree

shape and relative node ages by saturated data, a phenomenon described by Phillips (2009) and Brandley *et al.* (2011).

Rates of diversification within the African Platycleura

To visualise the temporal pattern of diversification within the main African clade of *Platycleura*, a lineage-through-time (LTT) plot was calculated in R v. 3.4.2 (R Development Core Team, 2011) from the BEAST chronogram using the APE (Analyses of Phylogenetics and Evolution) v.4.1 package (Paradis *et al.*, 2004).

Geography of diversification within the Platycleurini

Likelihood-based estimates of ancestral ranges were calculated using the dispersal-extinction-cladogenesis (DEC) model in Lagrange version 20130526 (Ree & Smith, 2008; Ree, 2013). Support values were weak for central nodes in the trees (see Results), so an exploratory analysis was conducted that compared results from three topologies: the MrBayes tree, the ML tree, and the BEAST tree. The MrBayes and ML phylograms were converted to chronograms using r8s version 1.81 (Sanderson, 2002) under the penalized likelihood criterion and a smoothing parameter determined from cross-validation analysis.

Areas were coded as follows: Southern Africa (S), Central/Equatorial Africa, including the Arabian Peninsula (E), Madagascar (M), India (I), and Asia/Europe (A). Comoros Islands were included with Madagascar. As peninsular India has been shown to be biogeographically distinct from the northern Himalayan region of India (Joshi & Karanth, 2013; Agarwal *et al.*, 2014; Kunte, 2016) only taxa that reach central or southern India were coded with (I). Thus, *Platycleura nobilis* (Germar), *Pycna repanda* (Linnaeus), *Oxypleura calypso* Kirby, and *Hamza ciliaris* (Linnaeus), found only in northern India, were coded with (A). The “Southern Africa” range was chosen to reflect patterns observed in Linder *et al.* (2012), including South Africa, Namibia, and Botswana, and excluding Mozambique and Zimbabwe if the distribution only reached the northern sections of those countries. Species included as the sole representative of more widespread genera or higher clades: *Cicada orni* Linnaeus; *Chremistica* sp.; *Hyalessa maculaticollis* (Motschulsky); *Kongota punctigera* (Walker) were coded with the generic distribution, in accordance with catalogue information (Sanborn, 2013) and a molecular tree of the SE Asian tribes (Hill *et al.*, in prep.). *Chremistica*, which is known from Asia, India, and Madagascar, was coded with A/I because data from an undescribed taxon suggests that the Madagascar *Chremistica* are unrelated to the Asian and Indian taxa (Marshall *et al.*, 2018a). All three *Cryptotympana* taxa were coded as A/I because, even though the three taxa are Asian, at least two *Cryptotympana* species extend to central and/or southern India and their phylogenetic positions within the genus are not known (Marathe *et al.*, 2018).

Lagrange was constrained to consider only states containing up to two of the fundamental areas shown. Areas M and S were considered not to be adjacent to either A or I, and dispersal between members of those four pairs was disallowed, following the implications of the divergence-time analysis (root age < 50 Ma). All other area-adjacencies and dispersals were allowed. A single time period, with one dispersal rate matrix (all values 1.0 or 0, as above), was assumed for the entire analysis. The options for symmetric data entry and estimated baseline dispersal and extinction rates were selected. Separate analyses were

run with M–I and S–I connectivity and dispersal allowed, to investigate the effect of their exclusion.

Results

Data characteristics

The final molecular data set comprised 2626 bp, of which 1921 bp were mitochondrial. The EF-1 α data (705 bp) contained portions of exons 3, 4 and 5 and their associated introns. All coding portions of the gene datasets aligned readily, while gaps corresponding to insertion or deletion events were included in the 16S and EF-1 α intron datasets. The initial 16S alignment was reduced from 497 bp to 350 bp to exclude A/T rich regions which could not be aligned unambiguously. PartitionFinder returned a seven-partition scheme with the substitution models shown in Table 2. Plots of transitions and transversions showed that substitution saturation was not present in 16S or EF-1 α introns, but codon-based plots suggested substitution saturation of 3rd base positions in COI, COII, and EF-1 α exons (data not shown). This suggests that more data and/or slower evolving genes, less prone to saturation at the tribal level, may be required to strengthen support for deeper nodes. Data characteristics and model selection for each partition are summarised in Table 2. Analysis setup files and resulting trees are available from the NHM data portal:

<https://doi.org/10.5519/0051823>.

Phylogenetic analysis

In the MrBayes and ML analyses (Fig. 1, Fig. S1), cicadas from the tribes Platycleurini, Orapini and Hamzini form a well-supported clade, sister to *Meimuna*+*Cicada*+*Hyalessa* (= Dundubiini+Cicadini+Sonatini). *Orapa numa* (Distant), representing the African tribe Orapini, is sister to *Pycna semiclara* (Germar) and nested within the earlier-diverging forest-associated clade of Platycleurini from Africa and Madagascar. *Hamza ciliaris* (Linnaeus), representing the tribe Hamzini, is nested within a well-supported clade of Asian Platycleurini.

All of the sampled Asian Platycleurini form a monophyletic assemblage, with the inclusion of the African taxon *Strumosella limpida* (Karsch) with weak support (Fig. 1). This Asian clade is well nested within the African genera. The included African and Asian species of the genera *Pycna*, *Oxypleura* and *Platycleura* do not form monophyletic clades.

The African members of *Platycleura* do not form a monophyletic assemblage because *Azanicada zuluensis* (see Villet, 1987) is deeply nested within the group and *P. signifera* Walker falls well outside the assemblage as sister to *Capcicada absimilis* (Germar). The AU test results (Table 3) show that constraining either all members of the genus *Platycleura* or only the African members of the genus *Platycleura* to be monophyletic had a significantly negative effect on the log-likelihood.

Including *A. zuluensis* as a member of the genus *Platycleura* improved the log-likelihood of the constraint analyses; however, these constraint analyses were still significantly worse than the most likely topology (Table 3). This is a result of *P. signifera* and the Asian members of *Platycleura* being more closely related to platycleurine genera other than *Platycleura sensu stricto* (Fig. 1).

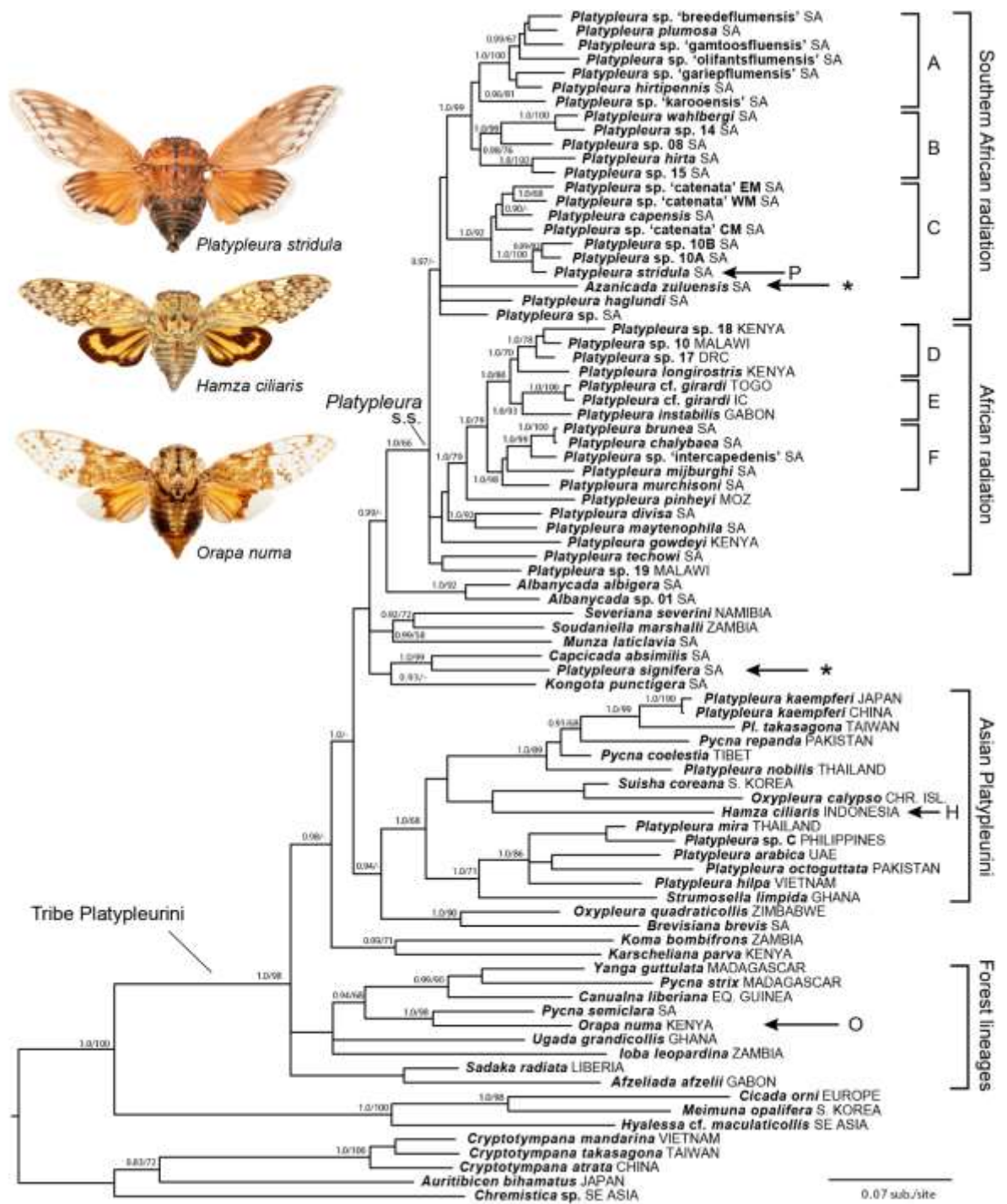


Fig. 1. Bayesian phylogram (50 % majority-rule tree) with posterior probability (≥ 0.95) and ML bootstrap (≥ 70 %) indicated for each node. Discrepancies in the placement of *Azanicada zuluensis* and *Platyleura signifera* with regard to *Platyleura* sensu stricto are highlighted using an asterisk. Collection localities are coded by country following each taxon name (DRC: Democratic Republic of the Congo; IC: Ivory Coast; MOZ: Mozambique; SA: South Africa). Labelled clades (A–F) are discussed in the text. The position of the type species of *Hamza* (H), *Orapa* (O) and *Platyleura* (P) are indicated with example images inset.

Table 3. Summary of topologically constrained likelihood analyses and corresponding probability values derived from the AU test. Significant values are in bold.

Monophyly Constraint	No. trees	-ln L	probability (p)
ML (no constraints)	1	33333.38552	0.98
African <i>Platypleura</i>	1	33398.00913	0.000147
African <i>Platypleura</i> and <i>A. zuluensis</i>	1	33364.44653	0.0214
All <i>Platypleura</i>	1	33612.461	2.2e-13
All <i>Platypleura</i> and <i>A. zuluensis</i>	1	33568.22635	8.66e-37

Within the African members of *Platypleura* sensu stricto (and including *Azanicada*) a large, exclusively southern African clade is apparent alongside weakly defined groups that inhabit savannah and forest regions of Africa (Fig. 1). The southern African lineage is subdivided into three well-supported clades corresponding to the Karoo (clade A), Grassland (clade B) and Fynbos (clade C) biomes. Also found in this clade are *Azanicada zuluensis* (South African coastal forests), *Platypleura haglundii* Stål (southern Africa savannahs), and an unidentified *Platypleura* "sp." (South African Afromontane grasslands).

Well supported forest and savannah clades are found in the remainder of the African radiation corresponding to a central and eastern African distribution (clade D), sister to the West African group (clade E). Sister to these northern clades is a clade restricted to the forest biome of South Africa (clade F).

Molecular dating - Platypleurini

The fossil- and clock-calibrated BEAST chronogram is shown in Fig. 2. The first 10⁶ generations were excluded as burn-in. The mean age of the most recent common ancestor of the sampled species is ca. 40 Ma, with a 95 % confidence interval spanning 32–50 Ma. The divergence between the tribe Platypleurini and the related tribes Cicadini (*Cicada*), Dundubiini (*Meimuna*), and Sonatini (*Hyalessa*) is estimated to the late Eocene (~ 39 Ma) with subsequent diversification within extant Platypleurini beginning in the later Oligocene (~ 25 Ma; Fig. 2).

The forest-associated clade within the Platypleurini, consisting of large-bodied cicadas widely distributed over sub-Saharan Africa, diverged from a common ancestor in the early Miocene (~ 22 Ma). The Asian taxa also diverged from the main body of African taxa in the early Miocene (~ 19 Ma). The genus *Platypleura* sensu stricto is estimated to have diverged from its South African Karoo sister genus, *Albanycada* Villet, in the mid-Miocene (~ 13 Ma), with subsequent diversification of *Platypleura* in the late Miocene (< 9 Ma; Figs. 2, 3B). The Madagascan cicadas *Yanga guttulata* (Signoret) and *Pycna strix* Amyot & Audinet-Serville share a common ancestor with the monotypic West African genus *Canualna* Boulard dated to the mid Miocene (~ 11 Ma).

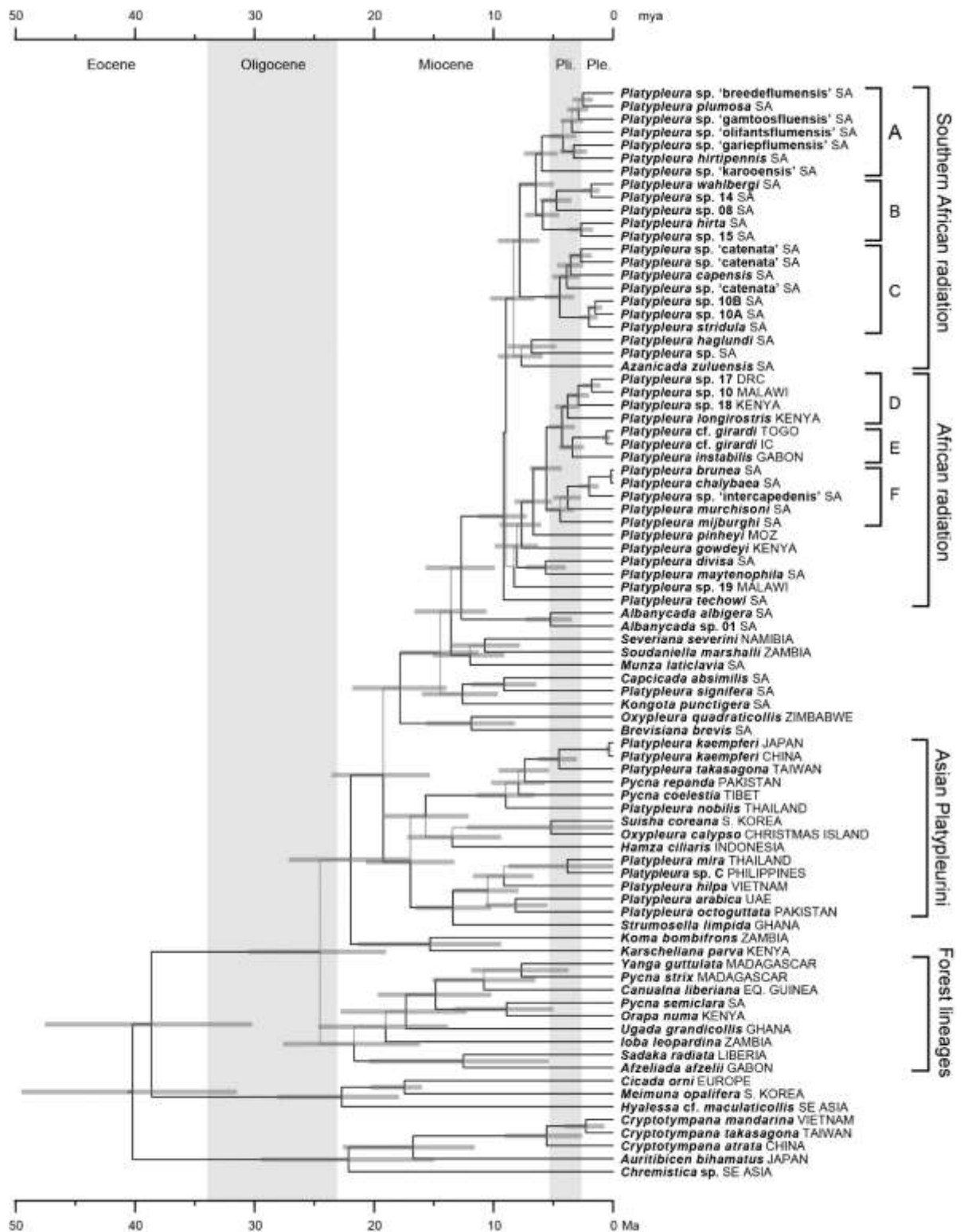


Fig. 2. Platyleurini chronogram from the BEAST divergence-time analysis. Nodes are centred on the mean MRCA with shaded bars indicating the distribution of the 95% HPD for each estimate. Branches in gray have < 0.95 posterior probability. Alternating vertical gray and white bars highlight geological epochs. Collection localities are coded by country following each taxon name. Pli = Pliocene; Ple = Pleistocene. See Fig. 1 caption for other abbreviations. Labelled clades (A–F) are discussed in the text.

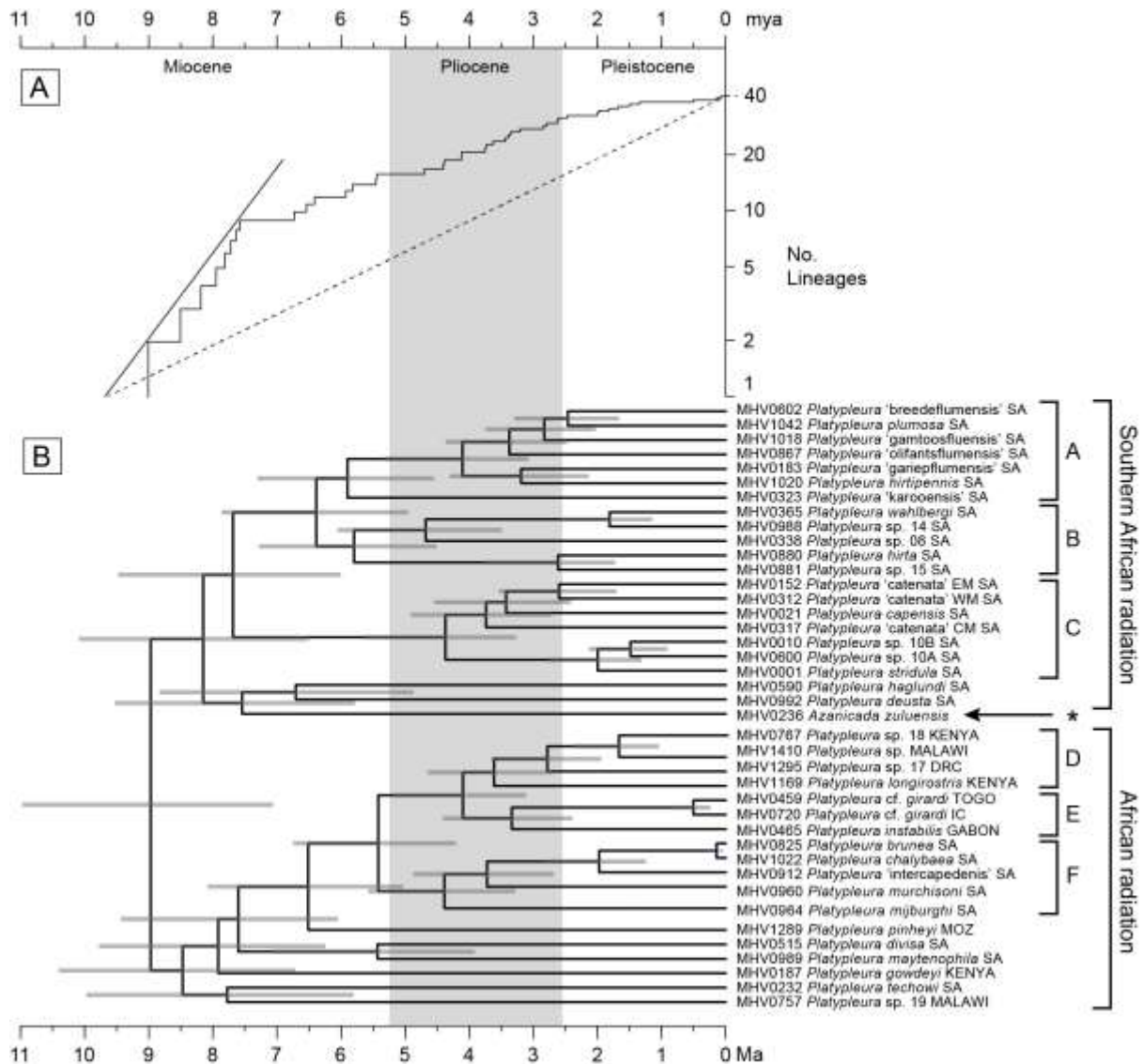


Fig. 3. (A) Lineage-through-time plot showing theoretical constant rate (dashed line) and initial rate (dotted line) of diversification, for *Platypleura* sensu stricto; (B) Section of Bayesian chronogram from Fig. 2 showing African representatives of *Platypleura* sensu stricto generated in BEAST. Nodes are centered on the mean MRCA with shaded bars indicating the distribution of the 95% HPD for each estimate. Discrepancies in the placement of *Azanicada* with regard to *Platypleura* sensu stricto are highlighted using an asterisk. Collection localities are coded by country following each taxon name. See Fig. 1 caption for abbreviations. Labelled clades (A–F) are discussed in the text.

Rates of diversification within African *Platypleura*

A lineage-through-time (LTT) plot of log-lineages against time and a hypothetical constant rate plot are shown in Fig. 3. The LTT plot shows a rapid initial diversification in the late Miocene (~9–7.5 Ma; dotted line in Fig. 3A) followed by an extended period of lineage accumulation at a reduced but consistent rate to the Pliocene-Pleistocene border (~2.6 Ma). The rate of lineage diversification decreases substantially during the Pleistocene.

Geography of diversification within the Platyleurini

For the chronogram derived from the MrBayes DEC analysis, an Equatorial African ancestor is inferred for the tribe Platyleurini (Fig. 4, Table S3) and for most nodes within the major clade containing the forest lineages. The deep-level ancestors in the larger half of the tree (*Platyleura* species + Asian platyleurines and related genera) are also inferred to be broadly African (Equatorial, Southern, or both). Despite the nested position of *Strumosella* Boulard (from Ghana) within the Asian Platyleurini clade, a single dispersal to Asia is consistent with the results, because the ancestor of the African genus and its sister-clade of four *Platyleura* species is inferred to have inhabited both Asia and Equatorial Africa. *Platyleura* sensu stricto is estimated to have had an African ancestor, most likely Southern Africa but some probability is assigned to Southern + Equatorial Africa. A similar reconstruction is found for the common ancestor of *Platyleura* sensu stricto + its closest sister genera in this topology (e.g., *Kongota* Distant, *Capcicada* Villet, *Munza* Distant, *Soudaniella* Boulard, and *Severiana* Boulard), except that the next most probable solution is Equatorial Africa alone. Only the ancestor of *Yanga* + *Pycna strix* is estimated to have lived in Madagascar.

The Lagrange results for the BEAST chronogram (Fig. 5), which place *Oxypleura quadraticollis* (Butler) + *Brevisiana brevis* (Walker) closer to *Platyleura* sensu stricto than in the MrBayes tree, are broadly similar but differ in two main respects. First, while Equatorial Africa remains the most likely ancestral area for the Platyleurini ancestor, there is some support for an Asian or Asian+Equatorial African range of this ancestor and support for a combination of solutions involving Equatorial Africa and Asia for the lineages leading to the main Asian clade. Second, Southern Africa alone is recovered as the most likely area for *Platyleura* sensu stricto and several of the preceding ancestors, among other minor changes. In the Lagrange analysis of the ML topology (Fig. S2), Equatorial Africa is again well supported for the tribe ancestor as with the MrBayes topology, along with southern Africa for *Platyleura* sensu stricto and the larger clade including *Kongota*, *Capcicada*, *Munza*, *Soudaniella*, and *Severiana*. Once again, a single geographic origin of the Asian *Platyleura* species is consistent with the results.

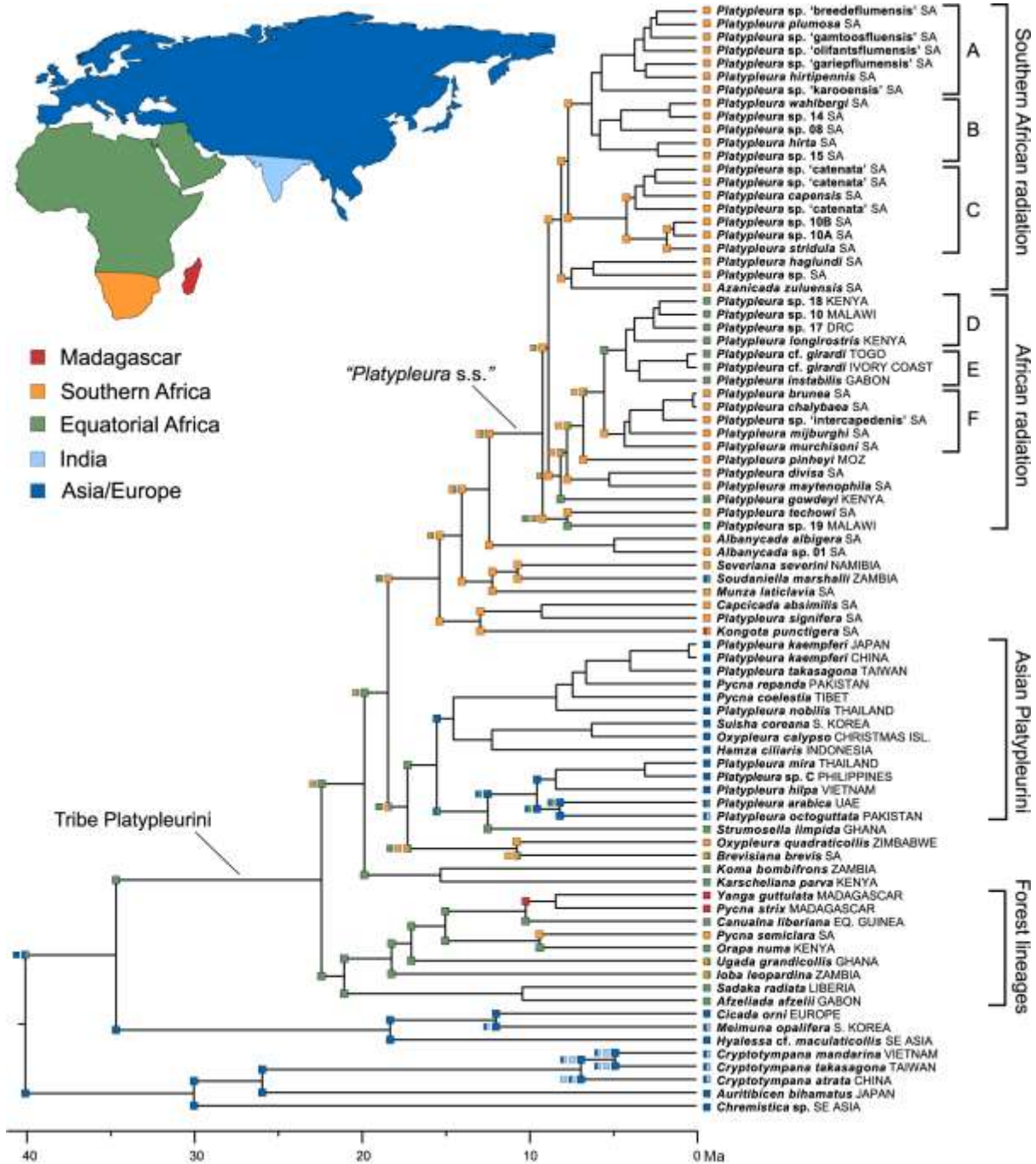


Fig. 4. Rate-smoothed Platyleurini tree from the MrBayes analysis with current taxon distributions coded at the tips of the tree and most probable ancestral geographic area plotted on each branch, as calculated by Lagrange (DEC method). Alternative reconstructions within two log-likelihood points of the most likely outcome are also shown as smaller boxes behind the symbol for the most likely solution. Collection localities are coded by country following each taxon name. Labelled clades (A–F) are discussed in the text.

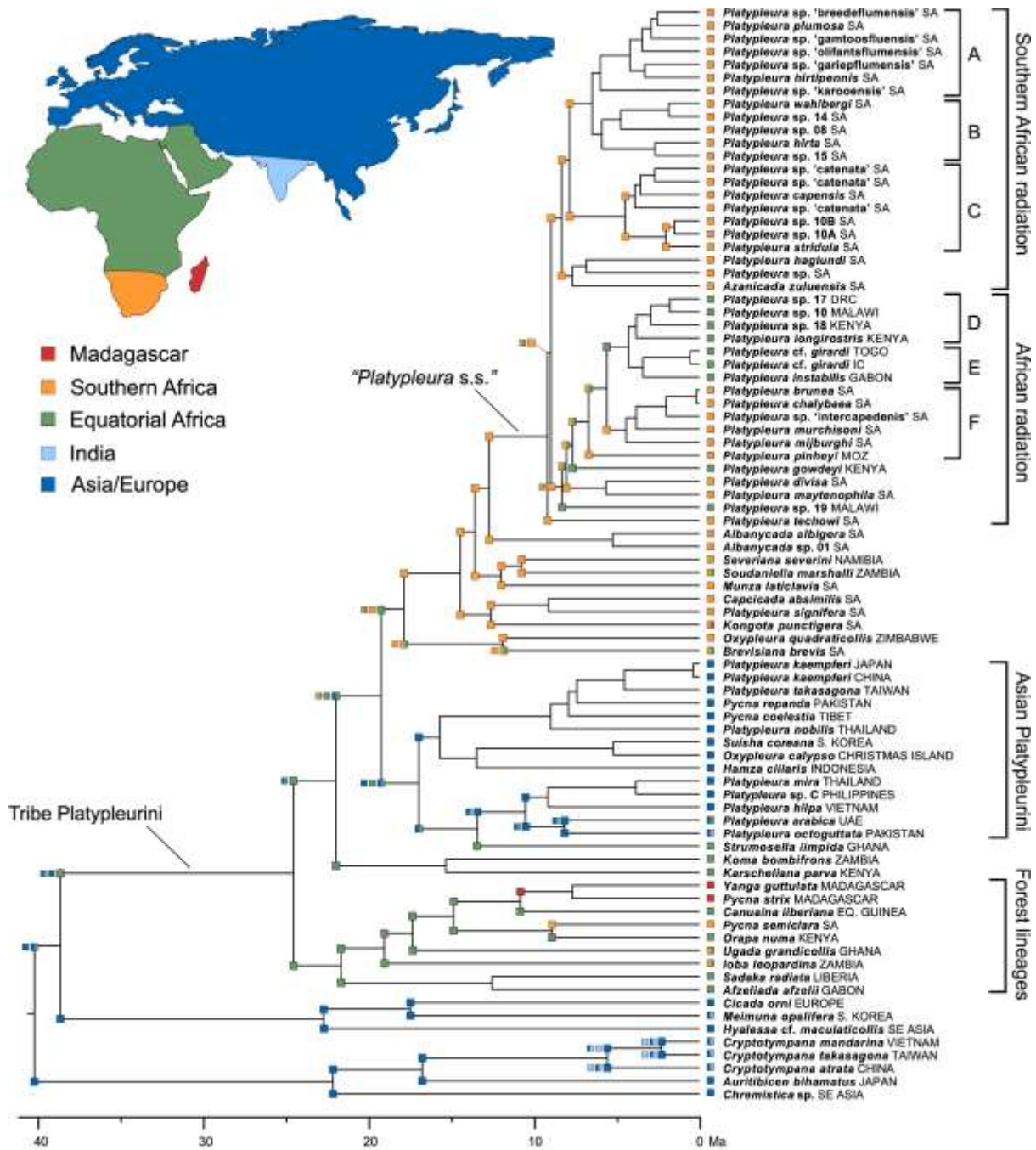


Fig. 5. Platyleurini chronogram from the BEAST analysis with current taxon distributions coded at the tips of the tree and most probable ancestral geographic area plotted on each branch, as calculated by Lagrange. Alternative reconstructions within two log-likelihood points of the most likely outcome are also shown as smaller boxes behind the symbol for the most likely solution. Collection localities are coded by country following each taxon name. Labelled clades (A–F) are discussed in the text.

Discussion

Family-group classification of the Platyleurini

The large sample of platyleurine genera in this analysis allows an assessment of the monophyly of the Platyleurini (Figs. 1, S2), which is upheld provided that the tribes Orapini and Hamzini are subsumed within it. The general appearances of *Orapa* (five species) and *Hamza* (one species) are essentially platyleurine, with extensively pigmented, camouflaged

wings, the abdomen as long as the head and thorax together, and the proximal profemoral spine inclined against the femur, confirming that Hamzini, Orapini, and Platyleurini are synonymous.

Hamzini was erected on the basis of the state of a character that dates back to Distant (1906) and earlier authors. In particular, when *Hamza* was described, the contemporary definition of higher taxa placed great emphasis on the degree to which the tymbal cover was developed. In *Hamza ciliaris* the dorsal rims of the otherwise well-developed tymbal covers lie well clear of the previous tergite, leaving the upper parts of the tymbal membranes unusually exposed from above (Distant, 1904; Duffels, 1991). To maintain consistency in the rest of his classification, Distant (1904, 1906) had to place *Platyleura bouruensis* Distant (= *Cicada ciliaris* Linnaeus) in a separate tribe. While this character-state is unusual, there are no other characters to indicate that Hamzini is distinct from Platyleurini, and several authors subsequently queried or rejected its independence (Duffels, 1991).

Orapini was similarly created on the basis of an autapomorphic character state of the uncus (Boulard, 1985), which is directed posteriorly rather than anteriorly as in most Platyleurini, and monophyly can be maintained by synonymisation on the basis of the molecular evidence.

Hamzini Distant has nomenclatural priority. However, because all of the genera here except *Hamza* have been classified in Platyleurini for 50–100 years, a petition has been submitted to the International Commission on Zoological Nomenclature to conserve the usage of Platyleurini over Hamzini (Marshall *et al.*, 2018b). Following Article 23.9.3 of the Code of Zoological Nomenclature, usage of Platyleurini is maintained while the case is pending. As a result, we here recognize the following classification:

Platyleurini Schmidt, 1918
= Orapini Boulard 1985 **syn.n.**

Biogeographical Hypotheses

Hypothesis 1: The tribe Platyleurini is of Gondwanan origin.

The preliminary dating analysis, based on an empirically derived molecular clock with minimum-age constraints from two fossils, suggests that the tribe Platyleurini diverged from related tribes in the late Eocene (ca. 40 Ma), with rapid diversification of crown group platyleurine genera beginning in the late Oligocene (~ 25 Ma; Fig. 2). The breakup of Gondwana began in the middle Jurassic (~ 170 Ma; Ali & Aitchison, 2008); thus, even given large confidence intervals on the dates, the divergence between the Platyleurini and related tribes in the subfamily Cicadinae is too recent to suggest Gondwanan vicariance driving allopatric differentiation between these tribes. The mean COI rate estimated in the BEAST analysis was 0.0084 substitutions per site per million years (sub./site/Myr). To extend the inter-tribal divergence back to the middle Jurassic would imply a COI mean rate of ca. 0.002 sub./site/Myr, much slower than the slowest COI rates observed for other insects (Papadopoulou *et al.*, 2010).

Hypothesis 2: Asian platypleurine taxa resulted from dispersal from Africa that post-dates the meeting of Africa and Eurasia in the mid-Oligocene (“Out of Africa”).

In all trees, the Asian fauna are nested within the radiation of African platypleurine genera (Figs. 1, 2), which suggests an African origin of the tribe. The date range for the divergence of the Asian clade (21–13 Ma) shown in the BEAST chronogram is consistent with a colonisation event occurring after the collision of Africa and Eurasia (30–25 Ma; Jolivet & Faccenna, 2000). This period was characterised by the closure of the Tethys Sea and the meeting of the African and Eurasian land masses (Hessami *et al.*, 2001; Willis & McElwain, 2002; Gheerbrant & Rage, 2006). Our results suggest that the Asian platypleurine fauna arose from dispersal into Eurasia post-dating the meeting of Africa and Eurasia—another example of “Out of Africa” geo-dispersal *sensu* Lieberman & Eldridge (1996) that has been seen in many taxa (e.g. Whitfield *et al.*, 2006; Kodandaramaiah & Wahlberg, 2007; Zhou *et al.*, 2012; Kunte, 2016; Sahoo *et al.*, 2018). This pattern is further suggested by ancestral area calculations in Lagrange (Figs. 4–6). However, since branch supports are weak in the central portion of the tree in all analyses, and since an Asian ancestor for the tribe was not completely excluded in the Lagrange analysis of the BEAST results, this conclusion remains tentative. The monophyletic origin of the Asian taxa (Fig. 1), including the African *Strumosella* and *Platypleura arabica* Myers (Arabian Peninsula and central Asia), could indicate a single dispersal event from Africa, but this inference is based on only four of the six Asian genera and excludes many Asian species within *Platypleura*, some of which are from India. Additional study will be necessary to conclude that the tribe originated in Africa.

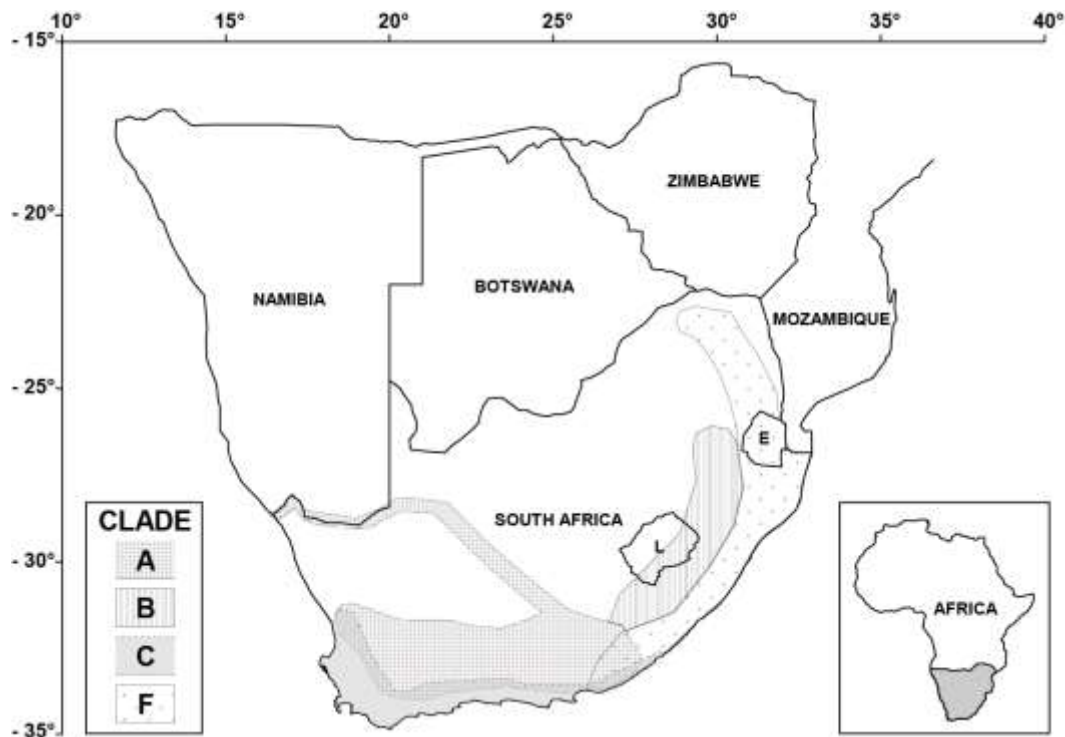


Fig. 6. Approximate distributions of selected species groups of *Platypleura* in southern Africa. Colours correspond to clade labels of Figs. 1–3 and denote Karoo (A), Grassland (B), Fynbos (C) and Forest (F) clades. The forest and savannah clades inhabiting East Africa (clade D) and West Africa (clade E) are not shown. Within South Africa letters denote Lesotho (L) and Swaziland (S).

It has been further suggested that “Out of Africa” dispersal is often followed by diversification in Asia and subsequent “Out of Asia” dispersal back into Africa (Folinsbee & Brooks, 2007; Kodandaramaiah & Wahlberg, 2007). This possibility is initially suggested by the nested position of *Strumosella limpida* (Karsch), a West African species, within the Asian clade, with an estimated date of divergence from the Asian species in the middle Miocene (~ 13 Ma). However, the Lagrange DEC results from all three phylogenetic trees are consistent with a single dispersal to Asia, with *Strumosella* originating from an ancestor that bridged Asia and Equatorial Africa.

Hypothesis 3: Platyleurine genera containing both Asian and African representatives (Platyleura, Pycna, Oxypleura) do not represent monophyletic assemblages.

This study represents a first estimate of the phylogeny of the tribe Platyleurini, and the results suggest that the taxonomy of the Asian Platyleurini require review. The included genera from *Platyleura*, *Oxypleura* and *Pycna* do not form reciprocally monophyletic lineages in our study (Fig. 1). Regarding *Platyleura*, the type species, *P. stridula* (Linnaeus), is included in this study and falls within the main clade of African *Platyleura* (+ *Azanicada*). Species from several other platyleurine genera fall between this African *Platyleura* clade and the Asian *Platyleura* taxa, or within the Asian clade. The AU test showed that analyses constrained to keep *Platyleura* monophyletic were inferior. However, a taxonomic revision is beyond the scope of this study and would require a more complete sample of Asian and African species (especially types of genera such as *Suisha*, *Soudaniella*, *Oxypleura*, and *Strumosella*) and further molecular and morphological attention, since key nodes in the central portion of the tree are not yet strongly supported.

The African species *P. signifera* Walker forms a well-supported sister relationship with *Capcicada absimilis* (Germar), which is confirmed by gross morphology of the genitalia (Villet & Price, in prep) and suggests that the former should be excluded from *Platyleura* sensu stricto (Fig. 7). We do not propose formally moving *Platyleura signifera* here, given the lack of a robust assessment of the other platyleurine genera.

Like Hamzini and particularly Orapini, the genus *Azanicada* was created on the basis of the autapomorphic structure of the urite (Villet 1989), which is distinct from the otherwise uniform urite found in African members of *Platyleura*. The remaining morphological characters of *Azanicada* are compatible with a placement in *Platyleura* sensu stricto (Fig. 7), which leads us to synonymize these genera and conserve the monophyly of *Platyleura* sensu stricto.

The type of *Oxypleura* is *O. clara* Amyot & Audinet-Serville, an African species from The Gambia that was not included in our analysis. Although we are confident that *Oxypleura* is polyphyletic as currently defined, it is not possible to conclude from current molecular evidence which (if either) position on the tree corresponds to the true *Oxypleura* until the type is sequenced. However, on the basis of highly similar genitalia we postulate that the type of *Oxypleura* would group with *O. quadraticollis*, rather than *O. calypso* (Fig. 8).

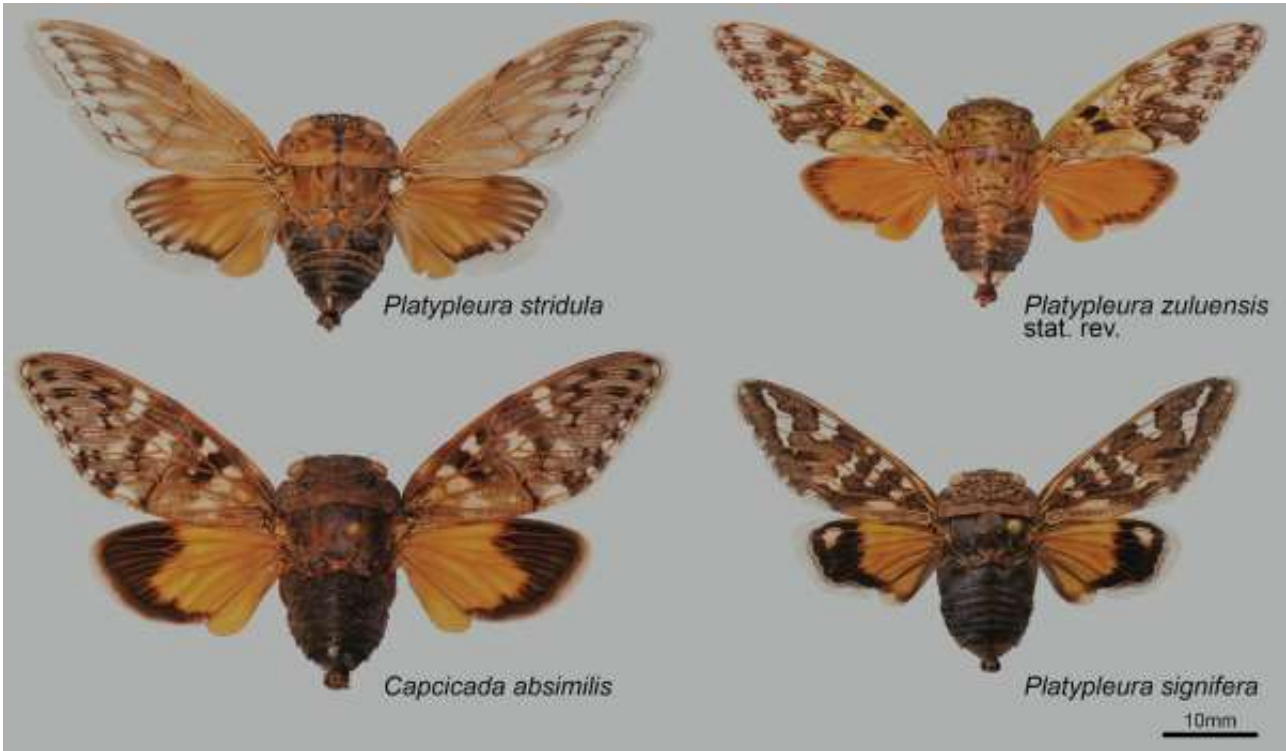


Fig. 7. Dorsal view of the type species *Platypleura stridula* with *P. zuluensis* stat. rev. and the type species *Capcicada absimilis* with *Platypleura signifera*.

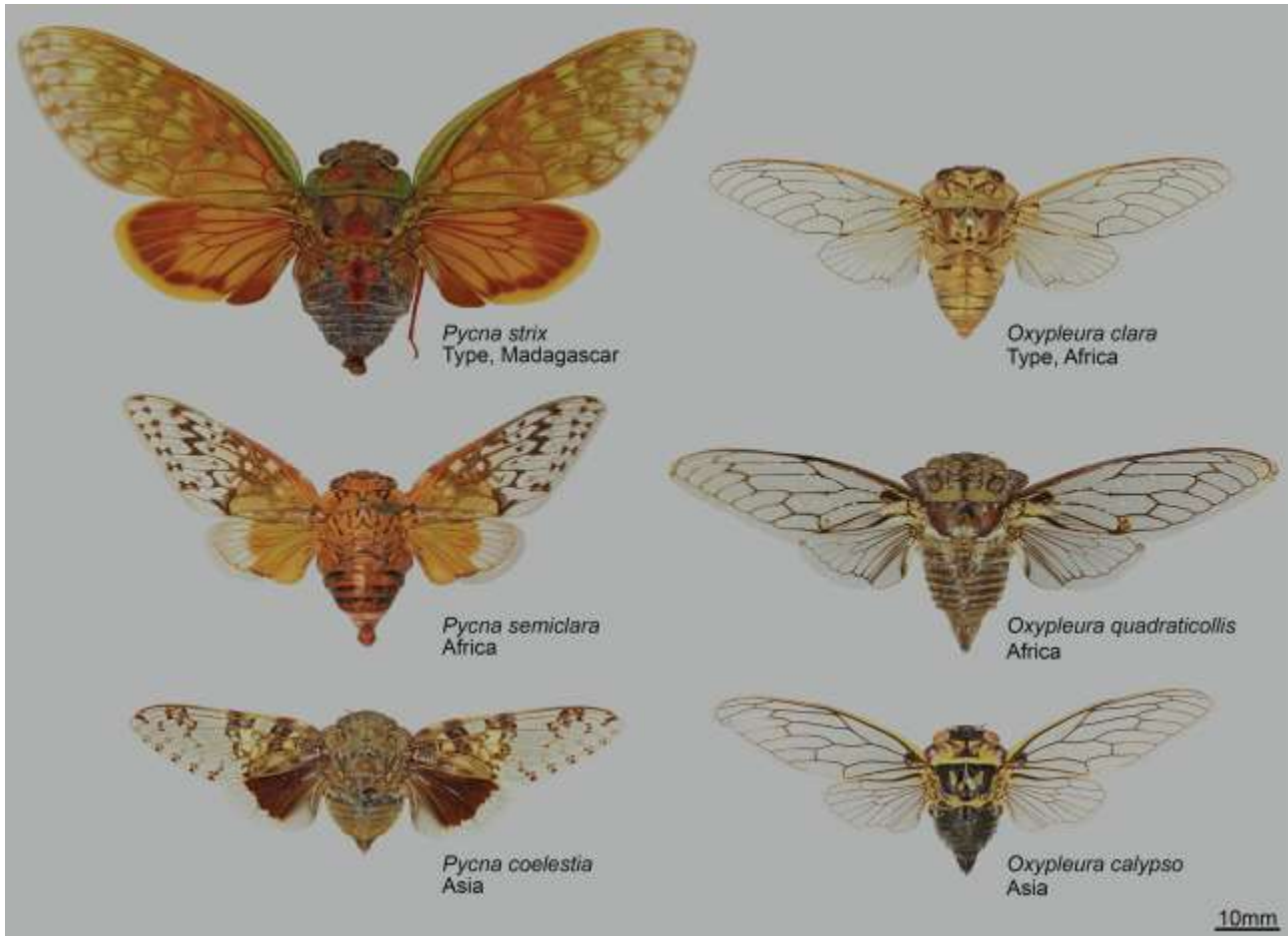


Fig. 8. Dorsal view of the type species and representative African and Asian species in the genera *Pycna* and *Oxypleura*.

The type species of *Pycna* is *P. strix* Amyot & Audinet-Serville. This Madagascan cicada is unrelated to *P. repanda* and *P. coelestia* from Asia in the molecular tree (Fig. 1), suggesting that the genus requires revision. More data are needed to strengthen the position of *P. semiclara* in the tree, but it also appears unlikely to form a monophyletic group with the *Pycna* type species (Figs. 1, 8).

Within *Platypleura* sensu stricto the following taxonomic changes are formally proposed:

Platypleura Amyot & Audinet-Serville, 1843

= *Azanicada* Villet, 1989 **syn.n.**

Platypleura zuluensis Villet, 1987 **stat.rev.**

= *Azanicada zuluensis* (Villet, 1989)

Hypothesis 4: Forest-associated platypleurine lineages predate the aridification of Africa.

Species from the forest-associated African platypleurine lineages (including *Ugada*, *Yanga*, *Pycna*, *Orapa*, *Canualna* and *Sadaka*) form one or two clades that diverged early in the Platypleurini tree (Fig. 1), probably before the aridification initiated by the closing of the Tethys and the initiation of the Antarctic Circumpolar Current (24 Ma: Pfuhl & McCave, 2005) (Fig. 2). Exploratory reconstruction of ancestral areas (Figs. 4–6) suggests an Equatorial Africa origin for the Platypleurini, which would also imply that early platypleurines inhabited forests, which are understood to have been the dominant African habitat before the collision between Africa and Eurasia (Jacobs, 2004; Aduse-Poku *et al.*, 2009). However, at least 40 described species from forest genera have not been sampled, and the weak support at central nodes of the trees means that this biogeographical inference is tentative.

The divergence of the large monophyletic assemblage of arid-adapted and largely South African Platypleurini, dated from the middle Miocene, can be correlated with the fragmentation of widespread forest habitat in Africa. This fragmentation was primarily due to dramatic global cooling and drying associated with the northward progress of Africa and South America, the consequent opening of the Drake Passage 41 Ma, the advent of the Antarctic Circumpolar Current, a drop in global CO₂, and the subsequent expansion of a permanent ice sheet in Antarctica (DeConto & Pollard, 2003; Couvreur *et al.*, 2008; Wahlberg *et al.*, 2009). Aridification and the dynamic nature of forests have been suggested as a possible cause for a number of lineage diversification events in the Oligocene and Miocene periods in Africa, including trees (Couvreur *et al.*, 2008), ctenoplectrine bees (Schaefer & Renner, 2008), nymphalid butterflies (Wahlberg *et al.*, 2009; Price *et al.*, 2010), rhytidid snails (Moussalli *et al.*, 2009), *Hyperolius* Rapp frogs (Wieczorek *et al.*, 2000), birds (Roy *et al.*, 2001) and various mammal lineages (Moritz *et al.*, 2000; Mayaux *et al.*, 2004; Willows-Munro & Matthee, 2009). The LTT plot for the South African radiation shows rapid lineage accumulation after 10 Ma followed by slower diversification in the Pliocene and a plateau during the Pleistocene (Fig. 3). Slower inferred diversification toward the present may be due to a combination of three factors: reduced rates of speciation, high rates of lineage extinction, and poor taxon sampling. Although the vast majority of South African taxa have been included in the analysis, the true number of species within *Platypleura* in the

remainder of Africa is presently unknown, thus insufficient taxon sampling cannot be discounted.

Hypothesis 5: Madagascan platypleurine taxa (Pycna and Yanga) are a result of recent dispersal from Africa, and not Gondwanan vicariance (cf. Yoder & Nowak, 2006).

Pycna strix (the type of *Pycna*) and *Yanga guttulata*, both from Madagascar, form a well-supported clade with *Canualna*, a monotypic genus found in West Africa and Annobon Island (Fig. 1). The common ancestor diverged in the late Miocene (~ 10 Ma; Fig. 2); this date is an order of magnitude smaller than required to imply Gondwanan vicariance rather than dispersal from Africa. Trans-oceanic dispersal in *Yanga* is not surprising when considering that the distribution of all of the 14 species in this genus are restricted to the African islands of the Seychelles, Comoros, Pemba and Madagascar (keeping in mind that the type of *Yanga* was not sampled), and some species are found on multiple islands. Indeed, many groups found on Madagascar and Africa are now thought to have dispersed between these landmasses relatively recently (Raxworthy *et al.*, 2002; Vences, 2004; Yoder & Nowak, 2006; Vences *et al.*, 2009).

*Hypothesis 6: The African members of Platypleura originated in northeastern Africa, with a southward radiation (Villet *et al.*, 2004).*

The ancestral lineage of *Platypleura* sensu stricto is proposed to have diverged from a common ancestor with *Albanycada* in the middle Miocene (~ 13 Ma). *Albanycada* is currently restricted to the thicket and Nama Karoo biomes in the Eastern Cape of South Africa, suggesting that *Platypleura* and *Albanycada* diverged from a common ancestor in southern Africa, not East Africa as previously suggested (Villet *et al.*, 2004). However, this hypothesis requires further attention as southern African thicket is part of a global thicket biome which was once widespread during the Palaeogene (65–23 Ma; Cowling *et al.*, 2005; Schrire *et al.*, 2005). Furthermore, the sister relationship between the tettigomyiine cicada genera *Xosopsaltria* Kirkaldy in the thicket biome in South Africa and *Paectira* Karsch in savannah in East Africa (unpublished data) and the disjunct distributions of multiple vertebrate taxa (e.g. Mathee & Robinson, 1996, 1997; Muwanika *et al.*, 2003) suggests a link between East and southern Africa that requires further attention.

Two main radiations of *Platypleura* within Africa are hypothesized; the first distributed throughout the forest and savannah biomes of sub-Saharan Africa (including clades D & E), and the second restricted to southern Africa (clades A–C & F) (Figs. 3B, 6). These radiations appear to have occurred concurrently and to have begun in the late Miocene (~ 8 Ma) with rapid lineage accumulation during the Pliocene (Fig. 3B).

The African radiation does not follow a pattern of regional monophyly (Fig. 3B), which is indicative of diversification in response to large-scale climatic shifts, but an absence of samples of species with a central African distribution precludes further analysis in this group. The pattern of initial high diversification in the late Miocene (~ 9–7.5 Ma) is congruent with a study of the chameleon fauna of southern Africa (Tolley *et al.*, 2008) and is suggestive of a common process in this region. One plausible mechanism in southern Africa is the development of the Benguela current in the late Miocene (Siesser, 1980; Dupont *et al.*, 2005) and the associated onset of cooler, more arid conditions (van Zinderen Bakker, 1975), which

would result in fragmentation of forest habitats. Indeed this has been suggested as a stimulus for lineage diversification in a number of southern African reptile taxa (Lamb & Bauer, 2003; Lamb *et al.*, 2003; Bauer & Lamb, 2005; Makokha *et al.*, 2007). Toward the end of the Miocene (~ 7 Ma), and into the Pleistocene, the rate of lineage accumulation in *Platypleura* decreased rapidly, indicative of increased extinction rates although the effect of incomplete taxon sampling cannot be completely discounted. This period is characterized by large-scale changes in the vegetation of southern Africa in response to climatic shifts (Linder, 2003). Within southern Africa, four well-supported clades correspond to the Karoo (clade A), Grassland (clade B), Fynbos (clade C) and Forest (clade F) biomes (see also Price *et al.*, 2010), suggesting biome-specific radiations in this region (Figs. 3B, 6).

Conclusions

As this is the first estimate of the relationships within the Platyleurini, with substantial numbers of species and some types remaining to be sampled, including a number of Indian taxa, the resulting inferences are necessarily tentative on the origin and diversification of this tribe in Africa. The dating analysis suggests that the divergence of the platyleurine cicadas from related tribes in Cicadinae is not a result of Gondwanan vicariance followed by dispersal into Asia. The Asian taxa are implied to have a single origin post-dating the meeting of the African and Eurasian landmasses, the taxonomic implications of which highlight the need for a formal review of the platyleurine genera. The evidence of diversification of *Platypleura* and closely related genera coincident with the late Oligocene and Miocene onset of aridification in Africa suggests that the fragmentation of forests and expansion of savannah may have contributed to the diversification of this tribe in Africa. The platyleurine cicada fauna of Madagascar are most likely not the result of ancient vicariance, but of recent dispersal from Africa. Finally, the origin of the genus *Platypleura* in Africa is not definitively placed within eastern or southern Africa and awaits a more complete sample, specifically from central and eastern African species (e.g. *P. machadoi* Boulard, *P. kabindana* Distant and *P. makaga* Distant) not available for this study. Within South Africa the majority of representatives of *Platypleura* form four distinct clades, each restricted to separate biomes: Fynbos, Karoo, Forest and Grassland respectively.

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Supplementary Material

Table S1. Approximate tally of tribe Platyleurini genera, species, and approximate distributions, ignoring undescribed species and subspecific taxa (generally following Sanborn, 2013). Genera in bold are sampled in this study. Nicobar/Andaman Islands included with Asia/India. Madagascar, Seychelles and Comoros included with African region. Note that unpublished genetic data indicates that *Kalabita* does not belong in Platyleurini.

Genus (# spp.)	African region	Asia/India (S./C. India)
<i>Afzeliada</i> (24)	24	
<i>Albanycada</i> (1)	1	
<i>Attenuella</i> (1)	1	
<i>Azanicada</i> (1)	1	
<i>Brevisiana</i> (3)	3	
<i>Canualna</i> (2)	2	
<i>Capcicada</i> (1)	1	
<i>Esada</i> (1)	1	
<i>Hanianosemia</i> (1)		1
<i>Hamza</i> (1)		1
<i>Ioba</i> (6)	6	
<i>Kalabita</i> (1)		1
<i>Karscheliana</i> (2)	2	
<i>Koma</i> (4)	4	
<i>Kongota</i> (2)	2	
<i>Muansa</i> (1)	1	
<i>Munza</i> (10)	10	
<i>Oxypleura</i> (11)	9	2
<i>Platyleura</i> (83)	41	43 (8)
<i>Pycna</i> (30)	22	8
<i>Sadaka</i> (6)	6	
<i>Sechellalna</i> (1)	1	
<i>Severiana</i> (3)	3	
<i>Soudaniella</i> (8)	8	
<i>Strumosella</i> (3)	3	
<i>Strumoseura</i> (1)	1	
<i>Suisha</i> (2)		2
<i>Tugelana</i> (1)	1	
<i>Ugada</i> (18)	18	
<i>Umjaba</i> (2)	2	
<i>Yanga</i> (12)	12	
Total (243)	186	58



Fig. S1. Platypleurini tree from the maximum-likelihood analysis, with bootstrap support values > 50% shown. Collection localities are coded by country following each taxon name.

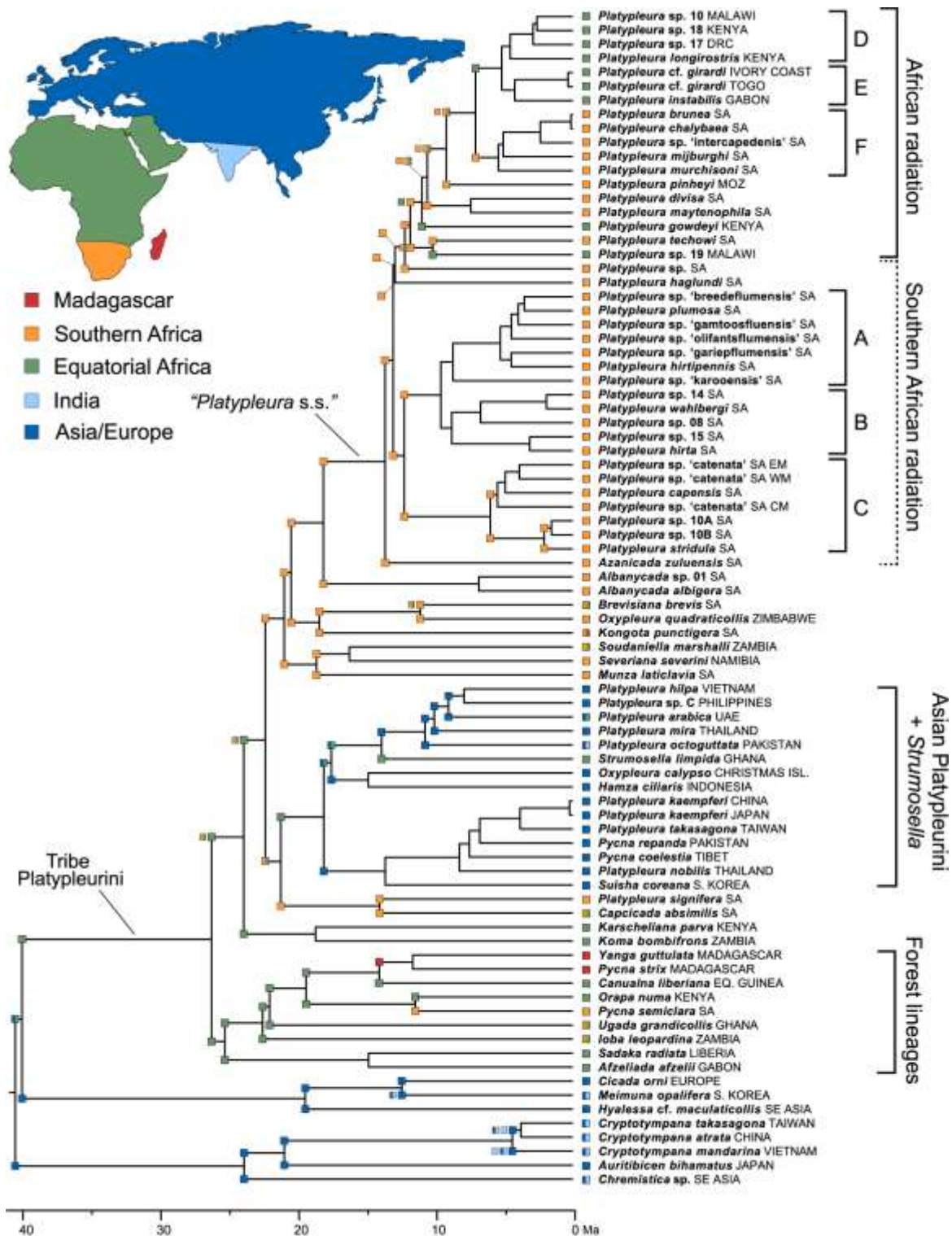


Fig. S2. Rate-smoothed Platypleurini tree from the maximum-likelihood analysis with current taxon distributions coded at the tips of the tree and most probable ancestral geographic areas plotted on each branch, as calculated by Lagrange. Alternative reconstructions within two log-likelihood points of the most likely outcome are also shown as smaller boxes behind the symbol for the most likely solution. Collection localities are coded by country following each taxon name. Labelled clades (A–F) are discussed in the text