



## Research

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# Multiple transgressions of Wallace's Line explain diversity of flightless *Trigonopterus* weevils on Bali

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The fauna of Bali, situated immediately west of Wallace's Line, is supposedly of recent Javanese origin and characterized by low levels of endemism. In flightless *Trigonopterus* weevils, however, we find 100% endemism for the eight species here reported for Bali. Phylogeographic analyses show extensive *in situ* differentiation, including a local radiation of five species. A comprehensive molecular phylogeny and ancestral area reconstruction of Indo-Malayan–Melanesian species reveals a complex colonization pattern, where the three Balinese lineages all arrived from the East, i.e. all of them transgressed Wallace's Line. Although East Java possesses a rich fauna of *Trigonopterus*, no exchange can be observed with Bali. We assert that the biogeographic picture of Bali has been dominated by the influx of mobile organisms from Java, but different relationships may be discovered when flightless invertebrates are studied. Our results highlight the importance of in-depth analyses of spatial patterns of biodiversity.

## 1. Introduction

The Indonesian island of Bali lies in a critical biogeographic position—on the edge of the Sunda shelf just west of the Lombok strait that demarcates Wallace's Line [1–4]. Yet, the numerous studies of the Indo-Australian fauna usually focus on the larger islands of Borneo and Sulawesi [5–8] and data on the fauna of Bali remain surprisingly scarce and scattered. Bali is essentially an extension of East Java, at the closest point only 2 km apart and repeatedly connected during lower sea levels [9]. This is reflected in Bali's presumably low degree of local endemism and fauna shared with Java.

Work in the first half of the last century focused mainly on lists of vertebrate species summarized by Rensch [10]: he concludes that there is only a single bird endemic to Bali, the now critically endangered Bali myna (*Leucopsar rothschildi* Stresemann). The majority of the remaining Balinese bird species also occur in Java, while only a very few are shared with islands to the East [10,11]. All native frog species except for one are shared with Java [12]. Large Asian mammals such as the banteng (*Bos javanicus* d'Alton) and the tiger (*Panthera tigris* Linnaeus) reached their easternmost area of distribution in Bali although the latter is now extinct on Bali [13]. The notion that the Balinese fauna is derived from Java relatively recently is confirmed by some rather mobile groups of invertebrates [14,15]. There are 14 species of land snails endemic to Bali and the neighbouring island of Nusa Penida [16], but the geographical provenance of their ancestors remains unknown.

There are no modern inventories of Balinese fauna, and molecular phylogenetic methods have never been applied to investigate macro-evolutionary processes explaining faunal origins or phylogeographic patterns within this island. Here, we conducted a comprehensive inventory of an Indo-Malayan–Australasian group of flightless weevils: *Trigonopterus* Fauvel is an ideal group

for studying the complex biogeographic history of the Indo-Australian archipelago. This genus has a marked tendency towards local species endemism, but despite the inability to fly, *Trigonopterus* has a wide range, from east Sumatra across Melanesia to the Samoan islands. It is hyperdiverse in New Guinea, with more than 300 species recorded [17,18]. Although only a single species has been described from Sulawesi to date, we have more than 100 new species awaiting formal description (A. Riedel 2014, unpublished data). Diversity decreases to the West, but is still substantial with more than 50 species recently discovered in Borneo, Sumatra and Java [19]. Species are confined to wet primary forests where they can be collected by sifting the leaf-litter. Many such habitats have been degraded or were converted to agricultural use as a consequence of the human population explosion on Java and Bali [20]. However, many lowland areas of East Java, Bali and the Lesser Sunda Islands currently support a seasonal type of monsoon forest, according to our experience not a suitable habitat for *Trigonopterus*. Therefore, these weevils are confined to remnants of wet primary forests, typically on mountainsides.

Here, we use molecular phylogenetic data coupled with ancestral area inference to show that *Trigonopterus* weevils have repeatedly colonized Bali from the East, thereby transgressing Wallace's Line. Surprisingly, there are no closer relationships with the fauna of nearby East Java, underpinning the need for comprehensively sampled, phylogeny-driven studies to better understand the region's faunal evolution.

## 2. Material and methods

### (a) Taxon sampling and DNA sequencing

Species for our analysis were selected from a preliminary phylogenetic reconstruction which we performed to identify major clades. This initial analysis contained 138 *Trigonopterus* species with an alignment of 4646 bps consisting of fragments from CO1, 16S rRNA, arginine kinase, CAD, elongation factor 1 $\alpha$ , enolase and histone 4. It included all 82 species from Sumatra, Java and the Lesser Sunda Islands found during a total of 212 days of fieldwork (17 days in Sumatra, 120 days in Java, 28 days in Bali, 27 days in Lombok, 12 days in Sumbawa and 8 days in Flores) covering 72 localities and resulting in 354 litter samples (electronic supplementary material, S6). A total of 3812 *Trigonopterus* specimens were available, and a full taxonomic treatment of this material is currently in preparation. All major areas of suitable habitat of the Sunda Arc were sampled. East Java, Bali and Lombok were sampled most intensely; we did not retrieve additional species upon repeated visits to the same localities. Areas of Sumatra, West Java and Flores may harbour additional species, but we are confident that all major clades from these areas have been discovered because additional visits did not reveal new lineages. A single clade containing most Balinese species together with a Lombok species (292) was well supported. Most species from Java and Sumatra, including species 299 and 348, are monophyletic with equally strong support. Eight species of *Trigonopterus* subgenus *Mimidotasia* from Java and Sumatra comprise an early diverging lineage that was omitted from the subsequent analysis because the group is missing from Bali and the Lesser Sunda Islands. The entire clade containing species 328 from Bali and species 317 from East Java was transferred to the subsequent analysis.

Our present dataset contains all Balinese species and their respective sister clades (electronic supplementary material, S3) along with a representative selection of the remaining fauna of the Sunda Arc comprising 40 species. Furthermore, the same number

of species was added, representing all major lineages from Borneo, and the hyperdiverse islands of Sulawesi and New Guinea. Four cryptorhynchine species from Australia, New Guinea and Java were included as outgroup representatives (*Critomerus iliacus* (Pascoe); *Microporopterus* cf. *setosus* Voss; *Ouporopterus squamiventris* Lea; *Miocalles* sp.). Some of the *Trigonopterus* species from New Guinea possess a valid name [21], while others are currently being revised and described. Undescribed species are referred to by unique species numbers that will be given in future taxonomic treatments. All the species were monophyletic in a phylogeny using CO1 data of multiple specimens per species, and also well delineated by male genital characters.

DNA was extracted non-destructively using the DNeasy and NucleoSpin 96 Tissue kits (Qiagen, Hilden; Macherey-Nagel, Düren, Germany). For PCR amplification (electronic supplementary material, S1), we used standard protocols ([http://zsm-entomology.de/wiki/The\\_Beetle\\_D\\_N\\_A\\_Lab](http://zsm-entomology.de/wiki/The_Beetle_D_N_A_Lab)). Sequences were edited using SEQUENCHER v. 4.10.1 (GeneCodes Corp., Ann Arbor, MI, USA).

### (b) Alignment and data matrices

Twelve fragments representing nine genes were sequenced (electronic supplementary material, S2 and S7). Alignments were performed with MUSCLE [22] and reading frames checked in MESQUITE v. 2.75 (<http://mesquiteproject.org>). Alignment length was 6800 bps (two assembled fragments of CO1 (1416 bps), 16S (579 bps), 18S (584 bp), 28S (534 bps), arginine kinase (720 bps), CAD1 (462 bp), CAD2 (594 bps), CAD3 (663 bps), elongation factor 1 $\alpha$  (372 bps), enolase (663 bps) and histone 4 (213 bps)).

### (c) Phylogenetic inferences

We used maximum likelihood (ML) as well as Bayesian inference (BI) to reconstruct the relationships among *Trigonopterus* species. ML analyses were performed in RAxML [23] with 1000 thorough bootstrap replicates using five different partitioning strategies: no partitioning, one partition for each gene, one partition for each genome (mitochondrial versus nuclear), one partition for each type (coding versus non-coding genes) and one partition for each codon position (for non-coding genes, one partition for each). The same strategies were used for BI analyses carried out in MrBAYES v. 3.2 [24] (electronic supplementary material, S4). We sampled 30 million generations of two independent runs consisting of eight Markov chain Monte Carlo (MCMC) sampling every 1000th generation. A burn-in of 5000 trees was chosen after investigation of split-frequencies and log-likelihood curves in TRACER v. 1.5 [25]. A 50% majority rule consensus tree was constructed afterwards based on the remaining trees. The best-fitting partitioning strategy for BI was selected using Bayes factors [26] tests based on marginal likelihoods estimated through stepping-stone sampling [27]. The most appropriate substitution model for each partition was selected using the Bayesian information criterion as implemented in jMODELTEST v. 2.1.3 [28].

### (d) Dating and ancestral area reconstruction

Divergence times were estimated with the Bayesian relaxed clock method implemented in BEAST v. 1.8.0 [29]. The only time-calibrated tree of Curculionidae available [30] did not recover Cryptorhynchinae as monophyletic, perhaps because more than 30% of the data was missing. The scant fossil record of this subfamily does not offer a taxon to which *Trigonopterus* could be safely attributed. As a result, we were not able to use a secondary calibration for the *Trigonopterus* radiation. In a first calibration, several substitution rates of Coleoptera have been used (calculated for the COI marker using multiple fossils and geological evidences [31–33]—see [34] for a rationale on the use of this interval). The early diversification of *Trigonopterus* would have taken place more than 60 Ma which appears significantly too old. The age of

flightless *Galapaganus* weevils older than the islands they inhabit was attributed to earlier, sunken islands [35]. In the case of *Trigonopterus*, no such land areas can be expected based on geological reconstructions of the New Guinea area more than 60 Ma [36,37]. The high interspecific divergences (mean 20% for CO1) previously reported for *Trigonopterus* [17] from supposedly young geological terranes might indicate accelerated molecular evolution, which has recently been linked to flightlessness [38]. Loss of flight in beetles not only promotes speciation, but also those flightless species retain a higher genetic differentiation on population level and show deeper genetic branching than flying species [39]. A life history that requires little movement is an equally important factor [40]. In *Trigonopterus*, both factors are given: these weevils, as well as all the other members of the subtribe Tylochina are fully wingless, and their habitat, the leaf-litter of humid forests, is a highly stable and relatively uniform resource. Groups of groundwater- and cave-dwelling Crustacea are also known for markedly accelerated evolutionary rates related to their fragmented populations and the frequent occurrence of bottlenecks [41].

Therefore, in order to obtain divergence time estimates, we used a geological calibration. We constrained the root of the tree not to be older than 30 Myr as a conservative estimate, because the early lineages in our phylogeny (figure 1) were all Papuan *Trigonopterus*. Present-day New Guinea has a highly complex orogenic history, but the most recent geological reconstructions of the region [36,37] suggest that at most small and low-lying islands were emergent before 30 Ma. If some land did exist before 30 Ma, it was lacking the horizontal and vertical dimension required to facilitate lineage diversification; habitats fully explaining the observed diversification patterns are more recent and likely of Miocene age ([34]; R. Hall 2013, personal communication). This was reflected in empirical studies (birds [42], rainbow fishes [43], diving beetles [34]) which estimate the onset of Papuan lineage diversification around 30 Ma or more recent. Thus, our calibration is likely to be conservative and may yield slightly overestimated ages. Using more recent root calibration dates (20 Ma and 10 Ma) had no impact on the biogeographic scenario inferred for Bali. The analyses were performed under a *Speciation: Birth–Death Incomplete Sampling* [44] using an estimated relaxed clock rate (uncorrelated lognormal) because the hypothesis of a strict molecular clock was tested and rejected ( $p$ -value < 0.001) in PAUP\* [45]. The MCMC parameters were fixed to 30 million generations with sampling every 1000th generation and discarding 5000 trees as burn-in. In order to reduce the computational time and the parameter space to explore, we fixed the best BI topology from which we removed all outgroups by manually editing the .xml file created in BEAUTi v. 1.8.0 [29]. A 50% majority rule consensus tree was created in TREEANNOTATOR v. 1.8.0.

Ancestral areas were inferred using the dispersal–extinction–cladogenesis (DEC) model in Lagrange [46,47] based on our BEAST topology. We defined seven areas: Bali, Kalimantan, Flores + Lombok + Sumbawa, Java, New Guinea, the Philippines and Sulawesi. No species of *Trigonopterus* occurred in more than one area. Palaeogeographic changes through time [36,37,48] were accommodated by two time slices encompassing the past 30 Myr. Rates of dispersal were based on distances between areas and geographical barriers (see the electronic supplementary material, S5). The maximum number of possible regions for each node was limited to three. We enforced all possible combinations of areas at the root and conducted likelihood comparisons to select the most likely ancestral area. A difference between potential combinations equal or greater than 2 log-likelihood units was considered significant [46,47].

### (e) Phylogeography

We analysed the phylogeographic pattern for the eight Balinese species by producing a haplotype network derived from 70

specimens, using the 5' CO1, 16S and CAD datasets. The sequences were collapsed into haplotypes using DNASP v. 5.10 software [49], and networks were inferred with HAPSTAR v. 0.7 [50] based on connection lengths calculated in ARLEQUIN v. 3.11 [51].

## 3. Results

### (a) Molecular phylogenetics

Phylogenetic inference using ML and BI recovered highly congruent topologies for the species of the Sunda Arc (figure 1); some differences exist at the backbone formed by New Guinea species. For BI analyses, the high ESS (effective sample size) values indicated convergence for all runs. Bayes factor analyses suggested that the best-fit partitioning strategy was the one comprising one partition for each genome (electronic supplementary material, S4).

### (b) Faunal evolution and biogeography

Balinese *Trigonopterus* are not monophyletic but belong to three separate lineages, each with its closest relatives outside of Bali. One clade comprises five species (sp. 285, sp. 334, sp. 289, sp. 340 and sp. 286), with its sister species found in Sumbawa (sp. 287); the second clade comprises the sibling species (sp. 280 + sp. 327) with its sister species in Lombok (sp. 282); the third clade represented by a single species (sp. 328) whose sister species is from Sumbawa (sp. 326).

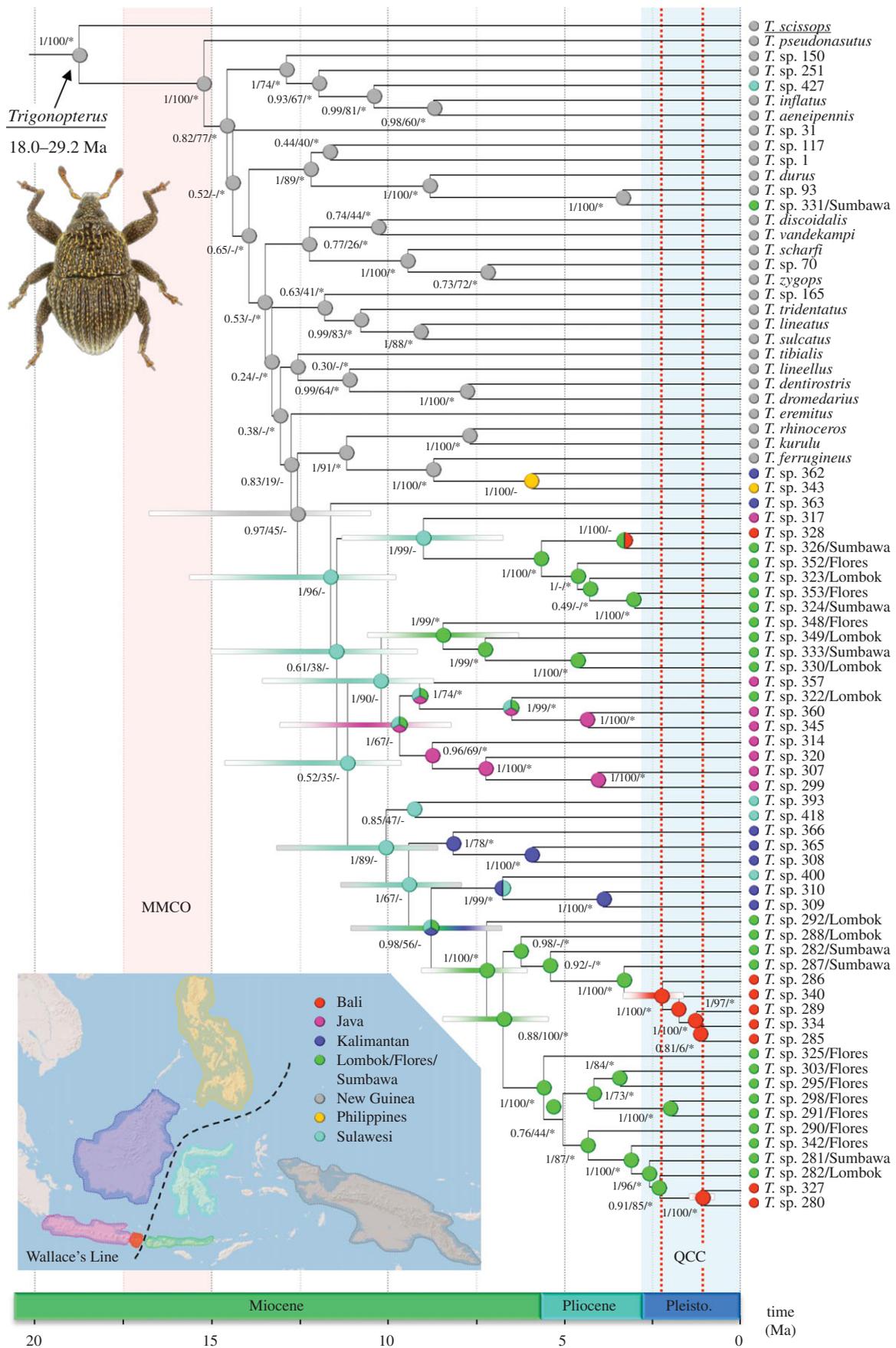
### (c) Dating and ancestral area reconstruction

High ESS values indicated that all dating analyses reached convergence. *Trigonopterus* had a median age of 22.59 Ma (95% HPD 18.04–29.16 Ma). For Balinese clades 1, 2 and 3, we estimate the following ages: 3.33 Ma (95% HPD 2.16–5.09), 2.24 Ma (95% HPD 1.69–3.19) and 1.15 Ma (95% HPD 0.73–1.86).

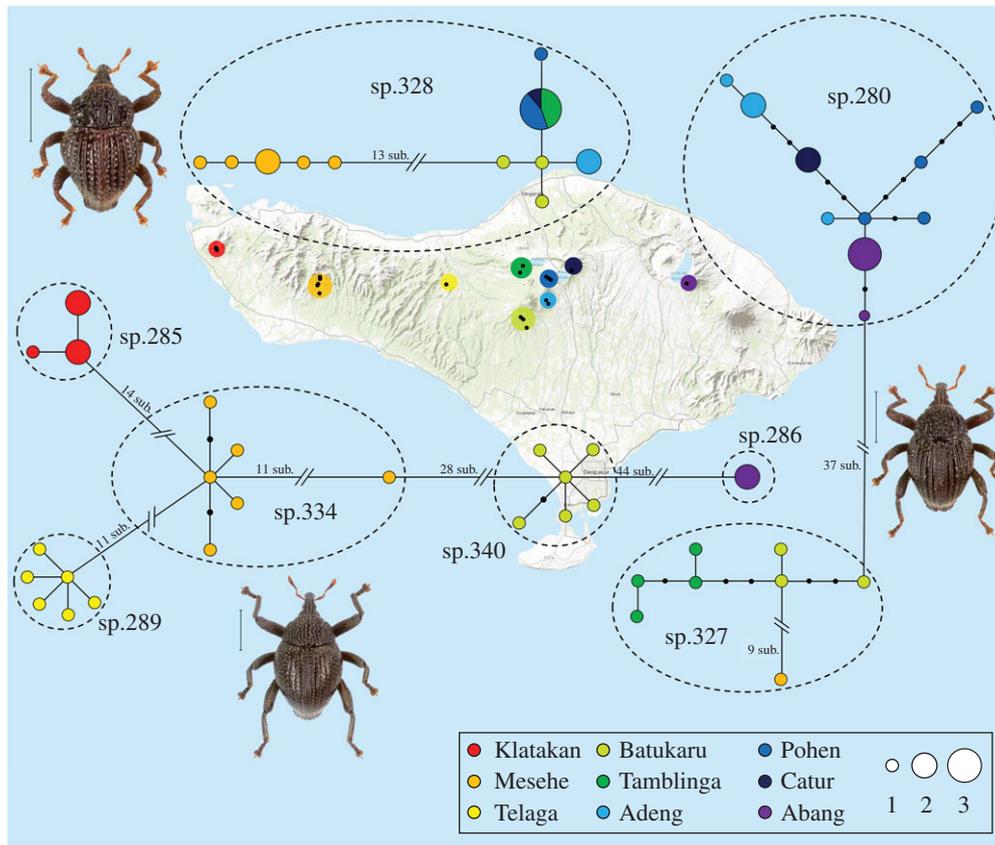
The AAR (figure 1) suggests that the early evolution of *Trigonopterus* was restricted to New Guinea until the Late Miocene. The New Guinea character state for the root was significantly recovered ( $\ln = -127.3$  against the second best root character state Sulawesi with  $\ln = -137.9$ ). Our results highlight a dispersal event towards Sulawesi at this period followed by the colonization out of this area of surrounding islands by the end of the Miocene and throughout the Neogene. Few lineages of basal clades reach Sulawesi, respectively, Sumbawa, another one the Philippines and Borneo. The Philippines might have served as a stepping stone for the colonization of Borneo from New Guinea as illustrated in the reconstruction (figure 1). All other species found on Borneo, Sumatra, Java and the Lesser Sunda Islands belong to one clade which also has a few Sulawesi endemics; the clade is completely absent from New Guinea. Evidence that the three clades of Balinese species have reached Bali coming from the Lesser Sunda Islands was found to be significant.

### (d) Phylogeography

The CO1-based network was the most informative one and fully compatible with the slightly less resolved 16S-based network; the one based on CAD fragment 3 was hardly resolved as that marker was not informative at this hierarchical level. All Balinese species of *Trigonopterus* weevils were genetically distinctive (1.7–24.3% smallest interspecific CO1  $p$ -distance,



**Figure 1.** Bayesian phylogenetic relationships and historical biogeography of Australasian flightless *Trigonopterus* weevils. Values at each node (a/b/c) are (a) posterior probability of BI analysis, (b) Bootstrap support value of ML analysis (a hyphen indicates that this node is not found in the ML-based topology) and (c) relative probability of splits. Values above 95% are indicated by an asterisk, values below by a hyphen. A 2.5-Myr timescale is provided at the bottom of the chronogram spanning the epochs since 20 Ma. Horizontal bars indicate the 95% credibility interval of the divergence times. The bottom-left corner map represents the Australasian region along with the biogeographic regions used in the DEC analysis. Present-day distribution of the species is given at the tips of the topology. Coloured pastilles at each node correspond to the most likely ancestral area recovered by the DEC model. The mid-Miocene climatic optimum (MMCO) and quaternary climatic change (QCC) are illustrated with vertical coloured bars. The red vertical bars indicate independent colonization of Bali.



**Figure 2.** Haplotype networks based on the CO1 dataset of 71 *Trigonopterus* specimens from Bali. Substitutions (sub.) are marked by black dots between the haplotypes. More than two substitutions are stated as numbers above the respective branches. For colour code of localities, see inset. Scale bar of specimens = 1 mm.

only 0.1–1.1% intraspecific mean  $p$ -distance; figure 2). Most species are micro-endemics, i.e. to date only retrieved from a single locality each, with all haplotypes of a given species unique to a single locality. The clade containing five species shows a marked geographical east–west sequence, with, for example, the easternmost species (286) is more closely related to the central species (340) than to the western ones. Similarly, species 285, 334 and 289 are more closely related to each other than to the central (340) and eastern (286) species. Interestingly, individuals of species 289, 334 and 340 all had different haplotypes.

The other putative species occur in multiple localities and exhibit a more complex haplotype structure especially in the central region of the island where populations are not clearly delineated geographically.

Species 280 and 327 each occur in more than one locality and exhibit a marked haplotype structure. Species 280 is restricted to the east and eastern centre of Bali and is immediately replaced by species 327 from the western centre of Bali towards the West; the species are allopatric, but nearest localities are merely 6 km separated from each other.

Species 328, in its own clade, has a clear east/west separation of its populations but the eastern part of its distribution presents a less structured pattern as highlighted by a shared haplotype found in different localities. This species was found in all central localities. A high number (13) of CO1 substitutions distinguish specimens from the central localities and those from western Mt. Mesehe, but no other evidence for the presence of cryptic species could be found based on external and genital morphology.

## 4. Discussion

Here, we empirically show that comprehensive phylogenetic studies can reveal complex evolutionary histories of organisms in the geologically equally complex Indo-Australian archipelago. Using a densely sampled molecular phylogeny, we shed light on the origin of Bali's indigenous fauna, especially the origins of the little-known endemics which may not be as sparse as hitherto believed. Counterintuitively, the flightless weevils we studied arrived from east of Bali and east of Wallace's line rather than from the much closer East Java.

Based on present-day geography, a sea-level lowering of 50 m would result in a land connection between Bali and East Java [9]. Such conditions have likely prevailed at times during the Pleistocene allowing the influx of mobile terrestrial organisms from Java [52]. However, Bali remained insular throughout more than 50% of its Pleistocene history [9]. More importantly, during periods of connectivity, the lowlands of East Java and Bali were most likely dominated by savannah vegetation [53], an unsuitable habitat for *Trigonopterus* weevils, as well as most other forest-adapted taxa. Even at times when Bali and Java formed one landmass, the fauna of ever-wet rainforests was confined to the upper elevations of mountains, just as it is today [54]. Thus, an insular evolution persisted for forest species at all times.

Flightless, edaphic weevils fall into the category of less dispersive taxa [55]. Their chances to be lifted up by strong winds should be negligible. Ocean currents as a means of dispersal, maybe as part of or contained in flotsam, appear as a more plausible cause of dispersal. In this particular case, the general

scenario does not look favourable, either: the Indonesian throughflow [56] passing from north to south would form a barrier carrying organisms into the Indian Ocean instead of helping them to cross the straits from east to west.

Under these circumstances, colonization of Bali from the Lesser Sunda Islands by a group of flightless weevils appears to be unusual. However, this happened at least three times independently, and the conspicuous absence of any colonization event from the West, notwithstanding the rich presence of *Trigonopterus* in Java, is more than unexpected and highlights the need for comprehensively sampled phylogenetic analyses if we are to unravel the complexities of faunal evolution of a given area. While we cannot rule out the possible discovery of East Javanese sister species of one Balinese species, this appears highly unlikely for all three lineages.

Within Bali, on the contrary, the observed population-level pattern reflects the expected clear geographical structure for flightless organisms between different tropical mountain ranges [57–59]. This confirms the very limited dispersal abilities of *Trigonopterus* weevils and questions chance as an explanation for the multiple transgression of Wallace's Line by *Trigonopterus*. Once other taxa of poor dispersers are examined with appropriate methods, a general pattern may emerge that paints a more accurate picture of the early zoogeographic history of Bali, when land connections or ocean currents might have been very different from how we see them today. Taxa worth a comprehensive study may be some genera of snails (i.e. *Asperitas* Gray, *Sasakina* Rensch [60]) and weevils of Celeuthetini (i.e. *Syntrophus* Marshall [61]) that have distributions suggesting close relationships between Bali and islands to the East instead of Java, but no phylogenetic data are available for these yet.

Our study of Balinese *Trigonopterus* provides the first robust phylogeny and state-of-the-art biogeographic analysis for any Balinese taxon. The early evolution of *Trigonopterus* apparently took place in the area of present-day New Guinea, most likely in an archipelagic setting, before the formation of the main New Guinean landmass [36,37]. The Sunda-arc and the Sunda shelf were colonized from the East by rather derived lineages (figure 1). Thus, the possible centre of origin of *Trigonopterus* apparently coincides with its

centre of diversity [62] in New Guinea/Australia. *Trigonopterus* was probably among the early groups to diversify on the proto-Papuan arc which was formed *ca* 30 Ma [36,63], a pattern also shown by some songbirds [42] and rainbow fish [43]. *Delias*, a diverse genus of butterflies, apparently also first diversified in the area of New Guinea and Wallacea, mainly during the Miocene [15,64].

The observed unexpected distribution patterns of *Trigonopterus* stress the importance of fine-grained and comprehensively sampled surveys in this biogeographic highly complex region. Relatively recent, largely Pleistocene processes of faunal exchange generated distribution patterns that include Bali along with Java as parts of the Sunda shelf contributing to what we perceive as Wallace's Line today [4]. However, the islands along the Sunda Arc from Sumatra to Flores are geologically heterogeneous and most likely emerged from the sea at very different times. Even Java is composed of a number of geologically distinct units [65]. The detailed study of less dispersive taxa undergoing endemic radiations during the Caenozoic will allow us to gain new insights into the development of a seemingly uniform chain of islands. Our present work highlights macro-evolutionary processes governing the biota of Bali and paves the way for future investigation of this frequently studied but still not fully understood area of the Indo-Australian Archipelago using molecular tools.

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**Data accessibility.** DNA sequences: European Nucleotide Archive (accession nos. FN429126 - FN429350, HE613858–613921; 615156–616164). Phylogenetic data: TreeBASE accession no. 15388. Final DNA sequence assembly uploaded as the electronic supplementary material.

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## References

- Wallace AR. 1860 On the zoological geography of the Malay Archipelago. *Zool. J. Linn. Soc.* **4**, 172–184. (doi:10.1111/j.1096-3642.1860.tb00090.x)
- Huxley TH. 1868 On the classification and distribution of the Alectoromorphae and Heteromorphae. *Proc. Zool. Soc. Lond.* **1868**, 294–319.
- Mayr E. 1944 Wallace's Line in the light of recent zoogeographic studies. *Q. Rev. Biol.* **19**, 1–14. (doi:10.1086/394684)
- Lohman DJ, de Bruyn M, Page T, von Rintelen K, Hall R, Ng PKL, Shi H-T, Carvalho GP, von Rintelen T. 2011 Biogeography of the Indo-Australian archipelago. *Annu. Rev. Ecol. Evol. Syst.* **42**, 205–226. (doi:10.1146/annurev-ecolsys-102710-145001)
- Whitmore TC (ed.) 1981 *Wallace's Line and plate tectonics*. Oxford, UK: Clarendon Press.
- Hall R, Holloway JD (eds). 1998 *Biogeography and geological evolution of SE Asia*. Leiden, The Netherlands: Backhuys.
- Stelbrink B, Albrecht C, Hall R, von Rintelen R. 2012 The biogeography of Sulawesi revisited: is there evidence for a vicariant origin of taxa on Wallace's 'anomalous island'? *Evolution* **66**, 2252–2271. (doi:10.1111/j.1558-5646.2012.01588.x)
- Klaus S *et al.* 2013 Out of Borneo: Neogene diversification of Sundaic freshwater crabs (Crustacea: Brachyura: Gecarcinucidae: *Parathelphusa*). *J. Biogeogr.* **40**, 63–74. (doi:10.1111/j.1365-2699.2012.02771.x)
- Voris HK. 2000 Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *J. Biogeogr.* **27**, 1153–1167. (doi:10.1046/j.1365-2699.2000.00489.x)
- Rensch B. 1936 *Die Geschichte des Sundabogens: Eine tiergeographische Untersuchung*. Berlin, Germany: Gebr. Borntraeger.
- MacKinnon JR, Phillipps K. 1993 *A field guide to the birds of Borneo, Sumatra, Java, and Bali, the Greater Sunda Islands*. Oxford, UK: Oxford University Press.
- McKay JL. 2006 *A field guide to the amphibians and reptiles of Bali*. Malabar, FL: Krieger Publishing Company.
- Whitten T, Soeriaatmadja RE, Afiff SA. 1996 *The ecology of Java and Bali*. The Ecology of Indonesia Series 2. Hong Kong, China: Periplus Editions.

14. Hendrich L, Balke M. 1995 Die Schwimmkäfer der Sunda-Insel Bali: Faunistik, Taxonomie, Ökologie, Besiedlungsgeschichte und Beschreibung von vier neuen Arten (Insecta: Coleoptera: Dytiscidae). *Faunist. Abh. Staatl. Mus. Tierk. Dresden* **20**, 29–56.
15. Braby MF, Pierce NE. 2007 Systematics, biogeography and diversification of the Indo-Australian genus *Delias* Hübner (Lepidoptera: Pieridae): phylogenetic evidence supports an 'out-of-Australia' origin. *Syst. Entomol.* **32**, 2–25. (doi:10.1111/j.1365-3113.2006.00349.x)
16. Vermeulen JJ, Whitten AJ. 1998 *Fauna Malesiana: guide to the land snails of Bali*. Leiden, The Netherlands: Backhuys Publishers.
17. Riedel A, Daawia D, Balke M. 2010 Deep *cox1* divergence and hyperdiversity of *Trigonopterus* weevils in a New Guinea mountain range (Coleoptera, Curculionidae). *Zool. Scripta* **39**, 63–74. (doi:10.1111/j.1463-6409.2009.00404.x)
18. Tänzler R, Sagata K, Surbakti S, Balke M, Riedel A. 2012 DNA barcoding for community ecology: how to tackle a hyperdiverse, mostly undescribed Melanesian fauna. *PLoS ONE* **7**, e28832. (doi:10.1371/journal.pone.0028832)
19. Riedel A, Suhardjono YR, Rahmadi C, Tänzler R. Submitted. Monograph of the *Trigonopterus* weevils from Sundaland and the Lesser Sunda Islands. *ZooKeys*.
20. Sodhi NS, Koh LP, Brook BW, Ng PKL. 2004 Southeast Asian biodiversity: an impending disaster. *Trends Ecol. Evol.* **19**, 654–660. (doi:10.1016/j.tree.2004.09.006)
21. Riedel A, Sagata K, Surbakti S, Tänzler R, Balke M. 2013 One hundred and one new species of *Trigonopterus* weevils from New Guinea. *ZooKeys* **280**, 1–150. (doi:10.3897/zookeys.280.3906)
22. Edgar RC. 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797. (doi:10.1093/nar/gkh340)
23. Stamatakis A. 2006 RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690. (doi:10.1093/bioinformatics/bt1446)
24. Ronquist F *et al.* 2012 MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542. (doi:10.1093/sysbio/sys029)
25. Rambaut A, Drummond AJ. 2007 Tracer v. 1.4. See <http://beast.bio.ed.ac.uk/Tracer>.
26. Kass R, Raftery A. 1995 Bayes factors and model uncertainty. *J. Am. Stat. Assoc.* **90**, 773–795. (doi:10.1080/01621459.1995.10476572)
27. Xie W, Lewis PO, Fan Y, Kuo L, Chen M-H. 2011 Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Syst. Biol.* **60**, 150–160. (doi:10.1093/sysbio/syq085)
28. Darriba D, Taboada GL, Doallo R, Posada D. 2012 jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* **9**, 772. (doi:10.1038/nmeth.2109)
29. Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012 Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973. (doi:10.1093/molbev/mss075)
30. McKenna DD, Sequeira AS, Marvaldi AE, Farrell BD. 2009 Temporal lags and overlap in the diversification of weevils and flowering plants. *Proc. Natl Acad. Sci. USA* **106**, 7083–7088. (doi:10.1073/pnas.0810618106)
31. Balke M, Ribera I, Hendrich L, Miller M, Sagata K, Posman A, Vogler AP, Meier R. 2009 New Guinea highland origin of a widespread arthropod supertramp. *Proc. R. Soc. B* **276**, 2359–2367. (doi:10.1098/rspb.2009.0015)
32. Papadopoulou A, Anastasiou I, Vogler AP. 2010 Revisiting the insect mitochondrial molecular clock: the mid-Aegean Trench calibration. *Mol. Biol. Evol.* **27**, 1659–1672. (doi:10.1093/molbev/msq051)
33. Andújar C, Serrano J, Gómez-Zurita J. 2012 Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. *BMC Evol. Biol.* **12**, 40. (doi:10.1186/1471-2148-12-40)
34. Toussaint EFA, Hall R, Monaghan MT, Sagata K, Ibalim S, Shaverdo HV, Vogler AP, Pons J, Balke M. Submitted. The towering oceanic orogeny of New Guinea as a trigger of arthropod megadiversity. *Nat. Commun.*
35. Sequeira AS, Lanteri AA, Scataglini MA, Confalonieri VA, Farrell BD. 2000 Are flightless *Galapaganus* weevils older than the Galapagos Islands they inhabit? *Heredity* **85**, 20–29. (doi:10.1046/j.1365-2540.2000.00690.x)
36. Hall R. 2009 Southeast Asia's changing palaeogeography. *Blumea* **54**, 148–161. (doi:10.3767/000651909X475941)
37. Hall R. 2012 Sundaland and Wallacea: geology, plate tectonics and palaeogeography. In *Biotic evolution and environmental change in southeast Asia* (eds DJ Gower, KG Johnson, JE Richardson, BR Rosen, L Rüber, ST Williams), pp. 32–78. Cambridge, UK: Cambridge University Press.
38. Mitterboeck TF, Adamowicz SJ. 2013 Flight loss linked to faster molecular evolution in insects. *Proc. R. Soc. B* **280**, 20131128. (doi:10.1098/rspb.2013.1128)
39. Ikeda H, Nishikawa M, Sota T. 2012 Loss of flight promotes beetle diversification. *Nat. Commun.* **3**, 648. (doi:10.1038/ncomms1659)
40. Vogler AP, Timmermans MJ. 2012 Speciation: don't fly and diversify? *Curr. Biol.* **22**, R284–R286. (doi:10.1016/j.cub.2012.03.015)
41. Bauzà-Ribot MM, Juan C, Nardi F, Oromí P, Pons J, Jaume D. 2012 Mitogenomic phylogenetic analysis supports continental-scale vicariance in subterranean thalassoid crustaceans. *Curr. Biol.* **22**, 2069–2074. (doi:10.1016/j.cub.2012.09.012)
42. Jönsson KA, Fabre P-H, Ricklefs RE, Fjeldså J. 2011 Major global radiation of corvid birds originated in the proto-Papuan archipelago. *Proc. Natl Acad. Sci. USA* **108**, 2328–2333. (doi:10.1073/pnas.1018956108)
43. Unmack PJ, Allen GR, Johnson JB. 2013 Phylogeny and biogeography of rainbowfishes (Melanotaeniidae) from Australia and New Guinea. *Mol. Phylogenet. Evol.* **67**, 15–27. (doi:10.1016/j.ympev.2012.12.019)
44. Stadler T. 2009 On incomplete sampling under birth–death models and connections to the sampling-based coalescent. *J. Theor. Biol.* **261**, 58–66. (doi:10.1016/j.jtbi.2009.07.018)
45. Swofford DL. 2002 PAUP\*. *Phylogenetic analysis using parsimony (\*and other methods)*, v. 4.0b10. Sunderland, MA: Sinauer Associates.
46. Ree RH, Moore BR, Webb CO, Donoghue MJ. 2005 A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* **59**, 2299–2311. (doi:10.1111/j.0014-3820.2005.tb00940.x)
47. Ree RH, Smith SA. 2008 Maximum-likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* **57**, 4–14. (doi:10.1080/10635150701883881)
48. Metcalfe I. 2005 Asia: south-east. In *Encyclopedia of geology*, vol. 1 (eds RC Selley, LRM Cocks, IR Plimer), pp. 169–198. Oxford, UK: Elsevier.
49. Librado P, Rozas J. 2009 DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452. (doi:10.1093/bioinformatics/btp187)
50. Teacher AGF, Griffiths DJ. 2011 HapStar: automated haplotype network layout and visualization. *Mol. Ecol. Resour.* **11**, 151–153. (doi:10.1111/j.1755-0998.2010.02890.x)
51. Excoffier L, Laval G, Schneider S. 2005 Arlequin v. 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1**, 47–50.
52. Miller KG *et al.* 2005 The Phanerozoic record of global sea-level change. *Science* **310**, 1293–1298. (doi:10.1126/science.1116412)
53. Bird MI, Taylor D, Hunt C. 2005 Palaeoenvironments of insular Southeast Asia during the last glacial period: a savanna corridor in Sundaland? *Q. Sci. Rev.* **24**, 2228–2242. (doi:10.1016/j.quascirev.2005.04.004)
54. Flenley JR. 1998 Tropical forests under the climates of the last 30,000 years. *Clim. Change* **39**, 177–197. (doi:10.1023/A:1005367822750)
55. Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK. 2012 Long-distance dispersal: a framework for hypothesis testing. *Trends Ecol. Evol.* **27**, 47–56. (doi:10.1016/j.tree.2011.08.009)
56. Gordon AL, Fine RA. 1996 Pathways of water between the Pacific and Indian oceans in the Indonesian seas. *Nature* **379**, 146–149. (doi:10.1038/379146a0)
57. Hewitt GM. 2004 The structure of biodiversity—insights from molecular phylogeography. *Front. Zool.* **1**, 1–16. (doi:10.1186/1742-9994-1-4)
58. Chatzimanolis S, Caterino MS. 2008 Phylogeography of the darkling beetle *Coelus ciliatus* in California. *Ann. Entomol. Soc. Am.* **101**, 939–949. (doi:10.1603/0013-8746(2008)101[939:POTDBC]2.0.CO;2)

59. Kajtoch L. 2011 Conservation genetics of xerothermic beetles in Europe: the case of *Centricnemus leucogrammus*. *J. Insect Conserv.* **15**, 787–797. (doi:10.1007/s10841-011-9377-8)
60. Hausdorf B. 1995 A preliminary phylogenetic and biogeographic analysis of the Dyakiidae (Gastropoda: Stylommatophora) and a biogeographic analysis of other Sundaland taxa. *Cladistics* **11**, 359–376. (doi:10.1111/j.1096-0031.1995.tb00095.x)
61. Marshall GAK. 1956 *The otiorrhynchine Curculionidae of the tribe Celeuthetini (Col.)*. London, UK: Trustees of the British Museum.
62. Briggs JC. 2000 Centrifugal speciation and centres of origin. *J. Biogeogr.* **27**, 1183–1188. (doi:10.1046/j.1365-2699.2000.00459.x)
63. Hall R. 2011 Australia–SE Asia collision: plate tectonics and crustal flow. *Geol. Soc. Lond. Spec. Publ.* **355**, 75–109. (doi:10.1144/SP355.5)
64. Müller CJ, Matos-Maraví PF, Beheregaray LB. 2013 Delving into *Delias* Hübner (Lepidoptera: Pieridae): fine-scale biogeography, phylogenetics and systematics of the world's largest butterfly genus. *J. Biogeogr.* **40**, 881–893. (doi:10.1111/jbi.12040)
65. Pacey A, Macpherson CG, McCaffrey KJ. 2013 Linear volcanic segments in the central Sunda Arc, Indonesia, identified using Hough transform analysis: implications for arc lithosphere control upon volcano distribution. *Earth Planet. Sci. Lett.* **369–370**, 24–33. (doi:10.1016/j.epsl.2013.02.040)