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Research

Transgressing Wallace's Line brings hyperdiverse weevils down to earth

Harald Letsch, Michael Balke, Emmanuel F. A. Toussaint, Raden Pramesa Narakusumo, Konrad Fiedler and Alexander Riedel

H. Letsch, R. P. Narakusumo and A. Riedel (<https://orcid.org/0000-0002-8291-4425>) (riedel@smnk.de), Museum of Natural History Karlsruhe, Karlsruhe, Germany. RPN also at: Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Inst. of Sciences (LIPI), Cibinong, Indonesia. – M. Balke, SNSB-Zoological State Collection (ZSM), Munich, Germany. – E. F. A. Toussaint (<https://orcid.org/0000-0002-8439-1285>), Natural History Museum of Geneva, Geneva, Switzerland. – K. Fiedler and HL, Univ. of Vienna, Dept of Botany and Biodiversity Research, Vienna, Austria.

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Wallace's Line, located in the heart of the Indo-Australian archipelago, has historically been hypothesized to strongly inhibit dispersal. Taxa crossing this barrier are confronted with different biota of Asian or Australian origin, respectively, but the extent to which these conditions have affected the evolution of the colonizing lineages remains largely unknown. We examined the potential correlations of body size, lifestyle and biogeographical distribution in the weevil genus *Trigonopterus*. These beetles are highly diverse both on foliage and in litter east of Wallace's Line but occur exclusively in leaf litter in the west. Based on a comprehensive, dated phylogeny of 303 species, we inferred nine crossing events of Wallace's Line, all from east to west. Five previously foliage-dwelling lineages changed their lifestyle to leaf litter habitats after crossing this barrier. Our results indicate that dispersal is not more likely in edaphic lineages, but rather that abiotic and/or biotic factors may be responsible for the exclusive leaf litter habitat of *Trigonopterus* in Sundaland. This includes differences in climate, and the different predatory faunas of Australia-New Guinea, Wallacea and Sundaland. A mimicry complex in New Guinea with *Trigonopterus* species as presumable model may be of relevance in this context.

Keywords: beetle evolution, community assembly, Indo-Australian archipelago, microhabitat evolution, mimicry complex, phylogeny of Curculionidae

Introduction

The biogeographic boundary coined 'Wallace's Line' lies in the heart of the Indo-Australian Archipelago (IAA), a region of extreme biological and geological diversity (Whitmore 1981, Lohman et al. 2011). Wallace's Line is situated between Bali and Lombok in the south, between Borneo and Sulawesi in the north, and northwards extends east of the Philippines. This biogeographic barrier has remained fascinating since it was initially suggested by Alfred Russel Wallace nearly 160 years ago to indicate a distinct break in animal distributions, separating Asian from Australian faunas (Wallace 1860). Since this discovery, our understanding of the geological history



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of the IAA has greatly improved (Hall 2009, 2012, 2013, 2017). The complex geological history of the archipelago, along with multiple Pleistocene sea-level changes (Voris 2000, de Bruyn et al. 2014), is responsible for the emergence of this entity that appears impermeable to some clades, but not to others. However, a paradigm shift has emerged in the past decades. Biogeographic analyses based on molecular dated phylogenetic trees revealed a number of scenarios where taxa managed to cross Wallace's Line in both directions. Examples include fanged frogs (Evans et al. 2003) and murine rodents (Rowe et al. 2019) coming from the west, as well as birdwing butterflies (Condamine et al. 2015), diving beetles (Balke et al. 2009) and weevils (Tänzler et al. 2014, Toussaint et al. 2015) coming from the east. Despite this faunal interchange, the biotas on either side of Wallace's Line maintain a distinct composition and differ greatly from one another.

The distributional range of the hyperdiverse weevil genus *Trigonopterus* of Cryptorhynchinae s.str. (Riedel et al. 2016, Letsch et al. 2020a) extends from Singapore in the west to Samoa in the east, encompassing the entire IAA and West Pacific Islands, with usually narrow endemic species, each occupying a range of less than 100 km in diameter (Tänzler et al. 2012, Riedel and Narakusumo 2019). All species are apterous and heavily sclerotized as adults (Van de Kamp et al. 2015). Therefore, colonization of new areas across the IAA has been hypothesized to result from passive dispersal in flotsam supported by ocean currents (Tänzler et al. 2014, Yeh et al. 2018). In their habitats inside primary rainforest, they tend to be relatively common and, according to our field observations, at times represent a significant fraction of the phytophagous beetle biodiversity.

There are two distinct lifestyles among adult *Trigonopterus*. While some species occur exclusively in the litter layer of forests, others are always found sitting on the foliage of small trees or shrubs. These two lifestyles are very stable within species; only very rarely are individuals found in the 'wrong' place. The species morphology usually gives clear indications as to which of the two lifestyles it is adapted to (Riedel 2010) (Fig. 1) and assignment of species to either one of these lifestyles is usually straightforward: Edaphic species generally have a broader body with distinct surface sculpture, and often with tactile scales; their coloration is usually somber or with a metallic sheen, but never bright orange. Species of the foliage are usually of subovate shape, with subglabrous cuticular surface, sometimes with dense punctuation, but without deep ridges, and without erect or suberect scales; their coloration is usually black, but in many unrelated species in New Guinea the elytra are of bright orange color. The bionomics of the 'foliage species' remains a mystery. While these weevils can be found sitting on leaves in numbers, sometimes copulating, they were thus far never observed feeding. Possibly, the leaves are primarily used as meeting points for mating. Their usual gut content is dead plant tissue, so the genus may be classified as saprophagous. Lifestyle has repeatedly switched between both types in the evolution of *Trigonopterus*, though

it remains stable within clades comprising 70 species or more (Toussaint et al. 2017b). This morphologically clear division of lifestyles is unusual among Cryptorhynchinae. Years of intense fieldwork across the IAA indicate that *Trigonopterus* weevils are absent from foliage in the arboreal or shrub layer in Sumatra, Borneo, Java, and largely so from the Lesser Sunda Islands, where they are nevertheless represented by many edaphic species. In Sulawesi, the Moluccas, New Guinea and Australia, in contrast, representatives of the genus are found both in the litter layer and on foliage (Fig. 1).

Previous studies suggested that *Trigonopterus* originated in Australia-New Guinea and repeatedly dispersed westwards, transgressing Lydekker's and Wallace's Lines (Tänzler et al. 2016, Toussaint et al. 2017b). Here, we reconstruct the lifestyle evolution of *Trigonopterus* species with respect to their biogeographical history. We focus on the following questions: 1) did the colonization of Sundaland only occur in edaphic lineages or did it cause a change in the lifestyle of originally foliage-dwelling lineages? 2) Are transitions in lifestyle associated with patterns of differential diversification? 3) Does body size differ between biogeographical regions? Regional differences in lifestyle and body size may indicate the impact of abiotic and/or biotic factors, such as differences in environment structure, climate and interactions among organisms (e.g. predation). As dispersal in flightless *Trigonopterus* weevils appears to occur mainly via drift by sea currents (Tänzler et al. 2014, 2016), it is also possible that smaller species and leaf litter inhabitants get easier swept to the sea with floating substrate and might therefore simply have a higher chance to get passively dispersed to other shores. To address these questions, we rely on a comprehensive phylogenetic dataset containing all relevant lineages of the Sundaland fauna, all species available from the source area, i.e. Sulawesi (Tänzler et al. 2016), and a selection of species representing major lineages from New Guinea, Oceania and Australia.

Material and methods

Taxon sampling

We sampled *Trigonopterus* species as follows: 128 species from Sulawesi, representing all species at hand at the beginning of this study and exceeding the number of currently described species (Riedel and Narakusumo 2019). Thirty-eight (38) species from Sundaland and the Lesser Sunda Islands (Borneo 11, Java 9, Sumatra 2, Lesser Sunda Islands 14, Palawan 2), representing 36% of the 99 described species (Riedel et al. 2014), with all relevant clades selected based on Tänzler et al. (2016), who had included all species from this region. The species selection from Australia, New Guinea and the Moluccas (127) may represent less than 10% of the existing fauna, but based on unpublished preliminary analyses containing > 400 species, their phylogenetic diversity is represented adequately. Furthermore, three species from Samoa and seven from New Caledonia were included;

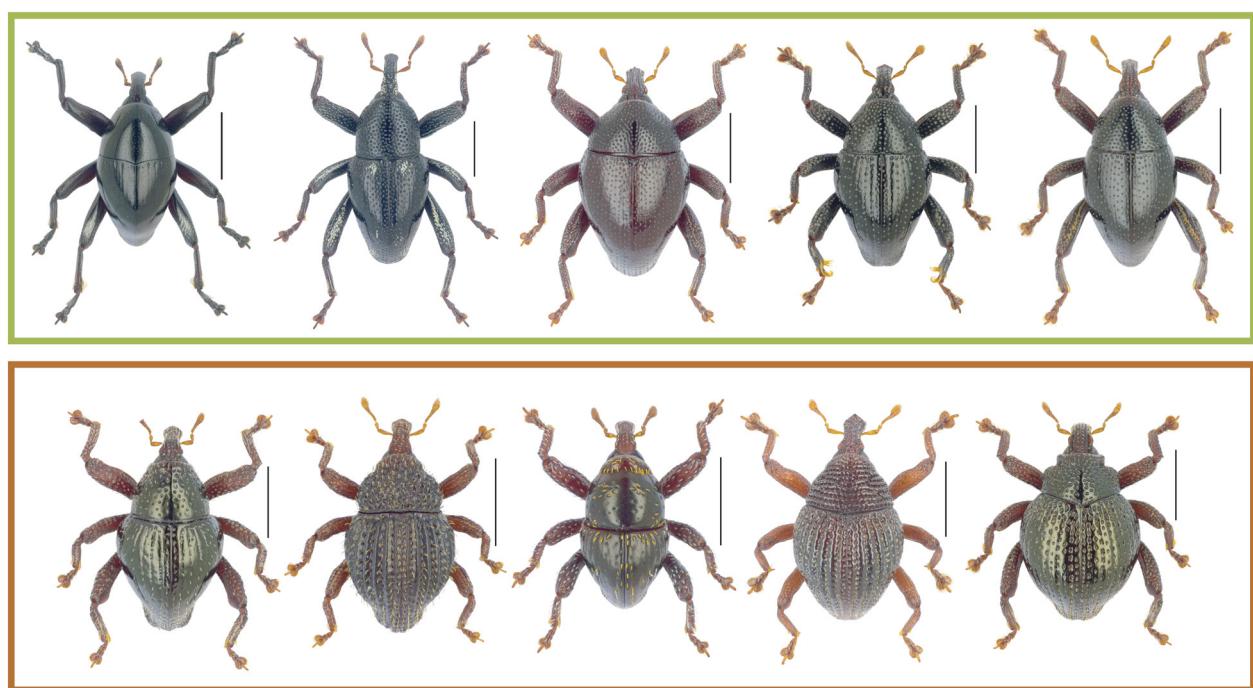
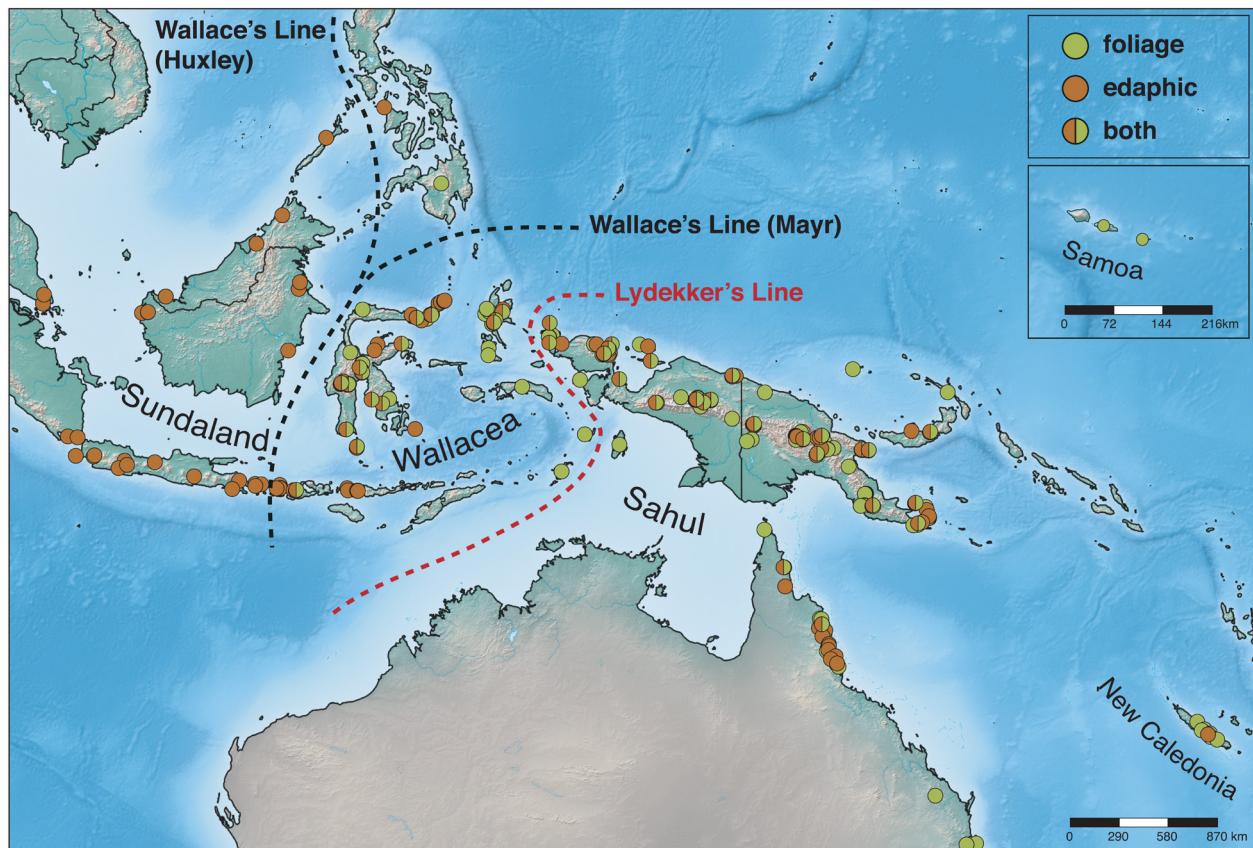


Figure 1. Map of the Indo-Australian Archipelago with records of *Trigonopterus*. Localities derived from the literature, museum specimens and sampled specimens. Edaphic species in brown; species on foliage in green; localities with both lifestyles split in brown-green. The base map was produced using SimpleMappr (<www.simplemappr.net>) *Trigonopterus* species on foliage (upper row): *T. pseudallotopus*, *T. inhomarus*, *T. posoensis*, *T. cirripes*, *T. celebensis*; in leaf litter (lower row): *T. artemis*, *T. wangiwangiensis*, *T. procurtus*, *T. crenulatus*, *T. lampros*.

this provided a test if the same lineages of potentially higher dispersal ability have colonized islands both to the west and east of New Guinea. As outgroup representatives, we used four species of other Cryptorhynchinae from Australia and New Guinea following the most recent phylogenetic hypotheses for this subfamily (Riedel et al. 2016, Letsch et al. 2020a). Genomic DNA of all specimens was extracted non-destructively (Riedel et al. 2010) using the DNeasy and NucleoSpin 96 Tissue kits (Qiagen, Hilden; Macherey-Nagel, Düren, Germany). We sequenced fragments of eight genes, including the ribosomal mitochondrial 16S rRNA, nuclear 18S rRNA and nuclear 28S rRNA, as well as five mitochondrial and nuclear protein-coding genes, Arginine kinase (ArgK), Carbamoyl-phosphate synthetase 2 (CAD), Cytochrome-c-oxidase I (COI), Elongation-factor 1 alpha (EF1 α), Enolase (EN) and Histone 4 (H4). Primers and PCR conditions principally followed Toussaint et al. (2017b). An overview of samples, markers and accession numbers can be found in Supplementary material Appendix 1 Table A1.

Phylogenetic analyses and divergence time estimations

All gene fragments were separately aligned using MAFFT v7.409 with default settings (Katoh and Standley 2013, Katoh et al. 2017). Ambiguously aligned positions in the rRNA alignments were excluded with ALISCORE v2.0 (Misof and Misof 2009) and all alignments were subsequently assembled with AMAS v0.94 (Borowiec 2016). The best-fitting partitioning scheme and corresponding models of nucleotide substitution were searched using ModelFinder as implemented in IQ-TREE v1.6.10 (Nguyen et al. 2015, Chernomor et al. 2016, Kalyaanamoorthy et al. 2017), restricted to models that can also be applied in the subsequent divergence time estimations. Codon positions of each protein-coding gene fragment, as well as each rRNA gene fragment were defined as distinct partitions a priori. Based on the detected partition-model-scheme, we performed 20 independent maximum likelihood (ML) tree searches in IQ-TREE v1.6.10 to avoid local optima. Nodal support was assessed using 1000 ultrafast bootstrap replicates (Minh et al. 2013), with the ‘bnni’ option (Hoang et al. 2018), and an increased maximum number of iterations to stop (-nm 10 000). Additionally, 1000 replicates of the SH-like approximate likelihood ratio test (Guindon et al. 2010) were performed.

We relied on the phylogenetic study of Letsch et al. (2020a) on Cryptorhynchinae evolution to calibrate the *Trigonopterus* phylogeny. The root of the tree was calibrated using the normally distributed 95% highest posterior density (HPD) interval inferred for the ‘Indo-Australian clade’ in Letsch et al. (2020a), which was estimated at 66.8–81.17 million yr ago (Ma), and the crown of *Trigonopterus* was calibrated with the respective HPD of 47.7–61.14 Ma. Divergence time estimates were performed in the program BEAST v1.10.4 (Suchard et al. 2018), using the topology from the best ML tree obtained by IQ-TREE as a fixed

input tree. To account for uncertainty in evolutionary rates, uncorrelated lognormal relaxed-clock models were assumed for each partition, with a Birth-death tree model prior, following the best BEAST analyses scheme in Letsch et al. (2020a). Substitution model parameters were set according to the ModelFinder results. The BEAST analysis was run for 50 million generations (sampling every 20 000 generations). Convergence and mixing of parameters, as well as the effective sample sizes (ESS) were inspected using Tracer v1.7.1 (Rambaut et al. 2018). Post-burnin samples were used to construct a maximum clade credibility tree with mean node heights in TreeAnnotator v1.10.4 (Suchard et al. 2018).

Biogeographical analyses

Biogeographical analyses were conducted in the R package (R Development Core Team) BioGeoBEARS v1.1.2 (Matzke 2013), which allows estimating ancestral ranges using the Dispersal Extinction Cladogenesis (DEC) model (Ree and Smith 2008), a maximum likelihood implementation of the dispersal–vicariance analysis (DIVA) model (Ronquist 1997) (i.e. DIVALIKE model) and a maximum likelihood implementation of the BAYAREA model (Landis et al. 2013) (i.e. BAYAREALIKE model). This program also implements a parameter describing founder-event speciation (+J) which permits to account for the colonization of a new range via jump dispersal. The latter has been criticized recently, as models using this parameter tend to explain radiations mainly by founder events (Ree and Sanmartín 2018). However, we think that the jump dispersal event parameter is appropriate in the geographical setup of our study, as *Trigonopterus* species are generally endemic with narrow distribution ranges and long-range dispersal probably mostly facilitated by oceanic drift (Tänzler et al. 2014). Ancestral range reconstructions were estimated using the maximum clade credibility (MCC) tree from the BEAST analysis, restricted to *Trigonopterus* species. The number of maximum areas per ancestral range was constrained to three. We selected the following five regions for the BioGeoBEARS analyses: A) New Caledonia, B) Samoa, C) Sahul including Australia, New Guinea and the Aru Islands, D) Sundaland, including Palawan, Borneo, Java and Sumatra and E) Wallacea, including the Lesser Sunda Islands, Sulawesi and the Moluccas. Due to the complex geological history of the IAA, we first applied a time-stratified biogeographical analysis with two time slices representing the major periods of geological rearrangements in this area. However, as several uncertainties remain about the appearance of islands in the IAA (Hall 2012, 2013), the timing of these geological rearrangements is still problematic and we therefore compared the time-stratified analysis with a non-time-stratified analysis, which allows unconstrained dispersal between all areas through time. The design of the dispersal rate matrices (Supplementary material Appendix 1 Table A2) principally follows Toussaint et al. (2017a), based on recent paleogeographic studies (Hall 2012, 2013, 2017). The AICc was used to compare the fit of models (Table 1) within the two model classes, as models with and without +J cannot be

Table 1. Results of the BioGeoBEARS analyses.

Model	LnL	#	d	e	j	AICc	AICw
DEC+j (non-strat.)	-138.9	3	0.000	0.000	0.012	284.00	0.23
DEC+j (strat.)	-139.8	3	0.000	0.000	0.070	285.60	0.10
DIVALIKE+j (non-strat.)	-138.9	3	0.000	0.000	0.012	284.00	0.23
DIVALIKE+j (strat.)	-139.8	3	0.000	0.000	0.069	285.60	0.10
BAYAREALIKE+j (strat.)	-138.9	3	0.000	0.000	0.012	284.00	0.23
BAYAREALIKE+j (non-strat.)	-139.8	3	0.000	0.000	0.066	285.60	0.10
DEC (non-strat.)	-193	2	0.001	0.000	0.000	390.10	0.00
DEC (strat.)	-199.5	2	0.004	0.000	0.000	403.10	0.00
DIVALIKE (non-strat.)	-182.6	2	0.001	0.000	0.000	369.30	1.00
DIVALIKE (strat.)	-189.3	2	0.005	0.000	0.000	382.60	0.00
BAYAREALIKE (strat.)	-277.9	2	0.001	0.005	0.000	559.80	0.00
BAYAREALIKE (non-strat.)	-275.6	2	0.005	0.005	0.000	555.20	0.00

Notes: model, strat.: time-stratified analyses, non-strat.: unconstrained analyses; #, number of parameters; AICc, bias corrected Akaike's information criterion; AICw, Akaike weight; d, rate of dispersal; e, rate of extinction; j, relative probability of founder-event speciation at cladogenesis.

directly compared by a statistical test, because they are not nested versions of each other (Ree and Sanmartín 2018).

Ancestral lifestyle reconstructions

To infer the ancestral lifestyle conditions in *Trigonopterus*, we applied stochastic character mapping (Huelsenbeck and Bollback 2001), using the function `make.simmap` in the R package `phytools` v0.6-27 (Revell 2012). We first used the `fitDiscrete` function in the R package `geiger` v1.3.1 (Harmon et al. 2008) to estimate ancestral habitat states using a one-parameter equal-rates model (ER) and a six-parameter all-rates-different model (ARD) and then compared the fit of these models to the given data with the Akaike information criterion corrected for small sample size (AICc). A value of $\Delta\text{AICc} > 4$ was considered an indication of support for one model over the other following Burnham and Anderson (2002). The ARD model ($\text{AICc} = 268.7$) fitted the data substantially better than the ER model ($\text{AICc} = 285.08$, $\Delta\text{AICc} = 16.38$) and was therefore used for the stochastic character mapping with 1000 simulations in `phytools`. The function `densityMap` in `phytools` was used to summarize the generated habitat evolution on the MCC tree.

Lifestyle dependent diversification

To infer whether diversification rates differed between litter and foliage dwelling lineages, we applied the Hidden State Speciation and Extinction (HiSSE) model (Beaulieu and O'Meara 2016). Following Laenen et al. (2016), we ran 44 different HiSSE models including original BiSSE models (Maddison et al. 2007), as well as character-independent diversification models, with two and four hidden character states respectively (CID-2 and CID-4). All these models estimated speciation, extinction and transition rates between observed states 0 (foliage) and 1 (edaphic) in combination with hidden states A and B, resulting in a total of four states: 0A, 1A, 0B and 1B. Since our sampling is incomplete, we used sampling fractions per state to account for unsampled species (foliage: 134/536; edaphic: 169/528). We used the R

package `hisse` v1.9.6 (Beaulieu and O'Meara 2016) to calculate the log-likelihood of each model, and results were evaluated using the weighed AIC (Burnham and Anderson 2002).

Body size comparisons

To infer changes in body sizes as an indicator for potential differences in the biotic and/or abiotic environment of *Trigonopterus*, we compared their body length among different biogeographical regions (Sundaland, Wallacea and Sahul) and lifestyles (edaphic and foliage dwelling). The length of the body was measured in dorsal aspect from the elytral apex to the front of the pronotum. As females are regularly smaller than males, we measured 2–5 individuals for each species, including both females and males. To deal with possible biases generated by phylogenetic dependency, we applied a phylogenetic ANOVA, using the function 'phylANOVA' in the R package `phytools`, with 10 000 simulations as recommended to obtain stable p-values and post-hoc tests (with Holm sequential Bonferroni correction for multiple groups) to evaluate the significance of differences among all pairs of groups. Additionally, we applied the `contmap` function of `phytools` to display the changes in the relative size of *Trigonopterus* weevils throughout the phylogenetic tree. `Contmap` maps a continuous trait onto the phylogeny by estimating the states at internal nodes with ML and interpolating the states along the edges.

Results

Phylogeny, divergence times and historical biogeography

The results of the phylogenetic tree reconstructions and divergence time estimates are summarized in Fig. 2. Detailed results, including the best ML tree with approximate likelihood ratio test (SH) and ultrafast bootstrap support (BS), as well as the MCC tree with mean node heights and the highest probability density (95% HPDs) computed from the post

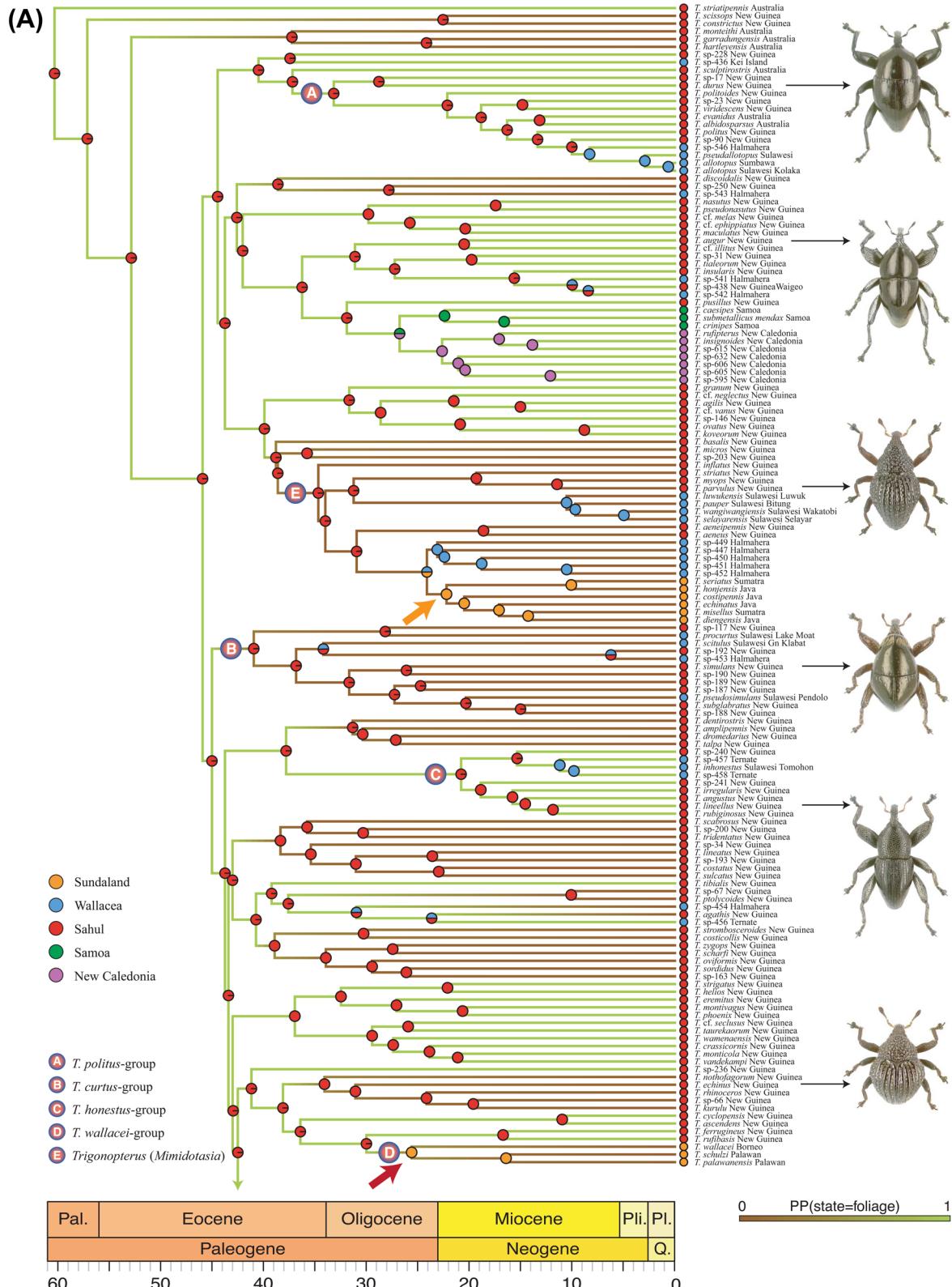


Figure 2. Time-calibrated phylogeny of *Trigonopterus* weevils and habitat use evolution. Lineages of edaphic species are presented in brown, lineages of foliage species in green. Ancestral state reconstruction generated through stochastic character mapping using phytools. Circles at the terminals of the tree indicate each species' locality. Pie charts at the internal nodes indicate the relative percentage of the reconstructed ancestral areas as estimated with BioGeoBEARS. Area composition: Sundaland: Palawan, Borneo, Java and Sumatra; Lesser Sunda Islands: Flores, Lombok, Sumbawa and Bali; Sahul: Australia, New Guinea and the Aru Islands; Moluccas: Halmahera, Ternate and Kei Island. Photos illustrate the morphological diversity of the genus *Trigonopterus*.

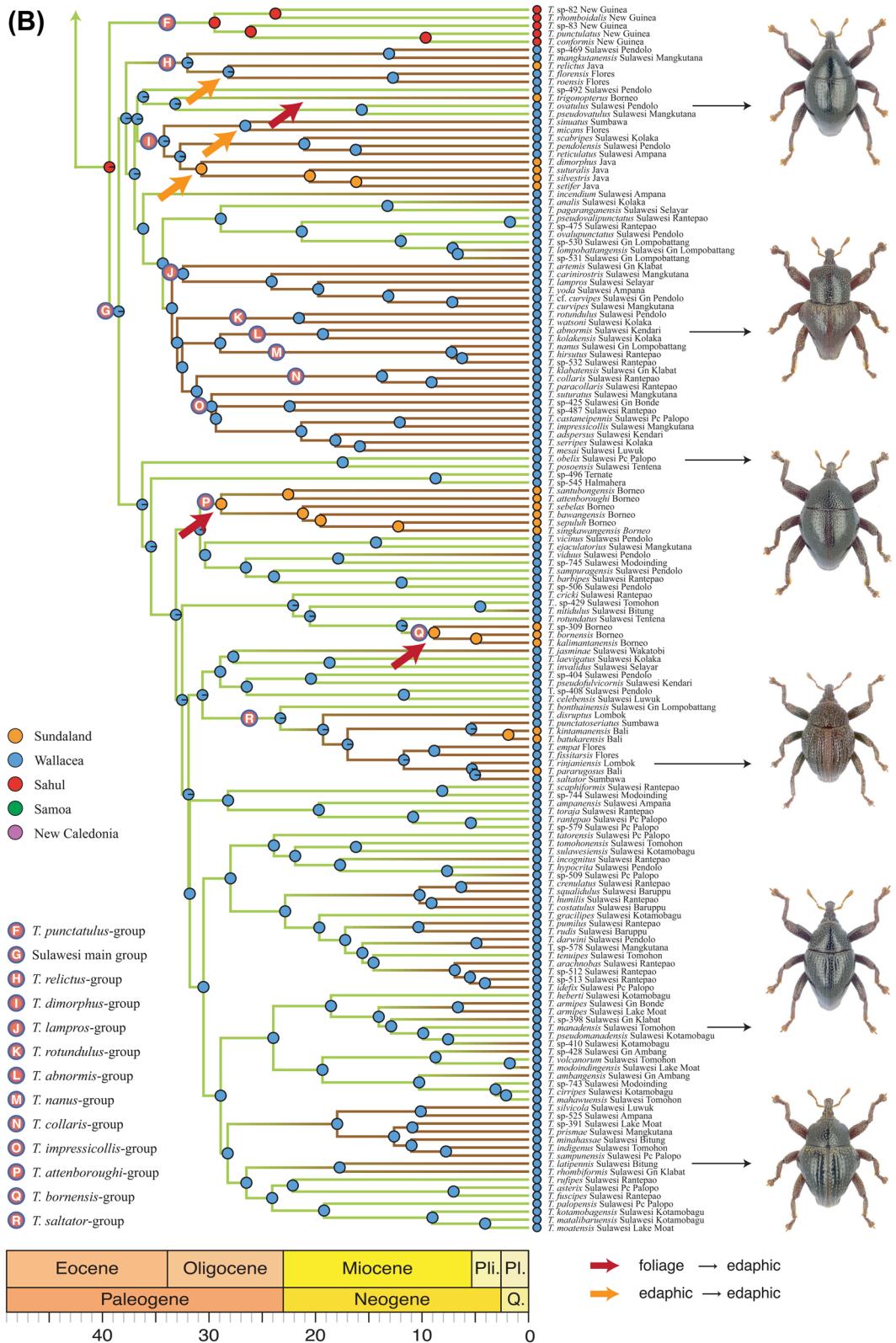


Figure 2. Continued

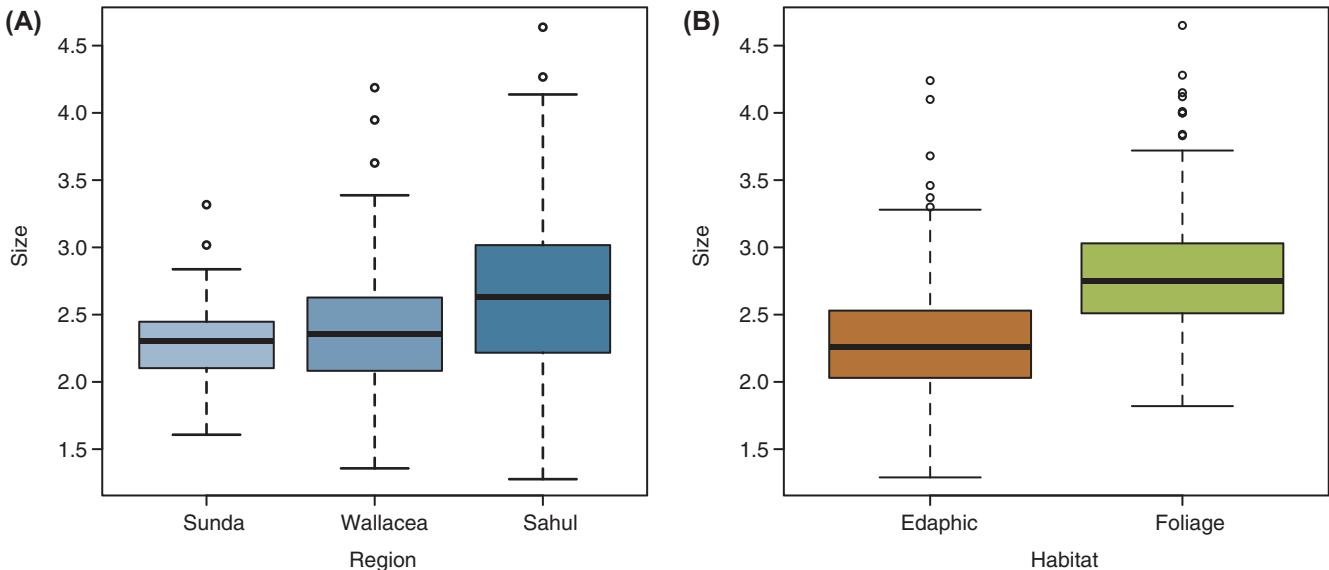


Figure 3. Body size comparisons among *Trigonopterus* weevils. Histograms represent the average body size of species among (A) different biogeographical regions, and (B) lifestyles. Error bars indicate standard error from the mean.

burn-in posterior samples, are provided in Supplementary material Appendix 1 Fig. A1, A2.

Our BEAST analysis recovers an origin of the crown *Trigonopterus* in the Paleocene at ca 60 Ma (95% HPD = 58–61 Ma), with a major radiation starting in the Middle Eocene at ca 45.9 Ma (95% HPD = 42–48 Ma). The subgenus *Trigonopterus (Mimidotasia)* ('clade E') was monophyletic (SH = 100, BS = 100; 34.64 Ma, 95% HPD = 32–38 Ma) and several previously defined species groups within *Trigonopterus* were also monophyletic, such as the *T. dimorphus*-group ('clade I') (SH = 98.5, BS = 91; ca 34 Ma, 95% HPD = 30–35 Ma) comprising species from both Sulawesi and Sundaland; the *T. politus*-group ('clade A'; SH = 99.1, BS = 100; ca 22 Ma, 95% HPD = 27–33 Ma) with species from Australia, New Guinea, Sulawesi and Sumbawa; and the *T. curtus*-group ('clade B'; SH = 98, BS = 96; ca 41 Ma, 95% HPD = 38–45 Ma) with species from New Guinea, Sulawesi and the Moluccas (cf. Fig. 2).

The biogeographical analyses in BioGeoBEARS led to consistent results, and the differences between specific runs were neglectable (Table 1, Supplementary material Appendix 1 Fig. A3). All models estimated the origin and early radiation of *Trigonopterus* in Sahul (Fig. 2, Supplementary material Appendix 1 Fig. A3). From there, multiple transgressions of Lydekker's Line occurred, resulting in the colonization of the Moluccas, Sulawesi and the Lesser Sunda Islands. One single clade, sister to the smaller *T. punctulatus*-group ('clade F') of New Guinea, is highly speciose ('clade G' of Tänzler et al. 2016). It reached areas that today comprise Sulawesi in the Late Eocene at 38 Ma (95% HPD: 35–40 Ma; SH = 100, BS = 100) triggering a major radiation. Most transgressions of Wallace's Line occurred in this clade, e.g. leading to the colonization of Borneo by species of the *T. attenuboroughi*-group ('clade P'; SH = 98.8, BS = 94) and the *T. bornensis*-groups

('clade Q'; SH = 100, BS = 100), and Java by species of the *T. relicitus*- ('clade H'; SH = 99.9, BS = 100) and the *T. dimorphus*-group ('clade I'; SH = 98.5, BS = 91). The *T. wallacei*-group ('clade D'; SH = 99.7, BS = 100) with three species in Borneo and Palawan, and six species of the subgenus *Trigonopterus (Mimidotasia)*, 'clade E' in Java and Sumatra transgressed Wallace's Line from Sahul much later in the Late Miocene and Pliocene. In total, we detected nine transgressions of Wallace's Line. A clade of three species endemic to Samoa is sister to the foliage-dwelling species of New Caledonia (Toussaint et al. 2017b). Together, they are sister to *T. pusillus* from Papua New Guinea (SH = 98.1, BS = 81), which is unrelated to any of the lineages colonizing the islands to the west. A detailed study of the relatively rich *Trigonopterus* fauna of Fiji and possibly Vanuatu would be needed for a detailed interpretation of the colonization patterns of the West Pacific Islands.

Lifestyle evolution

The ancestral lifestyle reconstruction analyses recovered foliage dwelling as the ancestral condition in *Trigonopterus*, with 40 independent transitions toward an edaphic lifestyle during the evolution of the genus (Fig. 2). We recovered eleven shifts from foliage to leaf litter in New Guinea alone, most of them in the late Eocene. In fact, less than one-third of the shifts occurred after the mid-Miocene. We also show that based on our taxon sampling and phylogenetic reconstruction, foliage dwelling was never regained secondarily (Fig. 2).

Lydekker's Line was crossed by five edaphic and six foliage-dwelling lineages, but only one transgression was followed by a habitat shift (Fig. 2). The early radiation of the species-rich Sulawesi clade 'G' (Fig. 2) occurred on foliage, but several groups later changed to a lifestyle in the forest litter, including

a clade of morphologically highly diverse species groups: the *T. lampros*-group ('clade J'), the *T. rotundulus*-group ('clade K'), the *T. abnormis*-group ('clade L'), the *T. nanus*-group ('clade M'), the *T. collaris*-group ('clade N' and the *T. impressicollis*-group ('clade O') (Riedel and Narakusumo 2019). In contrast, the transgression of Wallace's Line, i.e. the colonization of the Sunda Islands, occurred either out of exclusively edaphic clades (orange arrows in Fig. 2), such as *Trigonopterus* (*Mimidotasia*), or, was accompanied by a transition from foliage to an edaphic lifestyle (red arrows in Fig. 2). Therefore, the edaphic *Trigonopterus* fauna of Sundaland and the Lesser Sunda Islands is of multiple origins, stemming from both edaphic and foliage-dwelling ancestors.

Lifestyle dependent diversification

The results of the diversification rate analyses between the lineages of different lifestyles propose equal diversification rates among foliage dwelling and edaphic lifestyles when the assumed hidden trait is absent, but higher diversification rates in foliage dwelling lineages when the hidden factor is present (Supplementary material Appendix 1 Fig. A4, Table A3: HiSSE model M39). This indicates that a higher diversification in foliage dwelling weevils is associated with other unknown factors, but not lifestyle alone.

Body size evolution

The statistical comparisons of body sizes identified significant differences between lifestyles (phyLANOVA, $F=90.88$, $p<0.001$; Fig. 3A), but not biogeographical regions (phyLANOVA, $F=10.83$, $p=0.423$; Fig. 3B). With our current dataset, Sahul *Trigonopterus* are not significantly larger than those from Wallacea (Bonferroni post hoc test, $t=4.37$, $p=0.772$) or Sundaland (Bonferroni post hoc test, $t=2.93$, $p=0.526$). However, edaphic species are in general significantly smaller than species on foliage (Bonferroni post hoc test, $t=9.53$, $p<0.001$). Plotting *Trigonopterus* species' body sizes on the phylogeny (Supplementary material Appendix 1 Fig. A5), further shows a decrease in body size in several edaphic groups, e.g. the subgenus *Trigonopterus* (*Mimidotasia*), the *T. nanus*-group, the *T. rotundulus*-group, the *T. impressicollis*-group and the *T. saltator*-group. Increase in body size is rather rare and mainly occurs in foliage dwelling lineages from New Guinea.

Discussion

Our molecular phylogenetic inferences (Fig. 2) significantly expand our understanding of the evolutionary history of hyperdiverse *Trigonopterus* weevils (Tänzler et al. 2014, 2016, Toussaint et al. 2017b). We confirm that Sulawesi, as the central island of Wallacea, acted as a colonization hub for Borneo and the Lesser Sunda Islands (Tänzler et al. 2016). However, the recent node age estimations based on fossil calibrations shed a new light on the evolutionary history of one of the most

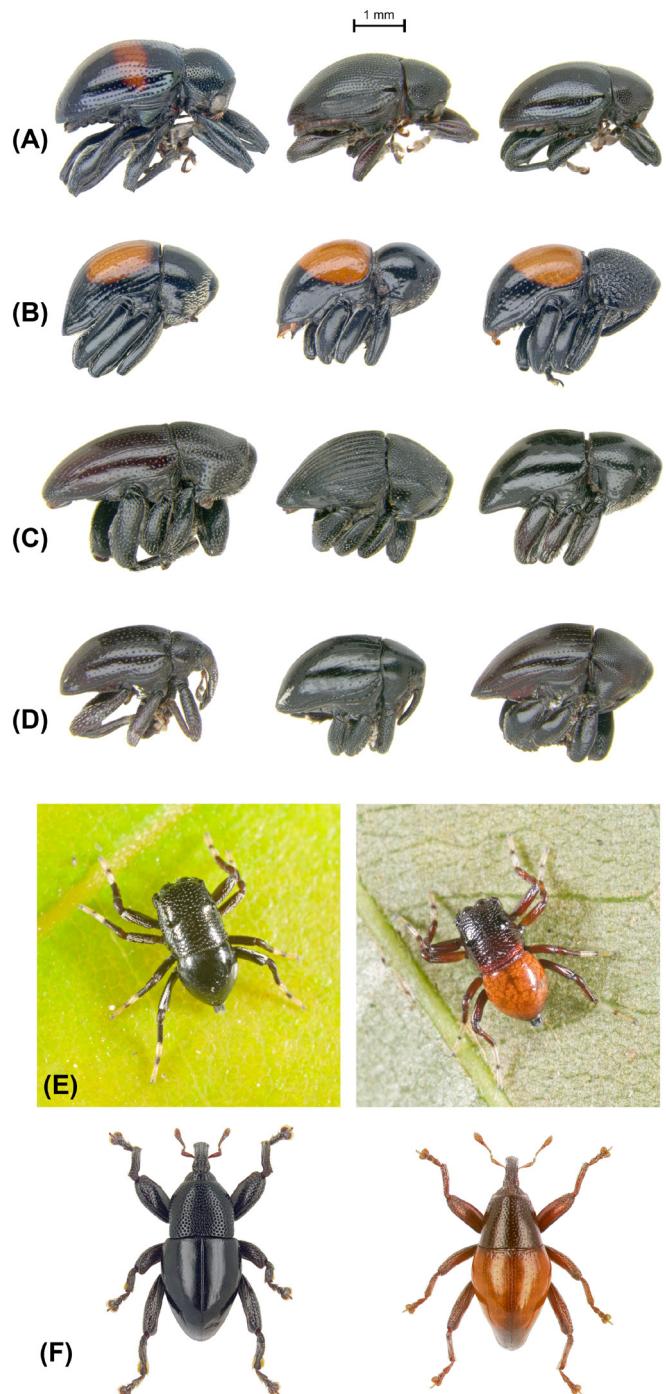


Figure 4. Mimicry complex involving species of the genus *Trigonopterus*. Rows (B, C, F) *Trigonopterus* models; row (A) *Stethotes* spp., Chrysomelidae; row (D), Curculionidae gen. sp., left *Imathia* sp., middle and right *Cryptorhynchinae*, unidentified genus; row (E) jumping spiders, *Coccochrestes* sp.; row (F) *Trigonopterus* sp. sympatric with spiders above.

diverse insect genera. Previous estimates recovering younger ages (Tänzler et al. 2014, 2016, Toussaint et al. 2017b) relied on biogeographical calibration points and/or nucleotide substitution rates, possibly introducing considerable noise. The

colonization of Sulawesi (i.e. of Wallacea) is now estimated to the Late Eocene (38 Ma), resulting in a large radiation with > 225 species (Fig. 2, Clade G). This age estimate suggests that *Trigonopterus* dispersed from Australia or a proto New-Guinean island setting to what is currently part of eastern Sulawesi and at the time likely the Sula Spur. Indeed, geological evidence indicates that Sulawesi is a composite landmass resulting from the agglomeration of geological elements from Sundaland, Sahul and the Pacific throughout the Cenozoic (Hall 2017, Nugraha and Hall 2018). In the Late Eocene, Sulawesi was rather an island setting with its eastern part, the Sula Spur, situated near the proto New Guinean region (Hall 2012, 2017), until it collided with the north Sulawesi volcanic arc in the Early Miocene (Nugraha and Hall 2018). Most colonization events of the Greater and Lesser Sunda Islands then occurred between the Oligocene and Miocene. The HiSSE analyses additionally inferred the highest diversification rate in *Trigonopterus* during the Middle and Late Eocene, when foliage dwelling species radiated in proto New Guinea and Sulawesi respectively, indicating that both colonization events may have initiated rapid local radiations (Supplementary material Appendix 1 Fig. A4). Colonization of landmasses west of Wallace's Line before the collision of the Sula Spur could be the result of passive oceanic dispersal. Our lifestyle reconstructions show that the nine transgressions of Wallace's Line were not confined to edaphic groups. Four transgressions occurred by foliage dwelling lineages, which subsequently switched completely to thrive in forest litter. This pattern indicates that species of litter and foliage have probably equal chances of dispersal, but upon arrival in Sundaland, an edaphic lifestyle was strictly favored over a foliage dwelling one. Furthermore, *Trigonopterus* weevils in Sundaland are not significantly smaller than in Wallacea and Sahul. However, they show a lesser variance in size, which may be due to a lesser average size of edaphic species compared to foliage species; or, the lesser phylogenetic diversity of the clades in Sundaland which excludes lineages of large body size present in New Guinea (Fig. 3). This rejects the idea that edaphic and/or smaller species have had a higher chance of passive dispersal by oceanic drift, simply due to their body size, lifestyle, and/or microhabitat use. The consistent shift of foliage dwelling lineages to litter habitats rather suggests that dispersers possibly encountered substantial changes of abiotic and/or biotic factors. Taxa crossing major biogeographic boundaries may be exposed to different environments to which they need to adapt to (Jablonski et al. 2006, Condamine et al. 2012, Owens et al. 2017). In the case of Wallace's Line, abiotic (i.e. geological and climatic) differences may play a role, as Sundaland (similar to Sahul) is nowadays characterized by a perhumid climate throughout most of the year, whereas most of Wallacea has a yearly dry monsoon climate with a pronounced dry season (Van Welzen et al. 2011). However, rainforests in the northern part of New Guinea are climatically similar to those on Borneo. Yet, foliage dwelling *Trigonopterus* abound in the former, while they are absent from the latter.

The exclusively edaphic lifestyle of *Trigonopterus* west of Wallacea might be explained best by different predatory faunas in the respective biogeographical regions. Due to their lifestyle, foliage-dwelling beetles are more exposed to small vertebrate predators, such as birds, frogs or lizards. Many of these beetle species possess an elaborate defense strategy composed of a drop-off reflex followed by death-feigning (thanatosis), frequently accompanied by mechanical blocking of their legs (Van De Kamp et al. 2014). Their cuticle is extremely thick and hard (Van de Kamp et al. 2015) withstanding considerable pressure (Riedel, personal observation). Furthermore, the elytra are densely interlocked along their median suture and laterally with the pterothorax and the abdomen (Van de Kamp et al. 2016). This extraordinary mechanical stability might be the key factor that provides protection against predators, as already demonstrated for the flightless genus *Pachyrhynchus* (Wang et al. 2018). Similar to *Pachyrhynchus* weevils, several foliage-dwelling species of *Trigonopterus* exhibit aposematic coloration with orange or red elytral patterns, which could function as effective warning signals. All these described characteristics are either less pronounced in edaphic species (blocking of legs), or exclusively shown by foliage dwelling species (coloration), which supports their possible function as defense mechanism against predators (Riedel unpubl.). In netwing beetles, dispersal to a different biogeographic region was followed by changes of aposematic coloration (Bocak and Yagi 2010). Interestingly, several arthropod taxa of New Guinea show striking resemblances to syntopic *Trigonopterus* weevils, such as in surface sculpture and specific color patterns. This includes leaf beetles of the genus *Stethotes* (Gressitt 1982) and other genera of Curculionidae (Setliff 2012, Riedel 2017) (Fig. 4A, D). Most intriguing are the quite diverse *Coccoctheses* jumping spiders (Fig. 4E), which also display a weevil-like walking behavior (Bohne and Hill 2011). This suggests the presence of a Batesian mimicry complex, where less armored arthropods have attained partial protection from visually oriented predators, presumably frogs, reptiles or birds. If biotic factors should force *Trigonopterus* weevils down to earth west of Wallace's Line, not only a regionally different predator fauna could be responsible, but also its lack of exposure to a largely unpalatable weevil fauna.

Conclusion

The present study confirms Wallace's Line as a rather porous barrier to the dispersal of a species-rich insect clade. As shown in previous studies (Tänzler et al. 2016), range expansion of *Trigonopterus* was unidirectional to Sundaland with a conspicuous absence of back-colonization to Wallacea and/or Sahul. This has also been shown for the flightless weevil genera of the tribe Celeuthetini (Toussaint et al. 2015), and was explained by the predominant sea currents in the IAA (Tänzler et al. 2016). Herein, for the first time we also demonstrate ecological consequences of crossing Wallace's Line by *Trigonopterus*, as the change of lifestyle was also unidirectional. Higher

dispersal chances for edaphic lineages of small body size are not supported. The evolution of an exclusive edaphic lifestyle in Sundaland was probably facilitated by yet unidentified ecological factors exerting differential selective pressures. To identify these selective forces, further studies are necessary. A better understanding of the role of *Trigonopterus*' defense mechanism in its colonization history, and the potential mimicry complex at play, would need experimental studies, which should test the reaction of different potential predators on *Trigonopterus* weevils in different situations.

Data availability statement

Data available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.h70rxwdg6>> (Letsch et al. 2020b).

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Supplementary material (available online as Appendix ecog-05128 at <www.ecography.org/appendix/ecog-05128>). Appendix 1.