Historical biogeography and phylogeny of the pantropical Psychotrieae alliance (Rubiaceae), with particular emphasis on the Western Indian Ocean Region[1]

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PREMISE OF THE STUDY: The Western Indian Ocean Region (WIOR) is a biodiversity hotspot providing an ideal setting for exploring the origins of insular biodiversity and dynamics of island colonization. We aimed to investigate the origins of the WIOR Psychotrieae alliance (Rubiaceae) with typically small, probably mainly bird-dispersed drupes, and the timing and direction or sequence of its colonization events in the region.

METHODS: We used the program BEAST to estimate divergence times and Lagrange for biogeographic reconstruction.

KEY RESULTS: The alliance has reached the WIOR at least 14 times via dispersals from Africa along with Asia and the Pacific mostly during the last 10 My, with at least one back-colonization to Africa. We inferred the earliest dispersal to Madagascar from the Pacific or Asia in the Miocene and numerous out-of-Madagascar dispersals to the nearby archipelagos but no dispersal out of those archipelagos. *Gynochthodes* with multiple fruits reached Madagascar twice from the Pacific possibly via ocean drifting. *Psychotria* with dry fruits (schizocarps) colonized Madagascar from the Pacific or Asia before reaching the Comoros from Madagascar possibly via wind dispersal.

CONCLUSIONS: This study reinforces the pivotal role of dispersal in shaping the WIOR biodiversity and as the critical initiating step in the generation of endemic biodiversity on its islands. The WIOR alliance shows strong Asian and Pacific affinities despite the proximity of the region to Africa. Madagascar has served as a stepping-stone for subsequent dispersal to the rest of the region. The Afro-Malagasy-Seychelles genus *Craterispermum* and the Malagasy *Puffia* may represent relictual lineages.

KEY WORDS Biogeography; Comoros; dispersal; divergence time estimation; Madagascar; Mascarenes; Rubiaceae; Seychelles; systematics

The Western Indian Ocean Region (WIOR) *sensu* Myers et al. (2000), also known as the Madagascar region (e.g., Warren et al., 2010), is an insular biodiversity hotspot (Bellard et al., 2014) encompassing Madagascar and the nearby archipelagos of the Comoros, Mascarenes, and Seychelles. The region contains many islands of various sizes and ages with high levels of diversity and endemism of plants and animals. The largest and oldest island in the region, Madagascar, lies about 400 km off the southeast coast of mainland Africa, and is located east of Mozambique, across the Mozambique Channel. This continental island together with East Antarctica, Australia, the granitic parts of the Seychelles, and India were all part of East Gondwana, which separated from Africa ca. 160 Ma (see e.g., Royer and Coffin, 1992) and subsequently broke up 160–120 Ma (Storey, 1995; Storey et al., 1995). India, the granitic Seychelles, and Madagascar were joined together to form the Indigascar landmass, which split up from Australia and Antarctica ca. 132 Ma (Reeves, 2014) and broke up ca. 65 Ma (Plummer and Belle, 1995; Reeves, 2014). Madagascar remained more or less in its current position, whereas the granitic Seychelles separated from India, and remained in the northern part of the WIOR. India drifted

toward the Eurasian plate, and became part of Asia (Plummer and Belle, 1995; Storey et al., 1995). The remaining islands of the Seychelles archipelago are of younger volcanic origins. The Comoros archipelago is equidistant between Madagascar and the southeastern coast of mainland Africa, and consists of four major volcanic islands: Mayotte (7 Ma), Moheli (5 Ma), Anjouan (3.9 Ma), and Grande Comoro (0.01 Ma) (Nougier et al., 1986). Finally, the Mascarene archipelago is situated about 900 km east of Madagascar, and comprises Mauritius (7.8 Ma), Reunion (2.1 Ma), and Rodrigues (1.5 Ma) Islands. It should be noted that the submarine Rodrigues Ridge is dated to 7.5–11 Ma (Duncan, 2009).

The biodiversity of the WIOR constitutes a mix of species that have been evolving there as a result of vicariance and those that arrived there via dispersals during the Cenozoic, 65 Ma to present (e.g., Yoder and Nowak, 2006; Agnarsson and Kuntner, 2012). Teasing apart these biogeographic scenarios for a particular lineage requires the knowledge about the phylogeny of the group, timing of divergences, and biogeographic distribution of ancestral taxa at different time intervals. With all this information at hand it is possible to put forward new biogeographic hypotheses on the timing and direction or sequence of colonization events between the islands of the WIOR and also between this region and the rest of the world.

Rubiaceae (or coffee family) is the largest woody flowering-plant family in the WIOR with at least 1200 species (ca. 9.20% of the total species richness of the family), which are unevenly distributed across the region. The Psychotriae alliance (subfamily Rubioideae) is the most species-rich lineage of Rubiaceae in the WIOR, with about 255 species currently classified in five of its nine tribes (Craterispermeae, Gaertnereae, Morindeae, Palicoureeae, and Psychotrieae). This information indirectly indicates that the alliance has reached the region numerous times. However, it does not tell us anything about the timing and direction or sequence of the island colonization within the WIOR, as well as the colonization events between the region and the rest of the tropics. Further, the high level of endemism and wide distribution of the alliance across the WIOR make this group of plants and the region an ideal model and geographic setting, respectively, for investigating the origins of insular biodiversity and dynamics of island colonization (see also Wikström et al., 2010; Kainulainen et al., 2017).

A broad sampling of the Psychotrieae alliance across its entire geographic ranges is required to study the colonization history of the group in the WIOR. The whole alliance constitutes a monophyletic group with no less than 3450 species of mostly understory shrubs (Razafimandimbison et al., 2008). The majority of these species belong to *Psychotria* L., one of the most speciose of flowering-plant genera (with about 1800 species, Razafimandimbison et al., 2014). The group is distributed pantropically, with only a few species in temperate America and East Asia, all from the tribe Mitchelleae (Razafimandimbison et al., 2008). Members of the alliance are ubiquitous components of many terrestrial ecosystems, and typically produce small, fleshy, drupaceous fruits, which are an important resource for numerous frugivorous, tropical birds and mammals. Therefore, frugivores are the primary seed dispersers of its species (e.g., Herrera, 1989; Galetti et al., 2011). The majority of the species-richness of the alliance in the WIOR is found in Madagascar, which has been shown to be the main source of dispersal to the neighboring archipelagos (e.g., Wikström et al., 2010; Strijk et al., 2012).

The Psychotrieae alliance is the sister group of the predominantly herbaceous Spermacoceae alliance within the subfamily Rubioideae (e.g., Bremer and Manen, 2000; Robbrecht and Manen, 2006), and is currently subdivided into nine well-supported lineages (Razafimandimbison et al., 2008; Rydin et al., 2008), corresponding to the tribes: Craterispermeae (found in Africa, Madagascar, and Seychelles); Gaertnereae (pantropical, except the Pacific and Australia); Mitchelleae; Morindeae, Palicoureeae, and Psychotrieae (pantropical); Prismatomerideae (tropical Asia); Schizocoleeae (tropical Africa); and Schradereae (Asia-neotropics). Schizocoleeae is sister to the rest of the alliance and the sister-group relationships between Palicoureeae and Psychotrieae and between Mitchelleae and Morindeae have been confirmed (see e.g., Razafimandimbison et al., 2008; Rydin et al., 2008; Wikström et al., 2015). However, there are conflicts regarding the phylogenetic positions of Craterispermeae, Gaertnereae, and Prismatomerideae (see e.g., Robbrecht and Manen, 2006; Razafimandimbison et al., 2008; Wikström et al., 2015). The phylogenetic relationships within Gaertnereae (Malcomber, 2002; Malcomber and Taylor, 2009), Mitchelleae (Huang et al., 2013), Morindeae (Razafimandimbison et al., 2009, 2012), Palicoureeae, and Psychotrieae (Razafimandimbison et al., 2014) have recently been assessed, but so far no study has addressed the evolutionary relationships within Craterispermeae, Prismatomerideae, or Schradereae.

The main objectives of this study were to investigate the origins of the WIOR Psychotrieae alliance and the dynamics (i.e., timing, direction, or sequence) of its colonization events within the region. We also aimed to gain new insights into the phylogeny and new hypotheses about the biogeographic origins of the whole alliance and its tribes.

MATERIALS AND METHODS

Taxon sampling—Our taxon sampling covered the entire geographic range of the Psychotrieae alliance, with particular emphasis on the tribes occurring in the WIOR. As many species as possible of all genera of the alliance found in the region were included, and sampling density was particularly broad for the species-rich genera *Chassalia* Comm. ex Poir., *Gaertnera* Lam., and *Psychotria sensu* Razafimandimbison et al. (2014; including taxa under the names of *Amaracarpus* Blume, *Calycosia* A. Gray, and *Hydnophytum* Jack that are used in this paper only because the formal synonymization has not yet been dealt with). We also included four Asian species of the genus *Schradera* Vahl, one species of the Malesian genus *Lecananthus* Jack (Schradereae), and the monospecific, Malesian genus *Gentingia* J.T.Johanss. & K.M.Wong (Prismatomerideae). *Psychotria balfouriana* Verdc., endemic to the Rodrigues Island (in the Mascarenes), was not included due to the lack of sequenceable material. This species is thought to be extinct (C. Baider, National Herbarium of Mauritius, personal observation). In total, we sampled 376 taxa of the Psychotrieae alliance: 15 from Craterispermeae (ca. 50% of its species), 28 from Gaertnereae (ca. 25% of its species), four from Mitchelleae (ca. 29% of its species richness), 27 from Morindeae (17.5% of its species), 126 from Palicoureeae (8.4% of its species), eight from Prismatomerideae (ca. 32% of its species), 161 from Psychotrieae (ca. 9% of its species), one from Schizocoleeae (50% of its species), and eight from Schradereae (ca. 15% of its species). Finally, eight additional species of Rubioideae were also investigated and *Luculia* Sweet was used as outgroup (Appendix S1, see Supplemental Data with this article).

Molecular markers and laboratory procedures—We used sequence data from the nuclear ribosomal nrETS and nrITS regions and the plastid *atp*B-*rbc*L, *ndh*F, *rbc*L, *rps*16, and *trn*T-F regions, as they have been shown to be useful for inferring phylogenetic relationships within the Psychotrieae alliance (e.g., Razafimandimbison et al., 2008, 2014). A large part of the data are from Razafimandimbison et al. (2014) and the protocols used for DNA extraction, amplification, and sequencing of the 74 newly investigated taxa were the same as those outlined in that study.

Phylogenetic analyses—Sequence data were aligned using MUSCLE version 3.8.31 (default settings; Edgar, 2004). Phylogenetic reconstructions were done using Maximum Likelihood and Bayesian Markov Chain Monte Carlo methods (Yang and Rannala, 1997). Nonclock analyses were conducted using RAxML version 8.2.9 (Stamatakis, 2014) and MrBayes version 3.2.5 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and relaxed-clock analyses using BEAST version 1.8.2 (Drummond and Rambaut, 2007; Drummond et al., 2012). Data were treated as three separate partitions depending on the type of data: nuclear (1245 characters) and plastid coding (3467 characters) and noncoding (5615 characters). For each partition a GTR+I+G nucleotide substitution model was chosen based on the corrected Akaike information criterion (AICc) as calculated using MrAIC version 1.4.6 (Nylander, 2004). The RAxML analysis consisted of 2000 bootstrap replicates using the rapid bootstraping algorithm and CAT approximation for the standard GAMMA model of rate heterogeneity (Stamatakis, 2014). The MrBayes analyses comprised two runs of four chains each that were run for 10^7 generations sampling trees and parameters every 1000th generation.

Divergence times were estimated in BEAST version 1.8.2 using the uncorrelated lognormal clock model and the birth-death tree prior (Gernhard, 2008). The root node was assigned a normally distributed age prior with a mean of 90 Ma and a standard deviation of 5 Ma (78–103 Ma). The species outside of the alliance included in this study were represented by single specimens. This somewhat poor sampling of the outgroup taxa could potentially result in underestimated ages for basal nodes. Therefore, additional secondary calibration priors outside of the targeted group were included. Secondary calibration priors that were applied include stem nodes of the tribes Ophiorrhizeae (63–83 Ma), Coussareeae (56–73 Ma), and Morindeae (38–43 Ma). These age priors conform to the 95% highest posterior density (HPD) interval of corresponding nodes inferred in a study of the Asterids and Gentianales divergence times by Wikström et al. (2015). The Morindeae stem lineage age prior is also supported by fossil evidence, specifically by the occurrence of *Morinda chinensis* in the early Late Eocene (38 Ma) of China (Shi et al., 2012). The BEAST analysis comprised two independent runs, each including 100×10^6 generations sampling trees and parameters every 10,000th generation. Individual runs were combined and resampled every 20,000th generation and removal of the burn-in (50%) produced a final sample of 10,000 trees and parameter estimates. Effective samples sizes of the parameters were evaluated using the program TRACER version 1.6 (Rambaut and Drummond, 2013). Node age estimates and age density intervals were subsequently summarized on the maximum clade credibility tree using treeannotator version 1.8.2 (Drummond and Rambaut, 2007; Drummond et al., 2012).

Biogeographic analyses—Eight geographical areas were recognized in the biogeographic analyses: (1) Madagascar (A); (2) Comoros (B); (3) Mascarenes (C); (4) Seychelles (D); (5) the Pacific (including Australia) (E); (6) Asia (F); (7) Africa (G); and (8) neotropics (H). Distribution data were taken from the World checklist of Rubiaceae (Govaerts et al., 2013). We coded undescribed or undetermined taxa according to where they were collected. The geographic range evolution of the Psychotrieae alliance was reconstructed within a likelihood framework using the dispersal-extinction-cladogenesis (DEC) model as implemented in the program Lagrange version 20130526 (Ree et al., 2005; Ree and Smith, 2008). The DEC model used was unconstrained (transitions between all areas are equally likely) with a maximum of two areas per node. To account for topological uncertainty in the reconstruction we compiled the likelihood for each node across a subsample of 100 trees. The trees were randomly drawn from the posterior distribution of trees from the BEAST analysis using the standard random module in Python version 2.7.6. (van Rossum and de Boer, 1991).

RESULTS

In this study, we investigated a total of 376 taxa and 2278 sequences, of which 606 (ca. 27%) are newly published here: 82 nrETS; 89 nrITS; 92 *atp*B-*rbc*L; 88 *ndh*F; 88 *rbc*L; 86 *rps*16; and 81 *trn*TF sequences. All EMBL/GenBank accession numbers of sequences of the studied taxa are summarized in Appendix S1.

Trees from the separate analyses of cpDNA (*atp*B-*rbc*L, *ndh*F, *rps*16, and *trn*TF) and nrDNA (nrITS and nrETS) were congruent concerning the relationships among major lineages within the Psychotrieae alliance, and did not show any strongly supported conflicts within the tribes (results not shown). Therefore, we chose to combine the data sets. Figure 1 is a simplified tree from the BEAST analysis of the combined datasets of the Psychotrieae alliance. The summary of the inferred dispersal events toward the WIOR and the out-of-Madagascar dispersals to the nearby archipelagos is shown in Figure 2. The maximum clade credibility tree (i.e., the tree in the Bayesian sample with the highest sum of posterior probabilities from the BEAST analysis of the combined data) is shown in Figures 1 and 3–7 and Appendix S2 in the form of a chronogram calibrated against The Geologic Time Scale (Gradstein et al., 2012). Appendix S2 depicts the sampling and topology of the clades that were collapsed in Figs. 1 and 5). Table 1 summarizes bootstrap frequencies from the Maximum Likelihood analyses conducted in RAxML, bayesian posterior probabilities from the nonclock analysis in MrBayes, bayesian posterior probabilities, mean ages, 95% highest posterior density intervals (HPD) of the age estimates from the relaxed-clock analysis in BEAST, and probabilities of the two most likely biogeographic scenarios from the DEC analysis in Lagrange for the alliance, its tribes, and some well-supported lineages from the WIOR discussed in this study. Corresponding estimates for all nodes can be found in Appendix S3.

Historical biogeography of the pantropical Psychotrieae alliance—The ancestral lineage of the Psychotrieae alliance was inferred to have originated in Africa in the Upper Cretaceous-Paleocene 68–56 Ma (node 745; 62 Ma, HPD: 68–56 Ma; Fig. 1, Tables 1, Appendix S3). Within the alliance our biogeographic reconstruction of the ancestral lineages of the tribes is equivocal mainly due to the weak support for the nodes 724 and 741 and the phylogenetic positions of Gaertnereae and Prismatomerideae (Fig. 1). Nevertheless, we present below the most-supported
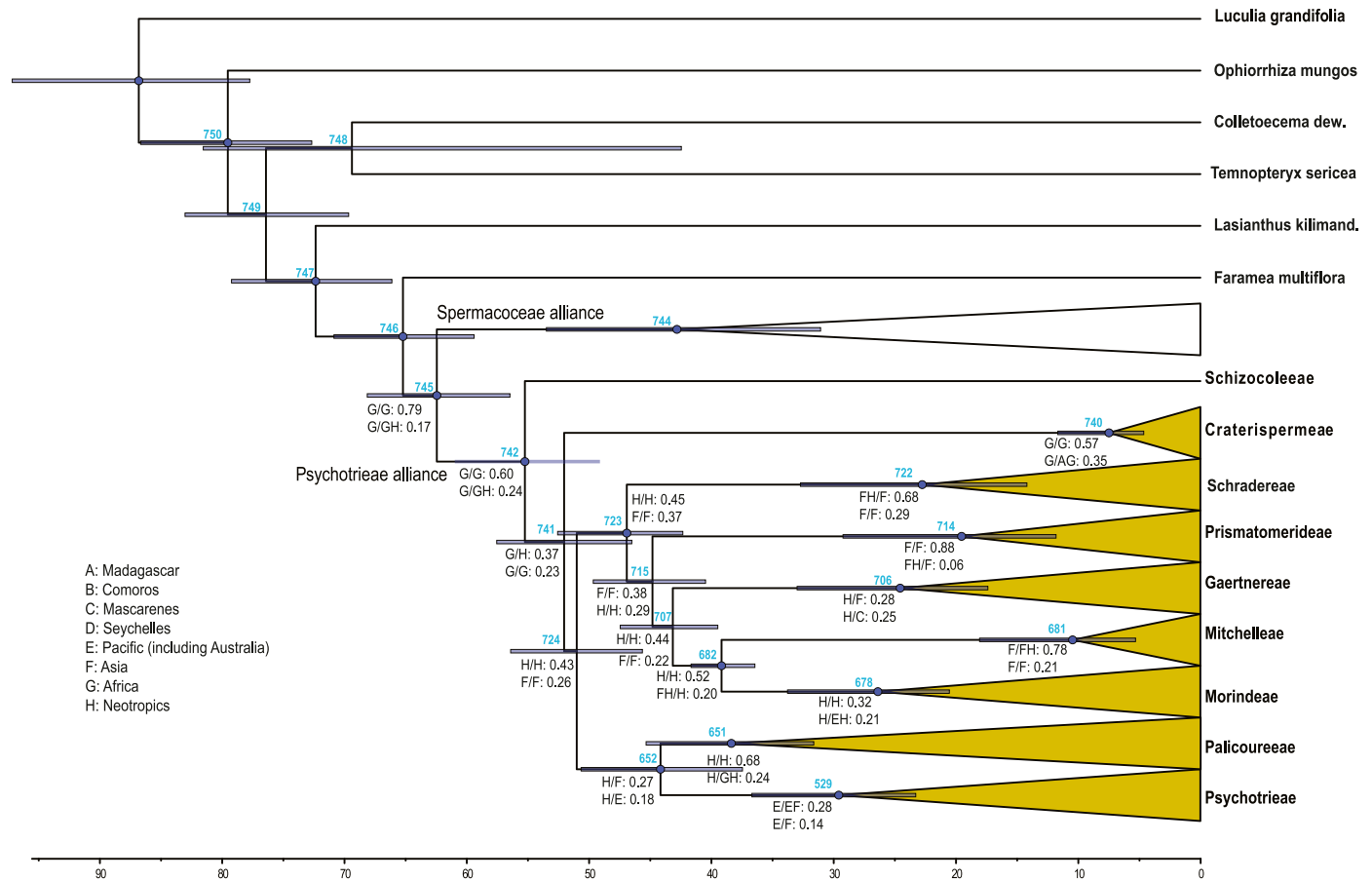
FIGURE 1 The Maximum clade credibility (MCC) tree from the BEAST analyses of the combined Psychotrieae alliance datasets. The tree is drawn as a chronogram calibrated against The Geologic Time Scale (Gradstein et al., 2012) and credibility intervals of the node age estimates are indicated by blue bars. Well-supported nodes (posterior probability ≥ 0.95) are shown as blue bullets. Relationships within the collapsed clades are detailed in Figures 3–7 and Appendix S2. Results from the Lagrange analyses are shown for nodes of interest.

biogeographic scenarios. The *Psychotria* alliance crown group dispersed from Africa to the neotropics between the Paleocene 61 Ma (node 742; 55 Ma, HPD: 61–49 Ma) and the Eocene 47 Ma (node 741; 52 Ma, HPD: 58–47 Ma). The alliance subsequently expanded its range in the neotropics, giving rise to the Palicoureeae crown group in the Eocene–Oligocene 45–32 Ma (node 651; 38 Ma, HPD: 45–32 Ma) and the Morindeae crown group 34–21 Ma (node 678; 26 Ma, HPD: 34–21 Ma) and the Gaertnereae crown group 33–17 Ma (node 706; 25 Ma, HPD: 33–17 Ma; Fig. 1; Table 1, Appendix S3), both in the Oligocene-Miocene. The Psychotrieae alliance colonized Asia from the neotropics in the Eocene 53–41 Ma (between node 723; 47 Ma, HPD: 53–42 Ma and node 715; 45 Ma, HPD: 50–41 Ma) (Fig. 1), and continued its range expansion there, giving rise to the crown groups of Schradereae 33–14 Ma (node 722; 23 Ma, HPD: 33–14 Ma) and Prismatomerideae 30–12 Ma (node 714; 20 Ma, HPD: 30–12 Ma), in the Oligocene–Miocene, and the crown group of Mitchelleae in the Miocene-Pliocene 18–5 Ma (node 681; 10 Ma, HPD: 18–5 Ma). The alliance reached the Pacific from the neotropics in the Eocene-Miocene 51–23 Ma (between node 652; 44 Ma, HPD 51–37 Ma and node 529; 30 Ma, HPD 37–23 Ma), giving rise to the Psychotrieae crown group in the Eocene-Miocene 37–23 Ma (node 529; Fig. 1; Table 1, Appendix S3). The stem and crown ages of the Psychotrieae alliance were inferred to be 68–56

Ma (node 745; 62 Ma, HPD: 68–56 Ma) and 61–49 Ma (node 742; 55 Ma, HPD: 61–49 Ma), respectively. The stem ages of the tribes of the alliance ranged from 61–49 Ma (Schizocoleeae node 742; 55 Ma, HPD: 61–49 Ma) to 42–36 Ma (Mitchelleae and Morindeae node 682; 39 Ma, HPD: 42–36 Ma), while their crown ages varied from 45–32 Ma (Palicoureeae node 651; 38 Ma, HPD: 45–32 Ma) to 12–5 Ma (Craterispermeae node 740; 7 Ma, HPD: 12–5 Ma; Table 1).

Colonization history of the Psychotrieae alliance in the Western Indian Ocean Region—As this study focuses on the biogeography of the Psychotrieae alliance in the WIOR, we concentrated on the results of the biogeographic and molecular dating analyses for the five tribes (Craterispermeae, Gaertnereae, Morindeae, Palicoureeae, and Psychotrieae) occurring in the region (Figs. 3–7).

The Psychotrieae alliance was estimated to have reached the WIOR via at least 14 independent dispersals from Africa, Asia, and the Pacific (Fig. 2) that gave rise to about 255 species. Six of these dispersals reached Madagascar from Africa starting in the Miocene and giving rise to: (1) the Malagasy *Craterispermum* lineage (Fig. 3: node 736); (2) the monospecific *Puffia* Razafim. & B.Bremer (Fig. 5: node 620); (3) the dry-forest, Malagasy *Chassalia* lineage (Fig. 5: node 586); (4) the rainforest *Chassalia* lineage (Fig. 5: node 583);
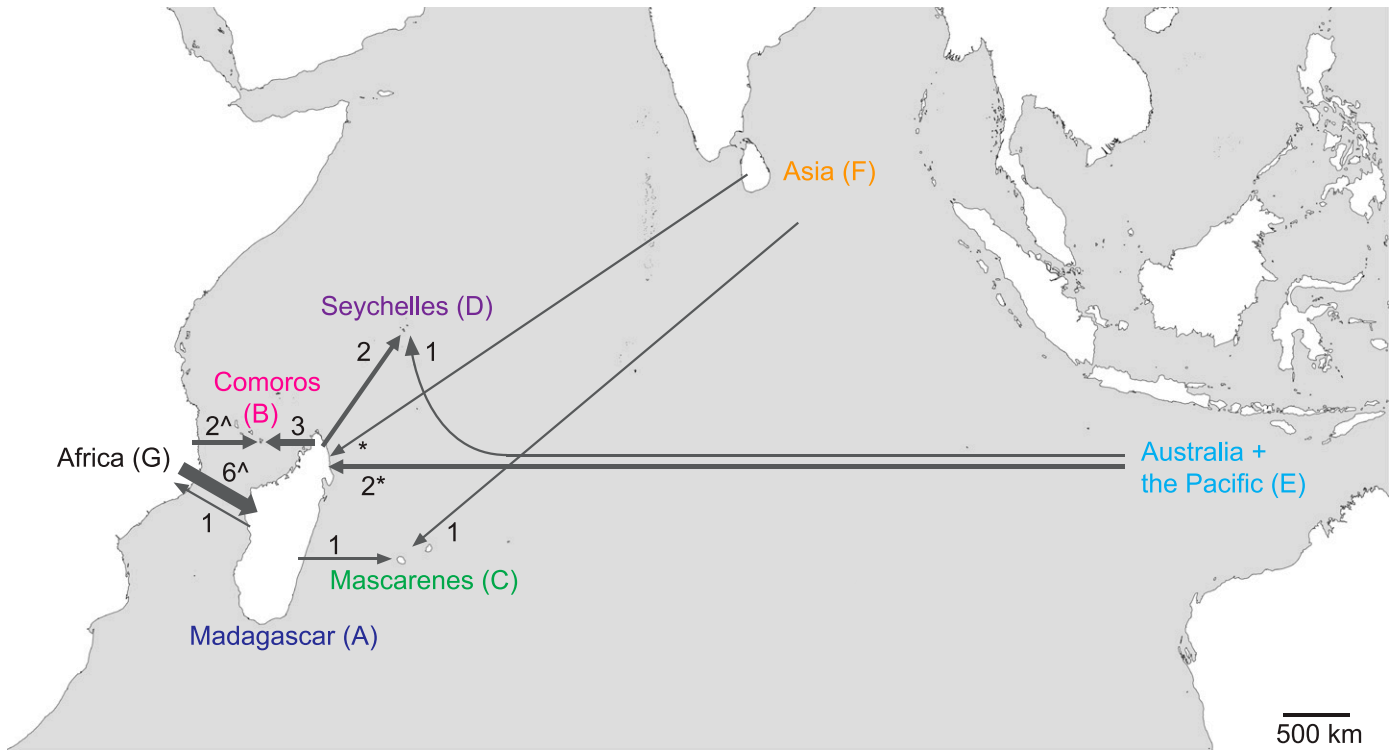
FIGURE 2 Summary of the inferred dispersal events toward the Western Indian Ocean Region and the out-of-Madagascar dispersals to the nearby archipelagos. "^" represents one dispersal from Africa to either Madagascar or the Comoros (Fig. 5: between nodes 607 and 606). "*" denotes one dispersal from either Asia or the Pacific to Madagascar (Fig. 7: between nodes 462 and 461).

(5) the Malagasy leaf-nodulate *Psychotria* lineage (Fig. 6: node 516); and (6) the Malagasy *Psychotria* lineage with yellow flowers (Fig. 6: node 485). Within the bacterial leaf nodulate *Psychotria* lineage (Fig. 6: node 521) two dispersals reached the Comoros from Africa during the Miocene, and gave rise to *Psychotria punctata* Vakte and *P. conocarpa* Bremek. (no crown ages available). *Geophila uniflora* Hiern also dispersed from Africa to Madagascar and the Comoros, although the sequence in which these islands were colonized was equivocal in our biogeographic analysis (Fig. 2: ^; Fig. 5: node 605). We inferred two dispersals from the Pacific to Madagascar within *Gynochthodes* Blume: the first giving rise to the Malagasy *Gynochthodes retusa* (Poir.) Razafim. & B.Bremer within the black-bluish-fruited *Gynochthodes* lineage (Fig. 4: node 660), and the second to a clade of the Malagasy species of *Gynochthodes* (Fig. 4: node 665) within the orange-fruited *Gynochthodes* lineage (Fig. 4: node 671). The latter dispersal event occurred during the Pliocene, whereas the former is no older than the Miocene (no crown age available for *G. retusa*). One dispersal from Asia to the Mascarenes starting in the Miocene was inferred, giving rise to the Reunion *Gaertnera vaginata* Lam. (Fig. 3, no crown age available). The ancestor lineage of "*Amaracarpus pubescens* Blume" reached the Seychelles from the Pacific (Fig. 6, no crown age available). Finally, one dispersal from the Pacific or Asia to Madagascar (Fig. 2: *; Fig. 7: node 461) was estimated in the Oligocene-Miocene, giving rise to a clade of the WIOR *Psychotria* species. Further, we inferred at least one back-colonization from Madagascar to Africa between the Miocene 8 Ma (node 477; 7 Ma, HPD: 8–5 Ma) and the Pliocene 4 Ma (node 471; 5 Ma, HPD: 7–4 Ma) in Psychotrieae (Fig. 6). At least six out-of-Madagascar dispersal events to the nearby archipelagos but

not a single dispersal event out of these archipelagos were inferred in the WIOR (Fig. 2).

Craterispermeae reached the WIOR via a single dispersal from Africa to Madagascar between the Pliocene 5 Ma (node 737; 4 Ma, HPD: 5–2 Ma) and the Pleistocene 1 Ma (node 736; 2 Ma, HPD: 3–1 Ma) (Fig. 3, Table 1, Appendix S3), and diversified there. The Seychelles were subsequently colonized from Madagascar between the Pliocene 3 Ma (node 731; 2 Ma, HPD: 3-1 Ma) and the Pleistocene (node 730; 0.5 Ma, HPD: 1–0 Ma), and gave rise to the Seychelles species *Craterispermum microdon* Baker (Fig. 3).

The ancestral area reconstruction for the Gaertnereae crown group and *Gaertnera* was equivocal (Fig. 3). A transoceanic dispersal of the tribe from the neotropics to the paleotropics was inferred, but the sequence of range expansion in Africa, Asia, and the Mascarenes is not conclusive. Nevertheless, it is clear that the tribe has reached the WIOR at least three times via dispersals, most likely from Asia: two times to the Mascarenes [the Reunion species *G. vaginata* and the core Mascarene *Gaertnera* (Fig. 3, node 699)] and once to Madagascar [Malagasy *Gaertnera* lineage, (Fig. 3, node 690)] (see also Table 1, Appendix S3).

Within Morindeae the sampled *Gynochthodes* species from Madagascar were resolved in two separate lineages and therefore the biogeographic analysis supported two independent arrivals to WIOR (Fig. 4, Table 1, Appendix S3). The Malagasy *Gynochthodes retusa* was nested within a *Gynochthodes* lineage with large black-bluish fruits (node 660), while another Malagasy *Gynochthodes* group (node 665) was resolved in a *Gynochthodes* lineage with small, orange fruits (node 671). The black-bluish-fruited *Gynochthodes* and the orange-fruited lineages were resolved as sisters, and were
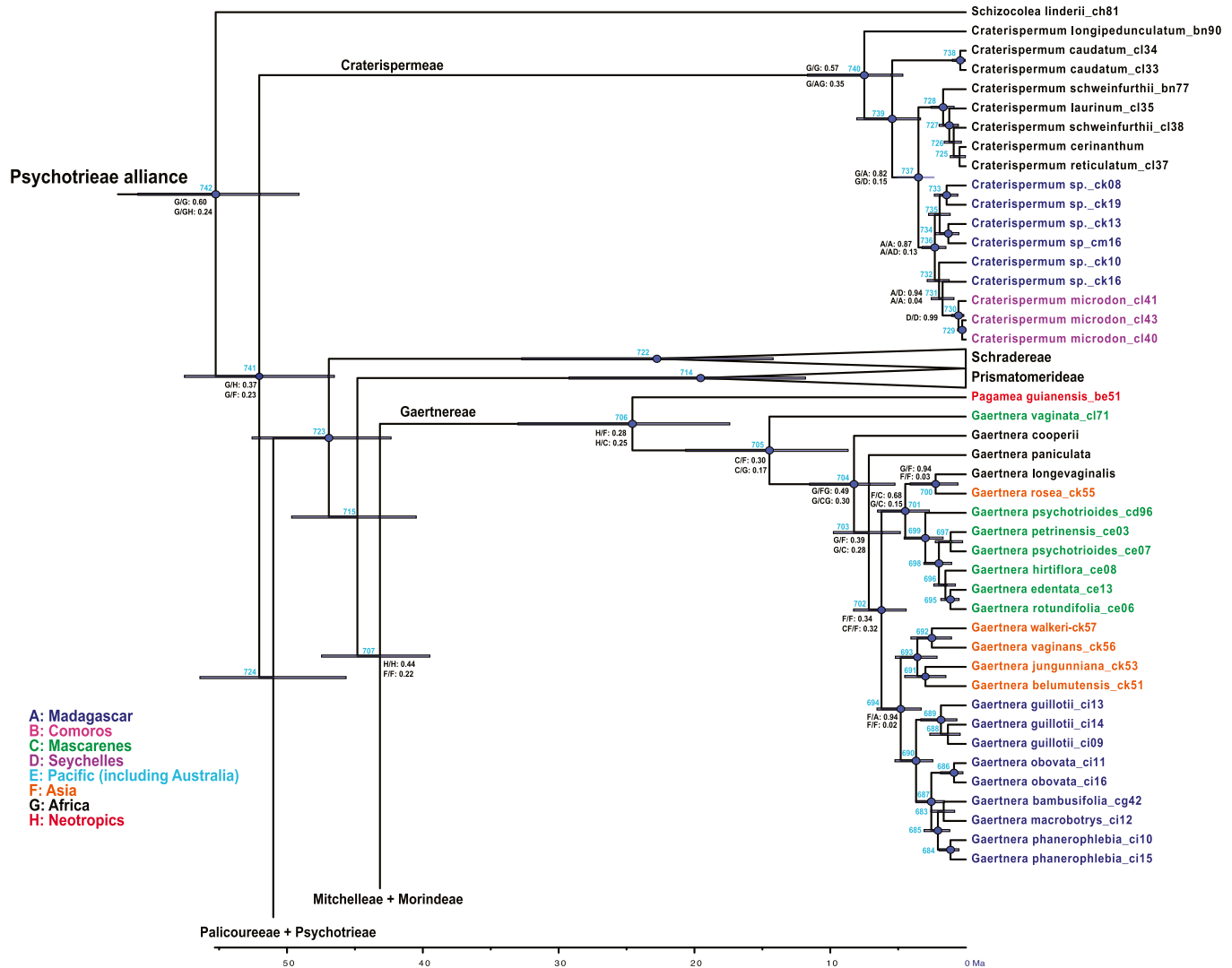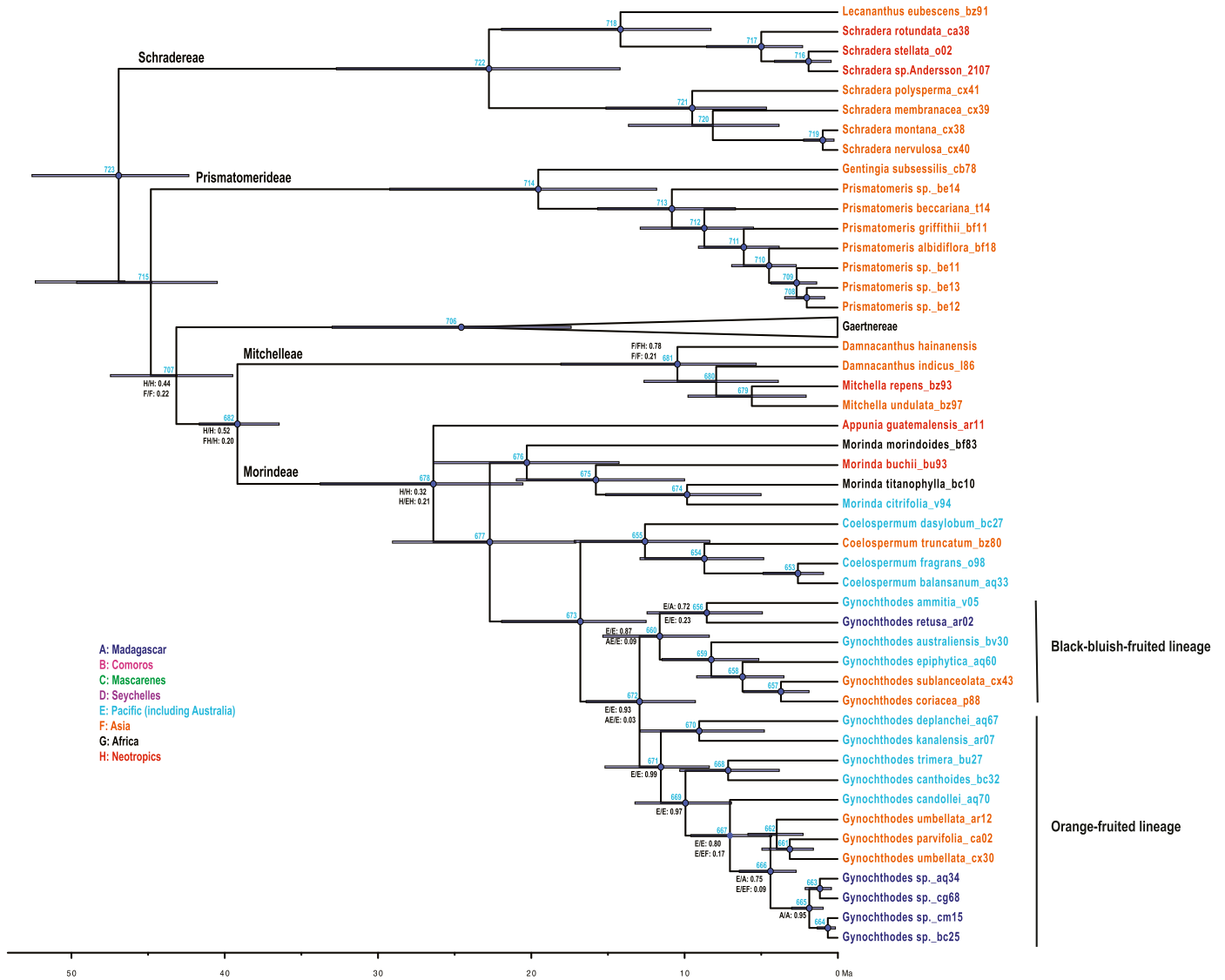
FIGURE 3 Part of the Maximum clade credibility (MCC) tree from the BEAST analyses of the combined Psychotrieae alliance data set, showing the tribes Craterispermeae and Gaertnereae. The tree is drawn as a chronogram calibrated against The Geologic Time Scale (Gradstein et al., 2012) and credibility intervals of the node age estimates are indicated by blue bars. Well-supported nodes (posterior probability ≥ 0.95) are shown as blue bullets. Results from the Lagrange analyses are shown for nodes of interest. The colors of the taxon names represent geographic origins: Africa = black; Asia = orange; Comoros = pink; Madagascar = blue; Mascarenes = green; neotropics = red; Pacific (including Australia) = cyan; and Seychelles = purple.

estimated to have originated from the Pacific, and diverged in the Miocene 16–9 Ma (node 672; 13 Ma, HPD: 16–9 Ma). Although no crown age is available for *G. retusa,* the dispersal of the clade represented by this species to Madagascar from the Pacific occurred at most 12 Ma (node 656; 9 Ma, HPD: 12–5 Ma); the orange-fruited *Gynochthodes* lineage dispersed from the Pacific to Madagascar between the Miocene 6 Ma (node 666; 4 Ma, HPD: 6–3 Ma) and the Pleistocene 1 Ma (node 665; 2 Ma, HPD: 3–1 Ma) (Fig. 4, Table 1, Appendix S3).

The tribe Palicoureeae reached the WIOR at least three times from Africa to Madagascar, giving rise to: (1) the Malagasy *Puffia* (node 620; 0.5 Ma, HPD: 1–0 Ma) in the Pleistocene 1–0.1 Ma; (2) the Malagasy and Comoros *Geophila uniflora* in the Pleistocene 1 Ma; and (3) the dry-forest, Malagasy *Chassalia* lineage (node 586; 2 Ma, HPD: 4–1 Ma) in the Pliocene 4–1 Ma (Fig. 5, Table 1, Appendix S3). *Puffia* and the African *Hymenocoleus* Robbr. were

inferred to have diverged in the Miocene 23–9 Ma (node 621; 16 Ma, HPD: 23–9 Ma). However, the crown group of *Puffia* was estimated to have diversified in Madagascar in the Pleistocene (node 620; 0 Ma, HPD: 1–0 Ma). The paleotropical *Geophila uniflora* has dispersed from Africa to Madagascar and the Comoros between the mid-Miocene 16 Ma (node 607; 12 Ma, HPD: 16–9 Ma) and the early Miocene 6 Ma (node 606; 6 Ma, HPD: 9–4 Ma), but it is not evident from our results, which of these areas was colonized first. Within the paleotropical genus *Chassalia sensu* Razafimandimbison et al. (2014), the three sampled *Chassalia* species from the deciduous dry forests of Madagascar (*Chassalia* sp.-cv96, bk67, and cm64) formed a clade (node 586; 2 Ma, HPD: 4–1 Ma) nested within an African *Chassalia* lineage (node 590; 8 Ma, HPD: 11–7 Ma). The ancestor of this Malagasy clade reached Madagascar from Africa between the Pliocene 7 Ma (node 588; 5 Ma, HPD: 7–3 Ma) and the Pleistocene 1 Ma (node 586). The sampled
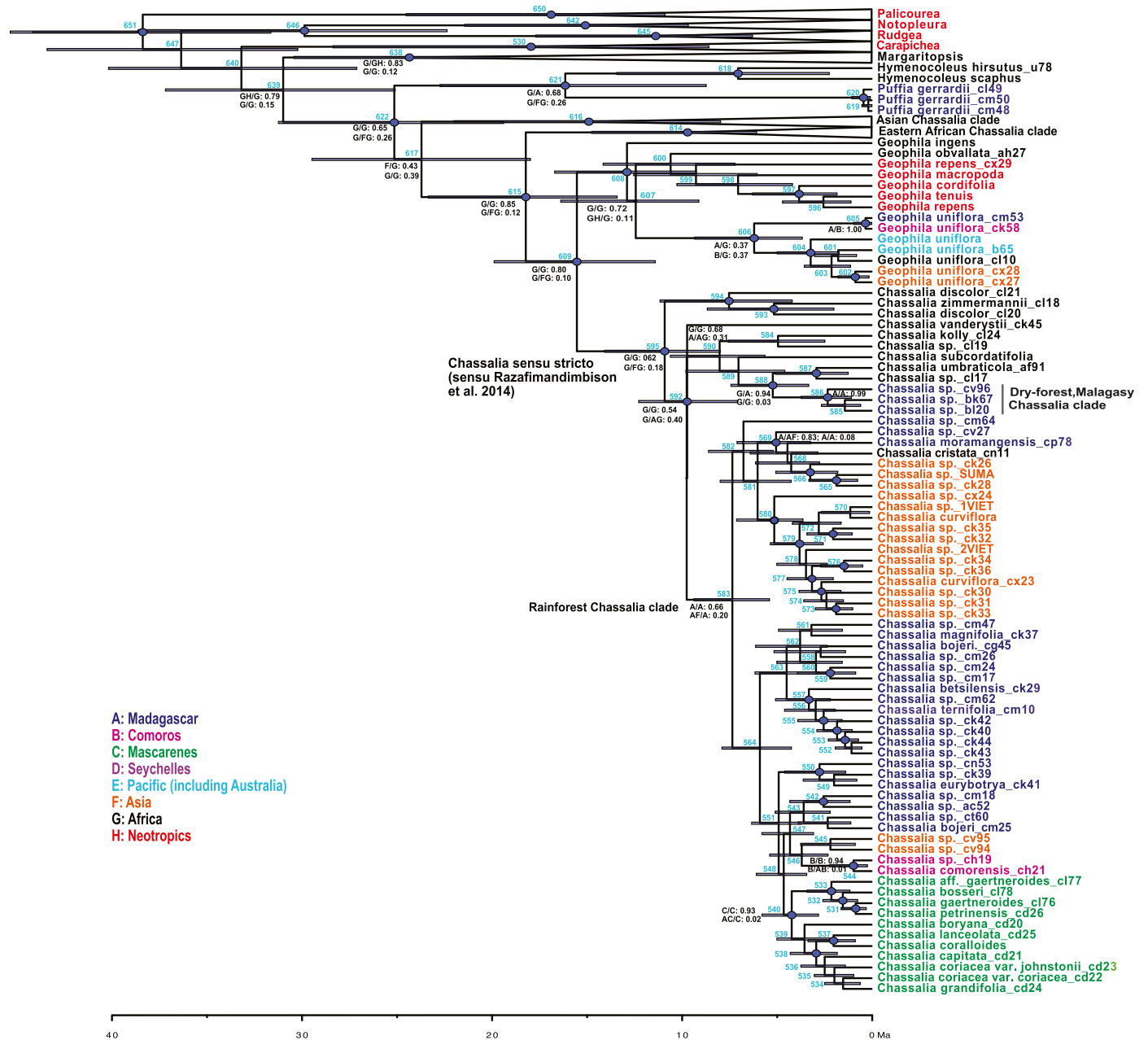
FIGURE 4 Part of the Maximum clade credibility (MCC) tree from the BEAST analyses of the combined Psychotrieae alliance data set, showing the tribe Morindeae. The tree is drawn as a chronogram calibrated against The Geologic Time Scale (Gradstein et al., 2012) and credibility intervals of the node age estimates are indicated by blue bars. Well-supported nodes (posterior probability ≥ 0.95) are shown as blue bullets. Results from the Lagrange analyses are shown for nodes of interest. Color representation as in Fig. 3.

species of *Chassalia* from the rainforests of Asia and the WIOR were poorly supported as monophyletic (node 583; 7 Ma, HPD: 9–5 Ma). Its ancestor was inferred to have reached Madagascar from Africa between the Miocene 12 Ma (node 592; 10 Ma, HPD: 12–7 Ma) and the Pliocene 5 Ma (node 583). The phylogenetic resolution and support within this rainforest clade was poor and consequently, the biogeographic reconstruction was ambiguous. However, it is clear that the group reached the Comoros once in the Pleistocene 2–0 Ma (node 544; 1 Ma, HPD: 2–0 Ma), the Mascarenes once in the Miocene-Pliocene 6–3 Ma (node 540; 4 Ma, HPD: 6–3 Ma), and Madagascar at least two times (Fig. 5, Table 1, Appendix S3).

Finally, the tribe Psychotrieae reached the WIOR at least six times, i.e., twice from the Pacific and four times from Africa (Figs. 6-7, Table 1, Appendix S3). Sampled representatives of the group from the WIOR were resolved in four of the seven major

Psychotria lineages (Razafimandimbison et al., 2014): (1) the Pacific *Psychotria* lineage (represented by one species in WIOR); (2) the Afro-WIOR *Psychotria* lineage (eight species in WIOR); (3) the Afro-Asian-WIOR *Psychotria* lineage (about 50 species in WIOR); and (4) the WIOR *Psychotria* lineage (about 100 species in WIOR) (Figs. 6–7). Within the Pacific *Psychotria* clade the Seychelles species "*Amaracarpus pubescens*" was strongly supported as having dispersed from the Pacific to the Seychelles within the last 3 Ma (Fig. 6; Table 1, Appendix S3). Within the Afro-WIOR *Psychotria* lineage (= Leaf nodulate *Psychotria* lineage) the ancestral lineage of the Malagasy *Psychotria* group was estimated to have originated from Africa, diverged in the Miocene 13–8 Ma (node 517; 10 Ma, HPD: 13–8 Ma), and dispersed to Madagascar between the Miocene 10 Ma and the Pliocene 4 Ma (node 516; 7 Ma, HPD: 10–4 Ma). Within the Afro-Asian-WIOR *Psychotria* lineage the group of the Malagasy *Psychotria*
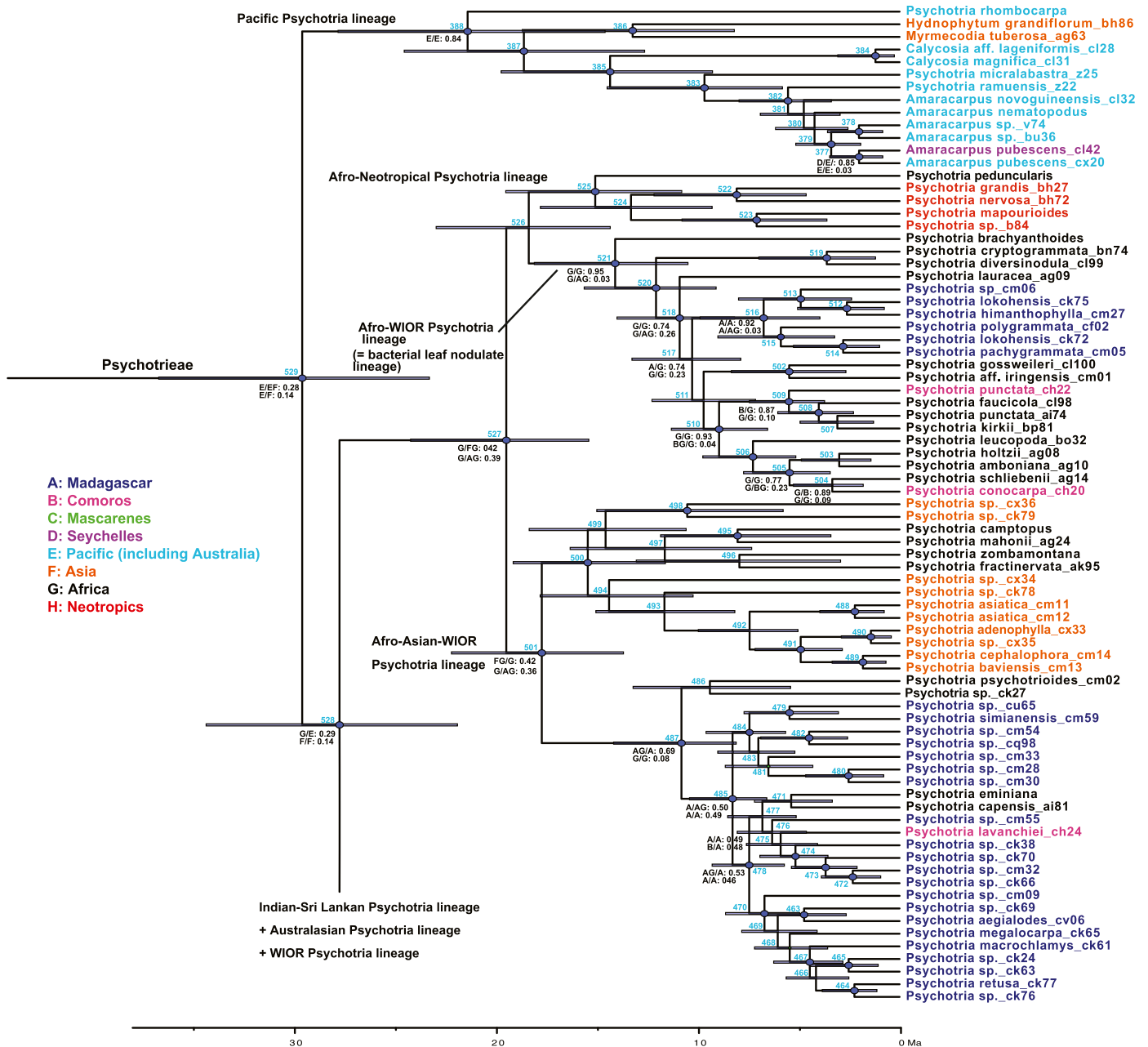
FIGURE 5 Part of the Maximum clade credibility (MCC) tree from the BEAST analyses of the combined Psychotrieae alliance data set, showing the tribe Palicoureeae. The tree is drawn as a chronogram calibrated against The Geologic Time Scale (Gradstein et al., 2012) and credibility intervals of the node age estimates are indicated by blue bars. Well-supported nodes (posterior probability ≥ 0.95) are shown as blue bullets. Results from the Lagrange analyses are shown for nodes of interest. Color representation as in Fig. 3.

with yellow flowers was inferred to have originated in Africa in the Miocene 14–8 Ma (node 487; 11 Ma, HPD: 14–8 Ma) and expanding its geographic range in Madagascar between the Miocene 14 and 7 Ma (node 485; 8 Ma, HPD: 10–7 Ma). Further, the ancestral lineage of the WIOR *Psychotria* lineage was estimated to have originated in the Pacific or Asia in the Oligocene 33–19 Ma (node 462; 25 Ma, HPD: 33–19 Ma), and expanded its range to Madagascar between the Oligocene 33 Ma and the Miocene 19 Ma (node 461; 23 Ma, HPD: 29–19 Ma) (Fig. 7, Table 1, Appendix S3). Independent out-of-Madagascar dispersal events to the neighboring

archipelagos followed (Fig. 2): (a) to the Comoros in the Miocene-Pliocene 8–4 Ma (node 475; 6 Ma, HPD: 8–4 Ma) (Fig. 6, Table 1, Appendix S3); (b) to the Comoros during the Miocene 11–4 Ma, or later (node 454; 7 Ma, HPD: 11–4 Ma) (Fig. 7, Tables 1, S1); (c) also to the Comoros from around the Miocene 11–6 Ma (node 435; 8 Ma, HPD: 11–6 Ma) (Fig. 7, Table 1, Appendix S3); (d) to the Seychelles between the Pliocene 3 Ma (Fig. 3: node 731) and the Pleistocene (Fig. 3: node 730); (e) also to the Seychelles between the Miocene 14 Ma (node 438; 10 Ma, HPD: 14–8 Ma) to the Pliocene 3 Ma (node 437; 6 Ma, HPD: 9–3 Ma) (Fig. 7,
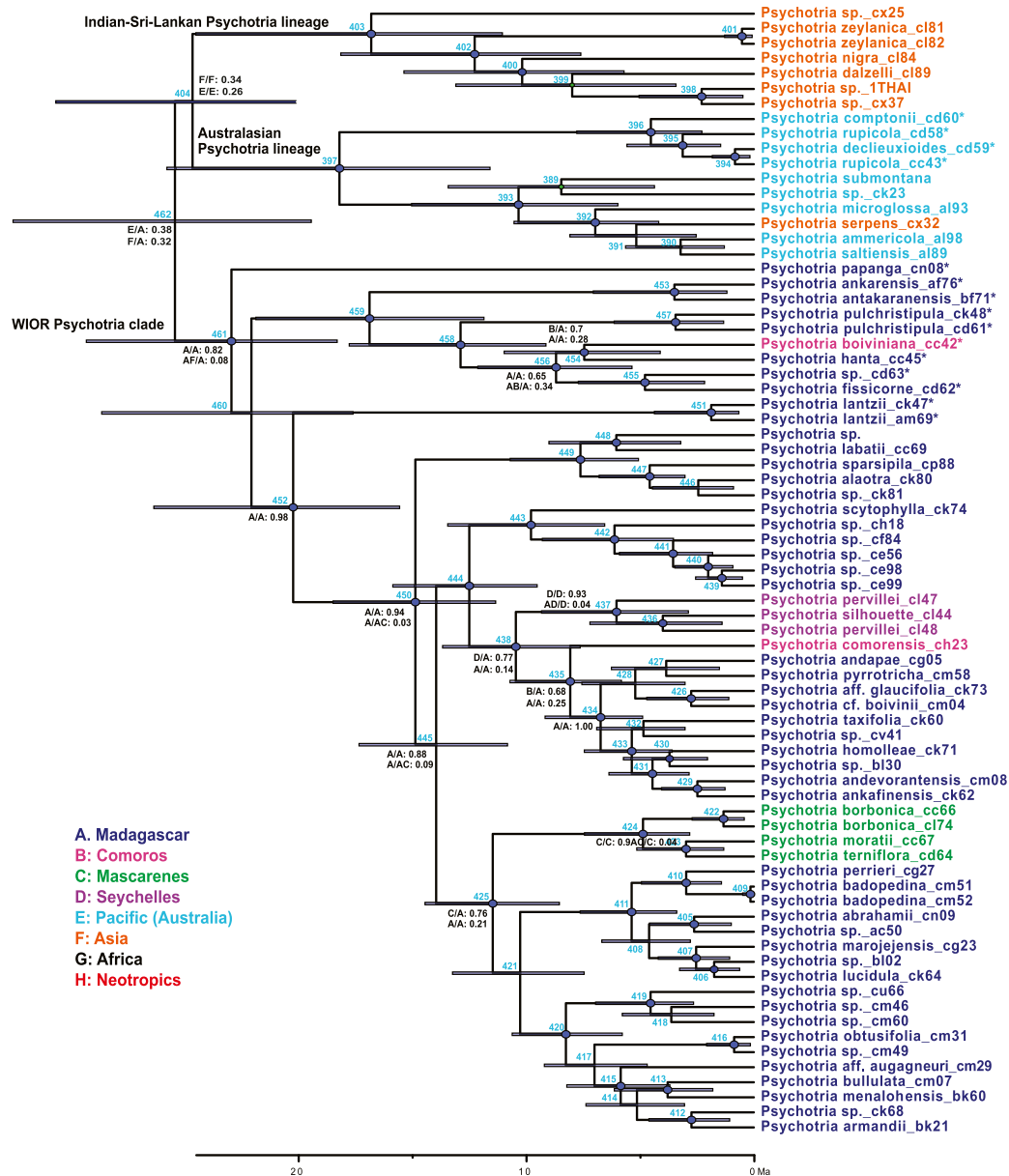
FIGURE 6 Part of the maximum clade credibility (MCC) tree from the BEAST analyses of the combined Psychotrieae alliance data set, showing the Pacific *Psychotria*, Afro-neotropical *Psychotria*, and Afro-Asian-WIOR *Psychotria* lineage of Psychotrieae. The tree is drawn as a chronogram calibrated against The Geologic Time Scale (Gradstein et al., 2012) and credibility intervals of the node age estimates are indicated by blue bars. Well-supported nodes (posterior probability ≥ 0.95) are shown as blue bullets. Color representation as in Fig. 3.

Table 1, Appendix S2); and (f) to the Mascarenes sometime between the Miocene 14 Ma (node 425; 11 Ma, HPD: 14–9) and the Pliocene 3 Ma (node 424; 5 Ma, HPD: 7–3 Ma) (Fig. 7, Table 1, Appendix S3).

***Phylogenetic relationships between and within the tribes of Psychotrieae alliance*—**The monophyly of the Psychotrieae alliance and the sister-group relationship between Schizocoleeae and the rest of the alliance are further supported by this study. The tribes Schizocoleeae and Craterispermeae, respectively, formed a basal grade

within the alliance (Fig. 1). The latter tribe was resolved as sister to a very large clade formed by the sister lineages the (Schradereae (Prismatomerideae (Gaertnereae (Mitchelleae-Morindeae)))) clade and the Palicoureeae-Psychotrieae clade. Prismatomerideae and Gaertnereae formed successive sister groups to the Michelleae-Morindeae clade, however the support for these relationships was low. Detailed results presenting the evolutionary relationships within the tribes Craterispermeae, Schradereae, Prismatomerideae, Gaertnereae, Palicoureeae, and Psychotrieae are given in Appendix S4.

FIGURE 7 Part of the maximum clade credibility (MCC) tree from the BEAST analyses of the combined Psychotrieae alliance data set, showing the Indian-Sri Lankan *Psychotria*, Australasian *Psychotria,* and WIOR *Psychotria* lineages of Psychotrieae. The tree is drawn as a chronogram calibrated against The Geologic Time Scale (Gradstein et al., 2012) and credibility intervals of the node age estimates are indicated by blue bars. Well-supported nodes (posterior probability ≥ 0.95) are shown as blue bullets. Color representation as in Fig. 3. An asterisk (*) denotes species of *Psychotria* with dry schizocarpous fruits.

indicated dispersal as the main biogeographic force responsible for the present biogeographic patterns of the extant members of the family. Our findings based on molecular dating and biogeographic analyses of the Psychotrieae alliance further support this conclusion.

Historical biogeography of the pantropical Psychotrieae alliance—The current study, which focuses on the colonization history of the Psychotrieae alliance in the WIOR, provides new insights into the biogeography of the whole alliance. Our results indicate that the alliance originated in Africa during the Upper Cretaceous-Paleocene (Fig. 1, Table 1, Appendix S3). The inferred range expansion to the neotropics began during the Paleocene-Eocene (nodes 742–741). This suggested presence of the alliance in the neotropics coincides with the existence of the North Atlantic Land Bridge (NALB). Therefore, it is possible that this was the route by which the Psychotrieae alliance reached the neotropics from Africa. The first two diverging lineages of the alliance, Schizocoleeae, and Craterispermeae, originated in Africa and diversified there. The old stem age of Craterispermeae (Paleocene-Eocene, Table 1) and the comparatively young crown age (Miocene) and those of the rest of the tribes (some time between Eocene and Miocene; Table 1, Appendix S3) suggest that Craterispemeae/*Craterispermum* may represent a relictual lineage (see e.g., Grandcolas et al., 2014) within the Psychotrieae alliance. The alliance continued to diversify in the neotropics, where Palicoureeae arose in the Eocene-Oligocene followed by Morindeae and Gaertnereae in the Oligocene-Miocene. The Psychotrieae alliance expanded its range to include Asia some time between the Eocene (node 723) and the Miocene-Pliocene (node 681). At least three major colonization events from the neotropics to Asia—represented by Schradereae, Prismatomerideae, and Mitchelleae—were inferred to have occurred during this period. Moreover, the alliance reached the Pacific from the neotropics some time between the Eocene (node 652) and the Miocene-Oligocene (node 529), giving rise to Psychotrieae (Fig. 1, Table 1, Appendix S3).

DISCUSSION

Wikström et al. (2015) estimated the crown age of the family Rubiaceae to be 87 Ma (HPD: 96–78 Ma), which is too young to be attributed to Gondwanan vicariance. The sequential break-up of Gondwana that resulted in the formation of the major continental landmasses (Africa, South America, Antarctica, the Australian continent, and Indigascar) occurred 180–120 Ma (e.g., Royer and Coffin, 1992; Reeves, 2014). Previous studies of the molecular dating and historical biogeography of various Rubiaceae lineages (e.g., Bremer and Eriksson, 2009; Manns et al., 2012; Wikström et al., 2010) have

Colonization history of the Psychotrieae alliance in Western Indian Ocean Region—The Psychotrieae alliance has reached the WIOR via at least 14 independent dispersals (Fig. 2), and is today represented by about 255 species. Nine of these inferred dispersals were from Africa (6 to Madagascar, 2 to the Comoros, and 1 from Africa to Madagascar or the Comoros), with five from Asia or the Pacific (2 to Madagascar from the Pacific, 1 to the Seychelles from the Pacific, 1 to the Mascarenes from Asia, and 1 to Madagascar from Asia or the Pacific). This biogeographic pattern is not surprising given the proximity of the WIOR to Africa. On the other hand, only about 30% of the 255 species seem to have had African origins, with 70% descending from Asian and Pacific ancestors. Interestingly, the earliest dispersal is that of the ancestor of the WIOR *Psychotria* lineage (Fig. 7: node 461) from the Pacific or Asia to Madagascar in the Miocene. This clade represents today about 39% of the species richness of the Psychotrieae alliance in the WIOR. Therefore, the current study reveals strong Asian and Pacific affinities for the Psychotrieae alliance in the WIOR despite this region's proximity to Africa; this biogeographic pattern is associated with the old age of the species-rich WIOR *Psychotria* lineage.

What factors may have promoted the repeated dispersals from Africa, Asia, and the Pacific toward the WIOR? Sea currents and winds in the WIOR blew west to east during the Tertiary (60–40 Ma) (Ali and Huber, 2010) and these eastward-blowing currents and winds may have facilitated dispersals of terrestrial plants and animals from Africa to Madagascar (see e.g., Townsend et al., 2010). However, for our study the earliest possible dispersal from Africa to the WIOR occurred later in the Miocene (= stem age of the Malagasy *Puffia*, Fig. 5: node 621). The majority of the colonization events from Africa to Madagascar took place in the Miocene-Pleistocene, when the sea currents and winds in the WIOR flowed in the reverse direction (since Miocene, Ali and Huber, 2010). This implies that sea currents and winds seem to have had a little influence on the dispersal of the WIOR Psychotrieae alliance, with the exception of the Malagasy *Gynochthodes* (see below).

Members of the alliance typically produce small, fleshy, drupaceous fruits, whose seeds are effectively dispersed mostly by avian frugivores (e.g., Herrera, 1989; Theim et al., 2014). While the colonization of Madagascar by plants with fleshy fruits has indeed been attributed to avian dispersal (e.g., Renner, 2004), only few frugivorous but nonmigrational bird species have been recorded in Madagascar (e.g., Hawkins and Goodman, 2003; and references therein). The seven species of forest-dwelling birds known to be frugivores in Madagascar have Asian and/or Australasian origins (see e.g., Warren et al., 2005). This biogeographic pattern seems to be consistent with the strong Asian and Pacific affinities of the extant species of the WIOR Psychotrieae alliance.

At least one back-colonization from Madagascar to Africa was inferred to have occurred in the Miocene-Pliocene (Fig. 6, node 471, i.e., the African clade comprising *P. capensis* (Eckl.) Vakte and *P. eminiana* (Kuntze) E.M.A.Petit) in the Afro-Asian-WIOR *Psychotria* lineage. This finding seems odd given the higher number (at least eight, Fig. 2) of the inferred dispersals from Africa to the WIOR (six, Fig. 2). Further, the high number out-of-Madagascar dispersals to the neighboring archipelagos is possibly associated with avian frugivores that are endemic to the WIOR (see also Warren et al., 2005). Irrespective of how these dispersals took place, this study further confirms that Madagascar is the main source of plants for its neighboring archipelagos (e.g., McArthur and Wilson, 1967; Wikström et al., 2010). In contrast, no dispersal event was estimated out of the three neighboring archipelagos to Madagascar (Comoros, Mascarenes, and Seychelles).

The Comoros, Mascarenes, and Seychelles have been postulated to serve as stepping-stones for recent dispersal events toward Madagascar (e.g., angiosperms, Schatz, 1996; chamaeleons, Raxworthy et al., 2002). Our analyses of the Psychotrieae alliance, consistent with those by Wikström et al. (2010) and Kainulainen et al. (2017), do not support this hypothesis, but instead reveal that Madagascar has served as a stepping-stone for subsequent colonization events to the rest of the WIOR (e.g., Craterispermeae, Fig. 3; the WIOR *Psychotria* lineage, Fig. 7). Except for within *Chassalia*, we find that the colonization events to the Comoros have yet to be followed by subsequent speciation. This pattern could partly be attributed to either the proximity of the Comoros to both Africa and Madagascar or to the young ages of the dispersal events to the Comoros from Africa. In contract, all colonization events to the Mascarenes and Seychelles have led to subsequent diversification.

As mentioned earlier, the Psychotrieae alliance is represented by five of its nine tribes (Craterispermeae, Gaertnereae, Morindeae, Palicoureeae, and Psychotrieae) in the WIOR. The monogeneric tribe Craterispermeae is an Afro-Malagasy-Seychelles group with about 31 species that has its center of species diversity in tropical Africa (20 species, Taedoumg and Hamon, 2013) and Madagascar (at least 10 species, De Block and Randriamboavonjy, 2015), with only a single species endemic to the Seychelles (Friedmann, 1994). The Malagasy *Craterispermum* are the result of a single colonization event from Africa that took place in the Pliocene-Pleistocene. The ancestor of the Seychelles genus *Craterispermum* had a Malagasy origin, and colonized the Seychelles during the Pleistocene (Fig. 3, Appendix S3).

The pantropical tribe Gaertnereae contains two genera and about 130 species, and is represented in the WIOR by the paleotropical genus *Gaertnera* whose center of species diversity is Madagascar (ca. 90 species; Malcomber and Taylor, 2009), with six species endemic to the Mascarenes (Verdcourt, 1989). No record of the genus is known from the Comoros or the Seychelles. The range expansion and ancestral area for *Gaertnera* could not be unambiguously resolved in this study. Our biogeographic analyses estimated two possible ancestral areas for the genus with almost the same probabilities: 0.28 for Asia and 0.25 for Mascarenes (Fig. 3: node 706). The Mascarenes [including the islands of Rodrigues (1.5 Ma), Mauritius (7.8 Ma), and Reunion (2.1 Ma)] are, however, much too young to represent an ancestral area for the genus and therefore we consider an Asian origin of *Gaertnera* more likely. We hypothesize that the early divergent clade represented by the Reunion species *G. vaginata* went extinct in Asia following a recent dispersal to Reunion Island. Examples of old lineages on the young islands of the Mascarenes have also been reported from other angiosperms (e.g., Dombeyoideae [Malvaceae], Le Péchon et al., 2010; *Monimia* [Monimiaceae], Renner et al., 2010; *Badula* [Primulaceae], Strijk et al., 2014). It could be argued that *G. vaginata* might also represent a relictual lineage, however more individuals need to be analyzed for a reappraisal. The ancestral area inference could be constrained by addition of time stratification to reflect the known ages of the volcanic islands of the Mascarenes or Comoros. However, our biogeographic analysis without enforcing such constraints show that the inferred age estimates of ancestral areas of all Comorian and Mascarene clades from which crown ages were available [e.g., ca. 3 Ma for the Mascarene *Gaertnera* lineage (Fig. 3: node 699; Appendix S3); ca. 4 Ma for the Mascarene *Chassalia* (Fig. 5: node 540;

TABLE 1. Bootstrap frequencies from the Maximum Likelihood analysis in RaxML, bayesian posterior probabilities (BPP) from the non–clock analysis in MrBayes, bayesian posterior probabilities, mean ages, 95% highest posterior density intervals (HPD) of the age estimates from the relaxed–clock analysis in BEAST, and probabilities of the two most likely biogeographic scenarios from the DEC analyses in Lagrange for the alliance, its tribes, and some lineages discussed in the text. Probabilities of the biogeographic scenarios are conditional on the node occurring and were calculated as an average across 100 trees randomly drawn from the posterior distribution of trees of the BEAST analysis. Node numbers correspond to those in Figs. 1 and 3–7. Geographic area codes – A: Madagascar; B: Comoros; C: Mascarenes; D: Seychelles; E: Pacific (Australia included); F: Asia; G: Tropical Africa; and H: neotropics.

Lineages	Bootstrap % (RAxML)	BPP (MrBayes)	BPP (BEAST)	Stem age (Ma)	Stem age 95% HPD (Ma)	Crown age (Ma)	Crown age 95% HPD (Ma)	DEC 1 area	DEC 1 prob.	DEC 2 area	DEC 2 prob.
Psychotrieae alliance (Fig. 1: node 742)	100	1.00	1.00	62	68–56	55	61–49	G|G	0.60	G|GH	0.24
Craterispermeae (Fig. 3: node 740)	100	1.00	1.00	52	58–47	7	12–5	G|G	0.57	G|AG	0.35
Malagasy *Craterispermum* Clade (node 736)	86	1.00	1.00	4	5–2	2	3–1	A|A	0.87	A|AD	0.13
Seychelles *Craterispermum* clade (node 730)	99	1.00	1.00	2	3–1	1	1–0	D|D	0.99	–	–
Gaertnereae (Fig. 3: node 706)	100	1.00	1.00	43	47–40	25	33–17	H|F	0.28	H|C	0.25
Reunion *Gaertnera vaginata* (stem; node 705)	100	1.00	1.00	25	33–17			C|F	0.30	C|G	0.17
Core Mascarene *Gaertnera* clade (node 699)	—	1.00	1.00	4	7–3	3	5–2	C|C	0.93	CF|C	0.02
Malagasy *Gaertnera* clade (node 690)	96	1.00	1.00	5	7–3	4	5–3	A|A	0.98	–	–
Mitchelleae (Fig. 4: node 681)	100	1.00	1.00	39	42–36	10	18–5	F|FH	0.78	F|F	0.21
Morindeae (Fig. 4: node 678)	100	1.00	1.00	39	42–36	26	34–21	H|H	0.32	H|EH	0.21
Black–bluish-fruited *Gynochthodes* clade (node 660)	71	1.00	1.00	13	16–9	12	15–8	E|E	0.87	AE|E	0.09
Malagasy *Gynochthodes retusa* (stem; node 656)	60	0.93	0.96	12	15–8			E|A	0.72	E|E	0.23
Orange–fruited *Gynochthodes* clade (node 671)	69	1.00	1.00	13	16–9	11	15–8	E|E	0.99	–	–
Malagasy *Gynochthodes* clade (node 665)	100	1.00	1.00	4	6–3	2	3–1	A|A	0.95	–	–
Palicoureeae (Fig. 5: node 651)	97	1.00	1.00	44	51–37	38	45–32	H|H	0.68	H|GH	0.24
Malagasy *Puffia* (node 620)	100	1.00	1.00	16	23–9	0	1–0	A|A	1.00	–	–
Malagasy–Comoros *Geophila* clade (node 605)	100	1.00	1.00	6	9–4	1	1–0	A|B	1.00	–	–
Rainforest *Chassalia* clade (node 583)	—	0.73	0.94	10	12–7	7	9–5	A|A	0.66	AF|A	0.20
Mascarene rainforest *Chassalia* clade (node 540)	63	1.00	1.00	5	6–3	4	6–3	C|C	0.93	AC|C	0.02
Comoros rainforest *Chassalia* clade (node 544)	100	1.00	1.00	4	5–3	1	2–0	B|B	0.94	B|AB	0.01
Malagasy dry–forest *Chassalia* clade (node 586)	100	1.00	1.00	5	7–3	2	4–1	A|A	0.99	–	–
Primatomerideae (Fig. 4: node 714)	100	1.00	1.00	45	50	20	29–12	F|F	0.88	FH|F	0.06
Psychotrieae (Fig. 6: node 529)	100	1.00	1.00	44	51–37	30	37–23	E|EF	0.28	E|F	0.14
Seychelles *Amaracarpus pubescens* (stem; node 377)	94	1.00	1.00	3	5–2			D|E	0.94	E|E	0.03
Malagasy leaf-nodulate *Psychotria* clade (node 516)	60	1.00	1.00	10	13–8	7	10–4	A|A	0.92	A|AG	0.03
Comoros *Psychotria punctuata* (stem; node 509)	100	1.00	1.00	9	11–7			B|G	0.87	G|G	0.10
Comoros *Psychotria conocarpa* (stem; node 504)	56	1.00	0.19	5	8–3	3	5–2	G|B	0.89	G|G	0.09
Malagasy *Psychotria* clade yellow flowers (node 485)	89	1.00	1.00	11	14–8	8	10–7	A|AG	0.50	A|A	0.49
Comoros *Psychotria lavancheri* (stem; node 475)	—	0.46	0.45	6	8–5			A|A	0.49	B|A	0.48
WIOR *Psychotria* clade (Fig. 6: node 461)	99	1.00	1.00	25	33–19	23	29–19	A|A	0.82	AF|A	0.08
Comoros *Psychotria boiviana* (stem; node 454)	76	0.97	0.97	9	12–5			B|A	0.70	A|A	0.26
Seychelles *Psychotria* clade (node 437)	75	1.00	1.00	10	14–8	6	9–3	D|D	0.93	AD|D	0.04
Psychotria comorensis (stem; node 435)	100	1.00	1.00	10	14–8			B|A	0.68	A|A	0.25
Mascarene *Psychotria* clade (node 424)	100	1.00	1.00	11	14–9	5	7–3	C|C	0.92	AC|C	0.04
Schradereae (Fig. 4: node 722)	100	1.00	1.00	47	53–42	23	33–14	FH|F	0.68	F|F	0.29

Appendix S3); ca. 5 Ma for the Mascarene *Psychotria* clade (Fig. 7: node 424; Appendix S3) ca. 1 Ma for the Comoros *Chassalia* (Fig. 5: node 544; Appendix S3)] are younger than their respective older islands [7.8 Ma for Mauritius (Mascarenes), 7.7 Ma for Mayotte, 5 Ma for Moheli, and 3.9 Ma for Anjouan (Comoros)]. Therefore, it was unnecessary to impose further constraint in the analysis. *Gaertnera* expanded its range from Asia to Madagascar between the Miocene-Pliocene and Pliocene (between nodes 694 and 690) and from Asia to the Mascarenes between the Miocene-Pliocene and the Pliocene-Pleistocene (between nodes 701 and 699), and subsequently diversified there (Fig. 3). The existence of a chain of islands stretching between the Mascarenes, the granitic Seychelles, Maldives, and India during episodes of drastically low sea levels between 1.3 Ma and 100,000 yr have been postulated (Miller et al., 2005). These islands, now submerged, could have facilitated the colonization of the WIOR from Asia via island stepping-stones (e.g., Warren et al., 2010). These low sea periods are, however, much younger than the arrival of *Gaertnera* in the Mascarenes in the Pliocene-Pleistocene (Fig. 3). Therefore, dispersal by birds feeding on the fruits of *Gaertnera* may be the most likely biogeographic factor responsible for the WIOR *Gaertnera* colonization.

The inferred crown age of *Gaertnera* (node 705: 20–9 Ma) (Fig. 3) in this study is notably much higher than that by Malcomber (2002), 5.21 Ma. This difference is partly attributed to the inclusion of the Reunion species *G. vaginata* in our analyses and its absence in Malcomber (2002). On the other hand, our inferred crown age of *Gaertnera* (excluding *G. vaginata*), 8.25 Ma, is still higher than Malcomber (2002)'s estimate. This is possibly due to differences in the methods used for inferring lineage ages [the use of molecular clock (Li and Graur, 1991) as the only means of inferring lineage ages for *Gaertnera* in Malcomber (2002) as opposed to the use of the uncorrelated lognormal clock model used for our study]. Further, Malcomber (2002) estimated absolute divergence times of the genus using three calibration dates. Firstly, the origin of the Mauritian lineage was assumed to have coincided with Mauritius emerging from the Indian Ocean 8 Ma (MacDougal and Chamalaun, 1969). Secondly, *Pagamea* was assumed to have diverged from *Gaertnera* as South America separated from Africa 95 Ma (Parrish, 1993). Thirdly, the divergence of *Pagamea* from *Gaertnera* was assumed to coincide with the first occurrence of Rubiaceae fossils during the Eocene 54 Ma (Roth and Dilcher, 1979). There are critiques against the use of island age for calibration, because island endemics can be much older than their islands (e.g., Heads, 2011; Le Péchon et al., 2015). Further, the second and third calibrations of Malcomber (2002) are erroneous, because the age of the break-up of South America and Africa (95 Ma) is even older than the age estimate of the family Rubiaceae (87 Ma, Wikström et al., 2015) and that the appearance of Rubiaceae fossils (54 Ma) is much older than the estimated crown age of the tribe Gaertnereae (Bremer and Eriksson, 2009; this study).

Morindeae is a pantropical tribe of about 160 species (in six genera) (Razafimandimbison et al., 2009) that is represented by the genera *Gynochthodes* and *Morinda* L. in the WIOR. *Gynochthodes* is distributed paleotropically with about 100 species, but has its center of species diversity in Australasia and tropical Asia (Razafimandimbison and Bremer, 2011). It occurs in Madagascar with four to five endemic but undescribed species, but is absent in mainland Africa. *Morinda citrifolia* L. is the sole species of *Morinda* in the region. The Malagasy *Gynochthodes* are the result of two independent dispersals from the Pacific to Madagascar: the first is the orange-fruited

Gynochthodes lineage dispersed during Miocene-Pleistocene (Fig. 4, between nodes 665-666); and the second is the clade represented by *G. retusa* some time in the last 11.6 Ma (node 656). While the *Gynochthodes* lineage with orange fruits has given rise to at least four or five extant species (all undescribed) in Madagascar (Razafimandimbison et al., unpublished data; Fig. 4), the black-bluish-fruited lineage has not diversified to the same extent. The pantropical *Morinda citrifolia* is widely distributed throughout the tropics (including the WIOR) and this large distribution is attributed partly to an adaptation of the seeds to oceanic dispersal (Razafimandimbison et al., 2010). Seeds of *M. citrifolia* remain viable after floating in seawater for several months (see e.g., Morton, 1992). The pyrene (i.e., seed and the associated endocarp) of this species contains a large air-filled cavity on its ventral surface, with the seed enclosed in its dorsal cavity (Razafimandimbison et al., 2010). The same type of pyrene has also been reported in other Morindeae species such as *Morinda royoc* L. and *Appunia guatemalensis* Donn.Sm. (Hayden and Dwyer, 1969) and at least two Malagasy species of *Gynochthodes* (Razafimandimbison, unpublished data). Therefore, it is possible that seeds of the ancestors of the two Malagasy lineages of *Gynochthodes* had the ability to float, allowing them to be transported by oceanic drifting. Sea currents and winds blowing across the Indian Ocean from Australia and Southeast Asia toward Madagascar during the Indian summer season (see e.g., Cheke and Hume, 2008) may have facilitated this route.

The pantropical tribe Palicoureeae comprises at least 1500 species classified in eight genera (Razafimandimbison et al., 2014), and is represented by the paleotropical genus *Chassalia*, the pantropical genus *Geophila* D.Don, and the Malagasy *Puffia* in the WIOR. The former genus, *Chassalia,* has its center of species diversity in tropical Africa with about 82 species, and is represented by at least 30 species in Madagascar, with six species in the Mascarenes and two or three in the Comoros (Govaerts et al., 2013). *Chassalia* is absent in the Seychelles (Friedmann, 1994). *Geophila* is mainly distributed in Africa and the neotropics (each with nine species), and is represented by the paleotropical *G. uniflora* Hiern in Madagascar and the Comoros (Razafimandimbison et al., 2014). The *Hymenocoleus-Puffia* clade (Fig. 5: node 621) originated from Africa. *Puffia* had diverged from *Hymenocoleus* already in the Miocene (node 621, Appendix S3), and subsequently dispersed to Madagascar. However, the crown age of *Puffia* is much younger, (Pleiostene, node 620, Fig. 5), indicating that the arrival to Madagascar may be quite recent. This monospecific genus is restricted to the lowland rainforests and littoral forests of eastern and southeastern Madagascar (from the Masoala Peninsula and Nosy Mangabe Island to the Manombo National Park), while *Hymenocoleus* is distributed in tropical Africa. This suggests that *Puffia* may well represent a relictual lineage. The sampled Malagasy representatives of *Chassalia sensu* Razafimandimbison (Fig. 5: node 595) appear to be derived from two distantly related lineages, which are the results of two independent dispersal events. A similar pattern has also been reported from the rubiaceous genus *Ixora* L. (Mouly et al., 2009; Tosh et al., 2013). The two *Chassalia* clades appear to be ecologically and geographically distinct, the first comprised three species restricted to the dry forests of Madagascar and the second formed by about 38 species from the rainforests of the Comoros, the Mascarenes, and central and eastern Madagascar. The rainforest *Chassalia* lineage is estimated to have had an African origin, which reached Madagascar between the Miocene 12 Ma and the Pliocene 5 Ma. However, we are unable to infer the biogeographic

reconstruction of the ancestral lineages of the rainforest species of *Chassalia* from the Comoros and Mascarenes.

Finally, the pantropical, monogeneric tribe Psychotrieae contains about 1800 species, and is the most diverse angiosperm group in the WIOR with 157 species and the only one found throughout the region, albeit with the majority of its species restricted to Madagascar (Razafimandimbison et al., 2014). The tribe reached the WIOR at least six times via dispersals: one from the Pacific, one from Asia, and four from Africa. The ancestor of the WIOR *Psychotria* clade dispersed to Madagascar from the Pacific or Asia (Fig. 7: node 462) in the Oligocene-Miocene, and diversified there. This lineage subsequently reached the Comoros, the Seychelles, and the Mascarenes via Madagascar, all in the Miocene-Pliocene (Fig. 7). This clade contains about 100 species, of which eight species (seven endemic to Madagascar and one to the Comoros) produce dry, schizocarpous fruits and constitute a basal grade within the group (see also Razafimandimbison et al., 2014). Each schizocarp is formed by two mericarps, which disperse separately. A character state reconstruction of the fruit type of *Psychotria* (fleshy drupes vs. dry dehiscent schizocarps) inferred the WIOR lineage to have had ancestors bearing schizocarps (Razafimandimbison et al., 2014). Bremekamp (1958) postulated that the mericarps of these plants are dispersed by simple gravity and Barrabé et al. (2014) postulated that they have lost their ability to disperse over long-distances after settling in Madagascar (and New Caledonia). However, the phylogenetic position of the Comoros species *Psychotria boiviana* (Bremek.) Razafim. & B.Bremer deeply nested within a Malagasy *Psychotria* clade (Fig. 7: node 459) with schizocarpous fruits indicates this is not the case. It is plausible that strong winds (e.g., tropical cyclones) could effectively disperse the mericarps of these *Psychotria* species over long-distances (see also Lacey, 1980). Winds blowing across the Indian Ocean from Australia and Southeast Asia toward Madagascar during the Indian summer season (see e.g., Cheke and Hume, 2008) may have facilitated this route.

The Malagasy *Psychotria* clade (Fig. 6: node 516) within the bacterial-leaf-nodulate lineage (Fig. 6: node 521) originated from Africa, and expanded its range to Madagascar between the Miocene (node 517) and the Miocene-Pliocene (node 516), where this group has since given rise to at least eight endemic species (Bremekamp, 1960). Bacterial endophytes of leaf-nodulated plants are known to be vertically transmitted to seeds (Lersten and Horner, 1976), and therefore are effectively dispersed along with the seeds of their plant hosts.

Intratribal relationships in the Psychotrieae alliance—As previously stated, the phylogenetic positions of Schizocoleeae, the sister clades Mitchelleae-Morindeae, and Palicoureeae-Psychotrieae have been supported in previous studies (e.g., Razafimandimbison et al., 2008; Wikström et al., 2015), and are further supported by our study. The phylogenetic relationships within Mitchelleae, Morindeae, and Palicoureeae have recently been addressed (Razafimandimbison et al., 2009, 2014; Huang et al., 2013), and will therefore not be further discussed here. The evolutionary relationships within each of the tribes Craterispermeae, Schradereae, Prismatomerideae, Gaertnereae, and Psychotrieae are discussed in detail in Appendix S5.

CONCLUSIONS AND PERSPECTIVES

The Psychotrieae alliance originated from Africa in the Upper Cretaceous-Paleocene. The overall pattern of the colonization of the alliance across its geographic ranges indicates that the group has dispersed efficiently across ocean barriers. This study reinforces the fundamental role of dispersal in shaping the WIOR biodiversity and as the critical initiating step in the generation of endemic biodiversity on its islands. The alliance has reached the WIOR at least 14 times probably via dispersals from Africa, Asia, and the Pacific mostly during the last 10 My, and with rare but at least one back-colonization to Africa. The earliest colonization event was inferred from the Pacific or Asia to Madagascar in the Oligocene-Miocene. The majority of dispersals to the WIOR were from Africa, but about 70% of the extant WIOR species of the alliance had Asian and Pacific origins. These latter biogeographic patterns are consistent with the Asian and Australasian origins of the extant Malagasy frugivorous birds. Several instances of out-of-Madagascar dispersals to the neighboring archipelagos were inferred, possibly associated with avian frugivores that are endemic to the WIOR. In contrast, no dispersal was inferred out of these archipelagos. Madagascar is further confirmed as the main source area, and has served as a stepping-stone for subsequent recent colonization events to the rest of the region. *Gynochthodes* with multiple fruits reached Madagascar twice from the Pacific possibly via oceanic drifting. *Psychotria* with dry fruits (schizocarps) colonized Madagascar twice before dispersing to the Comoros from Madagascar possibly via wind dispersal. Two ecologically and geographically distinct lineages of *Chassalia* in the WIOR, both with African origins, were identified, the first restricted to the Malagasy deciduous dry forests and the second rainforest *Chassalia* lineage confined to the Comoros, Mascarenes, and Madagascar, closely related to the Asia *Chassalia*. The Afro-Malagasy-Seychelles *Craterispermum* and the Malagasy *Puffia* may represent relictual lineages.

More data and more species of *Chassalia* (Palicoureeae) and *Gaertnera* (Gaertnereae) from Africa are needed to resolve the phylogenetic relationships and to better understand ancestral geographic areas within these genera. Matzke (2014) extended the standard DEC model of range evolution used in the analyses presented here, to also include founder event speciation (DEC+J), a process that appears to be particularly important in island clades. The extension is included in his R package BioGeoBEARS (Matzke, 2013). Equally important is that the package implements many models of range evolution in a common likelihood framework and this allow for standard statistical model selection procedures to be used for choosing the best model. Our current analytical setup for analyzing large number of trees, such as those from a posterior distribution, only allowed us to use the standard DEC model as implemented in Lagrange. Transferring this setup to BioGeoBEARS would allow us to both include a founder event speciation parameter in the analyses and to evaluate different models of range evolution against each other. Such a transfer should be a priority in future analyses.

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