The Panama microplate, island studies and relictual species of *Melipona* *(Melikerria)* (Hymenoptera: Apidae: Meliponini)

David W. Roubik

Smithsonian Tropical Research Institute
Ancon, Balboa, Republic of Panama

roubikd@si.edu

João Maria Franco de Camargo

University of São Paulo
Ribeirão Preto, SP, Brazil

(deceased)
Abstract. The endemic stingless honey-making bee *Melipona* (*Melikerria*) *insularis* sp. nov. on Coiba and Rancheria Islands in Pacific Panama is described, along with the proposed sister species, *M. ambigua* sp. nov. from NE Colombia. The Coiba Island group and Panama mainland were surveyed, yielding one meliponine endemic (*M. insularis* sp. nov.) and six meliponine genera and species. The poor Coiba fauna of amphibians and birds corresponds to the poor social bee fauna and suggests habitat barriers generally precluded re-colonization from the mainland during glacial periods. Many animals became extinct, yet some remain as relicts. *M. insularis* sp. nov. was isolated on accreted terranes of Coiba rain forest in the Panama microplate. Morphology suggests *M. insularis* sp. nov. is not a direct descendant of the San Blas-E. Panama endemic *Melikerria*, *M. triplaridis*. A phylogenetic hypothesis corroborates disjunct distributions. Rain forest endemics such as *Peltogyne purpurea* (Fabaceae) and *Ptilotrigona occidentalis* (Apidae, Meliponini) also occur as relictual, disjunct populations in Central and South America. These may have been isolated before accelerated biotic exchange began 2.4 Ma. Our work supports the geological findings of both a volcanic arc and the San Blas massif providing a substantial bridge for *Melikerria* from Colombia and Panama in Eocene to Miocene times. We suggest there have been taxon cycles permitting re-colonization during glaciations, whereby colonies of *M. insularis* sp. nov. were able to re-colonize Rancheria, a 250 ha island, 2 km from Coiba. However, rafting colonies nesting in trees, carried on vegetation mats, may have produced founding populations of *Melipona* in Central America and on oceanic islands such as Coiba.
**Introduction**

Because their species have strong dispersal limitations (Rasmussen & Cameron, 2010), meliponine bees are of particular interest for the study of relationships between islands and continents. Coiba Island is largely volcanic, Cretaceous, and after an origin in the Galapagos hotspot was pushed by the Cocos plate. The Eocene Coiba Island emerged far from present day South America but now lies 23 km from Panamanian mainland (Castroviejo, 1997). Here we describe two new species of Neotropical honey making bee, genus *Melipona*, one from Coiba Island and nearby Rancheria Island, and one from northern Colombia. Our studies on the *Melipona compressipes* (Fabricius) group (subgenus *Melikerria*) provide a comparative element of the biogeographically complex Caribbean region (Liebherr, 1988; Ricklefs & Bermingham, 2001; Morrone, 2006), including one of the oldest extant Neotropical stingless bee genera (Rasmussen & Cameron, 2010), also used by Maya people since antiquity for beekeeping and honey (Villanueva et al., 2005). Workers of subgenus *Melikerria* are the size of the western honey bee *Apis mellifera* but maintain much smaller colonies; all but one species build nests only in tree hollows. The distribution of *Melikerria* (26°S to 22°N) (Camargo & Pedro, 2007; Ramírez et al., 2010) including both the Amazon rim and basin also implies antiquity that should be useful in reconstructions of biotic evolution and diversification in tropical America.

When organizing the taxonomy of *Melipona*, Moure (1992) created subgenus *Melikerria* based on two apomorphies, following Schwarz (1932: 265): “The inner half
of the apex of the mandibles subdivided by strong emargination into two distinct contiguous teeth [the median tooth with sinuosity], and “The antero-lateral angles of the mesonotum with a rust-red patch of hair …”. Besides these features, and corroborating the monophyly of the subgenus, the males possess scythe-shaped genitalic gonostyles (JMC, pers. obs.). No new Melikerria has been described since Schwarz (1932) and all eight species of the subgenus known until now (Camargo & Pedro, 2007) are continental. Their distribution patterns are well defined and based on substantial field work: *Melipona beecheii* Bennett, 1831 from Mexico to Costa Rica (records for Cuba and Jamaica derive, probably, from introductions by indigenous peoples from continental areas [see Michener, 1982: 37] and a Panama record is likely erroneous [see Schwarz, 1934: 8 and Michener, 1954: 164], DWR, pers. obs.); *M. triplaridis* Cockerell, 1925 in eastern Panama; *M. salti* Schwarz, 1932, northern Colombia – Magdalena; *M. compressipes* (Fabricius, 1804), from Venezuela, Guyana, Suriname, French Guiana, Brazil – north of the Amazon and Negro Rivers, and Amazonian Colombia. Such a distribution is classified ‘northern Amazon’ (‘NAm’, of stingless bee distribution, Camargo & Pedro, 2003); *M. interrupta* Latreille, 1811, from Guyana, Suriname, French Guiana and Brazil, north of the Amazon River, east of the Branco River to the state of Amapá (overlapping with the eastern distribution of *M. compressipes*); *M. grandis* Guérin, 1834, southwest Amazonia (the ‘SWAm’ meliponine biogeographic component, Camargo & Pedro, 2003); *M. fasciculata* Smith, 1854, eastern Amazonia – Pará, Maranhão, Piauí, Tocantins, Mato Grosso; *M. quinquefasciata* Lepeletier, 1836, from northeastern Brazil to Argentina – Missiones, Paraguay and Bolivia (including an undescribed species, see Camargo & Pedro, 2007: 343). Of interest is the absence of this
subgenus along the Pacific mainland forest, from Ecuador to the northern Chocó, Colombia. We discuss the evidence, below, that this lineage colonized Coiba Island in the Miocene, went extinct on the mainland, but founded an ancestral population of the only *Melikerria* in Central America and Mexico, the ‘Maya honey bee’, *M. beecheii*.

**Materials and methods**

Coiba Island, in the Gulf of Chiriquí, is 23 km minimum distance from the Pacific coast of Panama, near Punta Gorda and the town of Pixvae (DWR, *pers. obs.*). Its area is 50,314 ha, between 7°10’4” - 7°53’27”N and 81°32’25” –81°56’15”W, with a maximum elevation 416 m. Annual rainfall is over 3000 mm and Coiba Island flora has major Darién affinities (*Velayos et al.*, 1997). Ranchería island, 21.7 km from the closest point to the mainland, with an area of 250 ha and 130 m at its highest point, is the second island where one of our two new bee species was found. Coiba is the largest island, and a group of eight lesser islands and 30 islets surround it. It was emergent (above sea level) during the late Eocene (*Cardiel et al.*, 1997). Coiba Island and the area we refer to as the ‘Panama microplate’ was transported with the Cocos Plate to the subduction zone of Panama and Costa Rica, and then incorporated in the continental border (*Meschede & Barckhausen, 2000; Lissinna et al., 2001; Trenkamp et al., 2002; Coates et al., 2004; Kerr & Tarney, 2005). Coiba Island originated as one of the exotic terranes, like the Azuero and Soná highlands flanking it on the mainland, and Osa and Nicoya Peninsulas in Costa Rica. During multiple glaciations that began over 3.0 Ma in the Pliocene, terrestrial connections to Coiba Island, lasting up to approximately 90,000 years, were
possible when sea levels lowered by as much as 130 m. The sea floor is presently at a maximum 100 m depth between Coiba and the mainland, both to the north and to the east.

Field collections were made for this study between 1979 and 2011, when the first author surveyed Coiba Island and nearby mainland biota 12 times and spent 50 field days there (Appendix S2). Playa Rosario, Playa Hermosa, Playa Venado, Playa Damas, La Central, La Salina and two points on western Coiba near its highest hills were used to assess the island forest biota, as we made trails inland to its hilly forest. Rancheria Island was studied intensively (nine visits, 18 days), Montuosa, Jicaron, Cébaco, the Outer Channel, and Santa Catalina Islands were surveyed for one or two days. Only the island of Cébaco is largely degraded or secondary forest. Collections on flowering plants, or using bee attractants (honey-water sprayed on vegetation; volatiles effective for both Meliponini and Euglossini, i.e. vanillin, methyl salicylate, skatole) were made during the morning and early afternoon. On the mainland, north and east of Coiba Island, the principal forest remnant resembling Coiba and Rancheria Islands was located in a 5 km² area at Punta Gorda, also near mainland closest to Coiba and Rancheria Islands. The peninsular Veraguas Province region has no other extensive lowland forest of closed canopy and is degraded. Two additional forest patches were surveyed between Pixvae and Soná. Collections in Colombia made by collaborators and beekeepers provided additional material (DWR collection, STRI).

Taxonomic methods include the terminology and measurements of Camargo & Pedro (2009). Abdominal terga and sterna are indicated as TII–TVIII and SII–SVIII respectively; antennal flagellomeres are fl.1, fl.2, etc., from the base to apex of the
flagellum; the first abdominal tergum, incorporated in the thorax, is referred to as the propodeum, and the ‘propodeal disc’ as the metapostnotum; legs and respective podomeres are followed by Roman numerals according to the segment, e.g. tibia III refers to the hind tibia. Alveolocellar distance is measured from the alveolus to the anterior margin of the lateral ocellus of the same side; interocellar distance between the lateral ocelli; clypeocellar distance from the apex of the clypeus to the anterior margin of the median ocellus; length of the head from the apex of the clypeus to the vertex aligned on the same focal distance; length of the forewing from the apex of the costal sclerite to the tip of the wing, except when indicated. Measurements were taken with an ocular reticule and are given in millimeters. Photos were taken with digital Pentax Camera K10D coupled onto with stereomicroscopy LEICA MZ APO.

We used limited molecular data provided by the Barcode of Life study (University of Guelph, Ontario, Canada), because the method supplies information concerning cryptic species, relative species ages and, because we lacked male specimens of the new species, an added means of testing species hypotheses. The mtDNA gene fragment CO1-5P was analyzed from specimens collected by the first author. These were combined in a distance matrix with sequences deposited in GenBank (Ramírez et al., 2010), after alignment using the ‘Clustal X algorithm’, for CO1 in Melikerria. Sequence data were used to produce values for a similarity matrix, using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Results
Melipona (Melikerria) insularis sp. nov.

(Figs. 1a,e,i, 2b; Appendix S1)

Trigona interrupta; Cheesman, 1929: 149

Melipona compressipes (distinct form of); Camargo, Moure & Roubik, 1988: 156

Diagnosis. Pilosity on head and thorax entirely whitish, with exception of yellow-ferruginous tufts on the anterior corners of mesoscutum (Fig. 1i); paraocular stripes expanded below (Fig. 1a), 1.3x scape diameter; yellowish bands on distal margins of metasoma TII-V, complete, with slight median indentation on TII and TIII and brief interruption on TIV and TV, width greater than or equal to scape diameter (Fig. 1e), slighter narrower and more broadly interrupted on TVI.

Holotype, worker.

Dimensions. Total length approximately 11.0 mm; forewing length, 8.32 mm (including tegula, 9.78 mm); maximum head width, 4.22 mm; TIII width, 4.18 mm.

Integument color. Predominantly black; anterior surface of scape, yellowish; posterior surface of scape, pedicel and posterior surface of flagellum dark ferruginous; anterior flagellar surface ferruginous. Mandible, except apex and base, and all tarsomeres, fusco-ferruginous; basitarsus III and distal part of tibia dark ferruginous. Wing membranes, ferrugineous. Tegula and wing veins, dark ferruginous, like tarsomeres. Head, pronotum and metasomal terga, with distinct yellowish markings; on face, paraocular stripe occupying inferior 2/3 of eye, pointed above and gradually widened below –lower 1/3 ca. 1.3x wider than diameter of scape; clypeus with complete median-longitudinal band (reaching epistomal suture, above, to distal margin of clypeus; some paratypes with band
faint superiorly), little wider than half the scape diameter, with subtriangular mark on each side, on the edges, largely separated from the median band (in one paratype barely a narrow marginal stripe, greatly muted, touching the median band at the edge markings); supraclypeal mark an isosceles triangle with very widened base. Labrum with yellowish band, diffuse edges along its base. A yellowish stripe, very narrow, on each side of pronotum. Bands on distal margin of TII-V, wide as scape diameter, complete on TII-III, having median indentation, and briefly interrupted on TIV-V; on TVI the band slightly narrower, reduced at edges and having slightly wider median interruption.

**Pilosity.** Whitish, pale silver, with ferruginous tufts on anterior corners of mesoscutum, ferruginous hairs on tarsomeres and internal face of basitarsi, and ventro-distal trochanter II tuft; black hairs on metasomal apex. Metasomal terga with very narrow fimbriae of small whitish hairs; TIII with simple hairs ca. 0.10 mm length; TIV-V with simple and branched hairs, 0.12 and 0.15 mm length, respectively; TVI without fimbria. TIII disc area with simple hairs, semi-erect, to 0.20 mm length; TIV-V having decumbent small hairs, ca. 0.10 mm length; TVI-VII with erect black hairs, interspersed with whitish plumose hairs, increasing in length and density, to 0.53 length on TVII margin, curving inward and mostly branched. SVII with black setae, longer at sides, with fulvous tips, narrower and denser on disc.

**Integument.** Finely dull- reticulate on head and thorax, slightly smoother and shiny on gena; scutellum with space between piligerous punctation, smooth and shining. Propodeum and metapostnotum dull-reticulate. Abdominal terga with fine, dull
micropuncturing, more shiny on TVI-VII. Tibia III (see Methods) very smooth and shining, basitarsus slightly less so.

**Shape and dimensions** (Appendix S1). Head 1.27x wider than long and 2.56x wider than clypeocellar distance. Compound eyes, 2.3x longer than wide, converging slightly below. Malar area short, ca. 0.58x diameter of fl.3 (see Methods). Clypeus 0.58x shorter than its maximum width, 0.68x clypeocellar distance. Mandible length 1.16x clypeocellar distance. Labrum normal, convex, with slight medial longitudinal sulcus. Scape 1.06x alveolocellar distance, approximately cylindrical and slightly wider than third fl.3. Fl.1 1.43x longer than wide. Interocellar distance 1.03x greater than ocelloorbital and 2.0x median ocellar diameter. Scutellum 0.48x shorter than wide, distal margin in slightly pointed semi-circle. Tibia III 0.82x width of head and 2.45x longer than wide, posterio-distal edge projected tooth like. Basitarsus III 1.75x longer than wide, posterio-distal edge slightly acute angled. Forewing 2.7x longer than wide and 1.97x longer than head width.

**Male.** Unknown.


**Etymology.** From Latin, *insularis*, isolated, island dwelling.
**Geographic distribution.** Coiba Island, Rancheria Island, Veraguas Province, Panamá.

**Nest.** Unknown.

**Remarks.** *Melipona (Melikerria) insularis* sp. nov. is distinguished clearly from *M. (Melikerria) triplaridis* Cockerell, 1925 – a bee endemic to eastern Panama, from the canal area to the Darién– by slightly smaller size (head width 4.18-4.27 mm, in *M. triplaridis* 4.27-4.46 mm), by larger yellow head markings, in particular the inferior paraocular stripe wider (Fig. 1d), by narrower and paler yellow metasomal bands (ca. 0.24 mm, in *M. triplaridis* 0.37-0.38 mm, Fig. 1h) and by the entirely whitish thoracic pilosity (except anterio-lateral margins of the mesoscutum), lighter than that of *M. salti* (Fig. 1j); *M. triplaridis* has yellowish ivory to fulvous pilosity (mesoscutal margins, axillae and scutellum; Fig. 1l). The ‘Barcode of Life’ results give a 98.31% similarity in the mtDNA sequence between *M. triplaridis* and *M. insularis*, based upon 657 base pairs. Another figure, 97.983% similarity, was obtained using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) with the Clustal X algorithm for p-values (see also Molecular Results, below). In addition, *M. insularis* sp. nov. differs from *M. ambigua* sp. nov. of northern Colombia by the thoracic pilosity, which in the latter is yellowish ivory, slightly lighter than in *M. triplaridis*, and by the yellowish clypeal markings– in *M. ambigua* sp. nov. the median clypeal band is attached to the side markings, making an ‘anchor’ shape; in *M. insularis* sp. nov. the arms of the anchor are interrupted below. Plumose pilosity of TVI in *M. insularis* sp. nov. is largely glabrous in the discal area and black simple hairs are more abundant and robust; on the other hand *M. ambigua* has denser plumose and simple whitish pilosity and few longer black hairs interspersed.
Evidence for a taxon cycle is given by a population of *M. insularis* on Rancheria Island. During glaciations colonies of the large bee were likely able to colonize this 250 ha island, 2 km from Coiba (see Discussion), and its superior flight and foraging ranges may confer survival ability.

*Melipona (Melikerria) ambigua sp. nov.*

(Figs. 1b,f,k, 2b; Appendix S1)

**Diagnosis.** Pilosity, on head and thorax, except ferruginous tuft at anterior corners of mesoscutum, entirely yellowish ivory, slightly more yellow at mesoscutal margins, axillae and scutellum (Fig. 1k). Paraocular bands expanded slightly below, ca. 1.1-1.2x scape diameter; clypeal marking anchor-shaped (Fig. 1b). Yellow bands on distal margin of TII-VI, complete, with slight median constriction, more evident on TIV-VI, as wide as greatest diameter of scape (Fig. 1f); TVI with complete band, as anterior segments.

**Holotype**, worker.

**Dimensions.** Total length, approximately 11.0 mm; forewing length, 8.70 mm (including tegula, 10.2 mm); maximum head width, 4.41 mm; TIII, width 4.56 mm.

**Integument color.** Predominantly black, like *M. insularis sp. nov.*, with the same variation, only legs with lighter coloration. Markings yellowish on head, pronotum and metasomal terga, pronounced; paraocular stripe occupying inferior 3/7 of compound eye, pointed above and gradually widening below – at the inferior extreme ca. 1.2x flagellar diameter; clypeus having anchor shaped marking – the median longitudinal stripe little wider than scape diameter, above not reaching the epistomal suture, below
touching lateral markings with narrowed stripes; supraclypeal marking an isosceles triangle, broad at base. Labrum with wide yellow band at base. Pronotal markings extensive, almost united at midpoint. Yellowish bands on distal margin of TII-VI, as wide as scape diameter, like those of *M. insularis sp. nov.*, band of TVI more complete.

**Pilosity.** Ivory yellow, except ferruginous tufts on anterior edges of mesoscutum, ferruginous setae on tarsomeres and internal surface of all basitarsi, a tuft on the ventro-distal trochanter II, and the black setae of metasomal apex, like *M. insularis sp. nov.* Mesoscutal margins and scutellum with slightly darker yellowish pilosity. Tergite III with fine, simple pilosity, semi-erect, longest ca. 0.20 mm, TIV-V with small decumbent simple hairs, reaching 0.10 mm length,; TVI with more erect hairs, longer and interspersed with plumose hairs, on the lateral margins, and some black setae; TVII clothed with long black branched hairs (to 0.53 mm), interspersed with abundant plumose ivory yellow hairs. Fimbriae on TIII-V borders exactly like *M. insularis sp. nov.* Sternite VII with black hairs.

**Integument.** Like *M. insularis sp. nov.***

**Shape and proportions** (Appendix S1). Head ca. 1.27x wider than long and 2.70x wider than clypeocellar distance. Compound eyes 2.41x longer than wide and slightly convergent below. Malar area 0.39x diameter of fl.3. Clypeus length 0.63x of its maximum width and 0.77x clypeocellar distance. Mandible 1.16x clypeocellar distance. Labrum normal, convex, with soft median sulcus. Scape 1.12x clypeocellar distance, approximately cylindrical and as wide as fl.3 diameter. Fl.1 1.6x longer than wide. Interocular distance 1.24x larger than ocelloorbital distance and 2.44x diameter of median ocellus. Scutellum 0.53x longer than wide, distal margin a slightly pointed semi-
circle. Tibia III 0.77x head width and 2.30x longer than wide, posterio-distal edge
slightly pointed tooth like. Basitarsus III ca. 1.60x longer than wide, posterio-distal edge
slightly closed. Forewing 2.82x longer than wide and 1.97x longer than head width.

**Male.** Unknown.

**Type specimens.** Holotype, worker, from “COLOMBIA, Bolivar Dpto. Cartagena. 15
km SW, September. 1980 – nest, D. Roubik”, at USNM. 24 paratypes, workers: 17 with
the same label as the holotype, at RPSP, STRI, USNM, National University of Colombia
at Bogota (LABUN), 7 additional workers, from “COLOMBIA, Bolivar Cart. Jardin
Bot. Piñeres, XII-17-79, 150 m, G. Parra coll.”.

**Etymology.** From Latin, *ambiguus*, uncertain, doubtful.

**Geographic distribution.** Known only from type locality.

**Nest.** Tree cavity, details unknown.

**Remarks.** Differing from *M. (Melikerria) salti* Schwarz, 1932, an endemic of the Santa
Marta region, Magdalena, Colombia, primarily in the tergal abdominal bands. In *M.
salti*, the band of TII is expanded at the lateral margins and slightly constricted in the
middle, bands on TIII-IV are more interrupted medially, and are absent, or present as
lateral markings, on TVI-VII (Fig. 1g); in *M. ambigua sp. nov.* bands are complete (see
Diagnosis). For comparisons with other species see “Remarks” for *M. insularis sp. nov.*

**Molecular results**

Seven species of *Melikerria* with CO1 sequences were analyzed, those accessioned
from GenBank (Ramírez *et al.*, 2010) were of 491 bp, and those from Barcode of Life
(DWR collection, STRI) were 657 bp. Two or three individuals were used for some species, and their similarities to the others were averaged. A mean similarity of 96.5% (SD 2.18%) exists among the seven taxa (Table 1). The Mexican and Central American species \textit{M. beecheii} was substantially less similar to its relatives. If \textit{M. beecheii} is excluded, average similarity among the other bees is 97.81%, SD 0.63%. Even considering this lower SD, the remaining six species show similar degrees of divergence.

**Discussion**

**Morphological and ecological perspectives.** Recognition of new \textit{Melikerria}, one endemic to Coiba and Rancheria islands, referred to in earlier literature by Cheesman (1929: 149, as \textit{T. interrupta}) and Camargo \textit{et al}. (1988) as “a distinct form of \textit{compressipes}”, and the other endemic \textit{Melikerria} of northern Colombia, brings new elements to the evolutionary history and biogeography of \textit{Melipona}, and lends credence to previous assertions on the biogeographic history and origin of Panamanian biota (Fig. 2a). We rejected the hypothesis of post-Pliocene dispersal by \textit{M. insularis sp. nov.} to Coiba and Rancheria islands in part because we did not find it in mainland forests, and in part because the other island meliponines are widespread (Appendix S2), which we interpret to signify recent, Holocene colonization. \textit{Melipona} visits many common forest edge flowers such as \textit{Cassia}, \textit{Mimosa}, \textit{Solanum} and melastomes, and \textit{M. insularis sp. nov.} actively seeks and collects vanillin crystals (DWR, unpublished data) and recruits well to honey water baits. This species, of large size, is particularly likely to be detected.
Its discovery on Rancheria Island—a single worker vibrating a flower of *Cassia* for pollen—was surprising. There has been no mainland population to colonize this small island, and it must periodically go extinct there (Wilson, 1961), which leads us to propose a taxon cycle; glaciation allowed colonies of *M. insularis* sp. nov. to re-colonize Rancheria from Coiba.

Colonies of Meliponini must be founded by groups of workers flying to and from nest sites, and considerable time is required to prepare the new nesting site, prior to colony reproduction, including nest occupation by a mated queen. Pollen and nectar are harvested from flowers and stored in the incipient nest, and honey transferred by the mother colony. Mother and daughter nests are united, sometimes over months or even years (Michener, 1974: 171; Roubik, 2006; Camargo, 2008). As a result, even small rivers and certainly oceanic gaps prevent meliponine colony dispersal. There is an intriguing specialization common both to *Apis* and meliponines, in that workers direct the queen to her new nesting site (see Seeley, 2010). Tree-nesting species may nonetheless disperse in floating trees or in trees on vegetation rafts (and honey bee swarms disperse many kilometers over open ocean, Roubik & Boreham, 1990). For marine islands, however, we favor the hypothesis of dispersal via terrestrial connections (Camargo et al., 1988; Roubik et al., 1997). How, then, did *Melikerria* arrive on Coiba Island; how and when did its ancestors spread among southern South America, Central America and Mexico? A *Melipona* on Coiba Island suggests that at some period it has been part of continuous mainland forest community, of sufficient duration to be used by founding swarms of meliponine bees, or that bee colonies numbering only a few to hundreds (see Nogueira-Neto, 2002; Lynch & Lande, 1997; Kerr & Vencovski, 1982;
Alves et al. (2010) were able to raft there on vegetation mats. Moreover, Melipona is preadapted to isolation in natural habitat by their tendency to replace the colony’s queen and curb diploid male production (Nogueira-Neto, 2002; Alves et al., 2010). Despite substantial investigation (Appendix S2), M. variegatipes was the only extant island endemic known for the Neotropics. Found on Guadeloupe and Dominica, it had no options for arrival there except from South America during glaciations (Camargo et al., 1988; Rasmussen & Cameron, 2010). We suggest that large body size and flight range, and exceptional communication ability (Michener, 1974; Roubik, 1989; Nieh & Roubik, 1995) increases survival on forested islands, forestalling extinction that we believe has been frequent among meliponines.

Based on morphology, the proposed general area cladogram for Neotropical meliponine bees (Camargo & Moure, 1996; Camargo, 1996; Camargo & Pedro, 2003) indicates three clades, and elucidates Neotropical biogeography in a time frame relevant to the present discussion (see also Amorim & Pires, 1996; Sigrist & Carvalho, 2009). One meliponine clade includes taxa that range from Pacific Ecuador and the Chocó to Panama, then to Mexico (called the ‘Chocó-Ca’ biogeographic component). Two biogeographically proximal clades are of the southwestern Amazon (SWAm) and northern Colombia and Panama to Mexico, the NAm biogeographic component (Camargo & Pedro, 2003). Camargo (1996) has evaluated Amazonian meliponine biogeography and finds congruence in three historically distinct areas, one that produced the southeastern South America and Atlantic Brazilian taxa, and two others, which were connected, in two distinct periods (Amorim & Pires, 1996; Camargo, 2008), to Central America. The logical implication is that Panama was the conduit of two major
continental stingless bee dispersal events. Furthermore, *Melikerria* lacked a corresponding component of the Chocó-Ca. That component is quite possibly represented by the relictual Coiba species that we describe, now extinct on the mainland, and descendants from northward dispersal, now called *M. beecheii*.

The proposed sister species of *M. insularis* sp. nov., *M. ambigua* sp. nov. allows us to make some inferences. The CO1 data indicated an average difference of 2.6% (SD 0.6%) among *Melikerria*. Variation within *M. beecheii* has been reported at 1.2% sequence divergence, but is analyzed from only one gene fragment (Quezada-Euán et al., 2007). The mtDNA similarities among taxa lead to no particular argument, save one: *M. beecheii* is a divergent taxon with a longer biogeographic divergence than found between its consubgenera, and this matches the conclusion from much more extensive data (Rasmussen & Cameron, 2010; Ramírez et al., 2010). Its within-species differentiation may be considered relatively high (see Magnacca et al., 2010). Detailed morphological examination of *Melikerria* provides an enhanced perspective. No comprehensive phylogeny of *Melipona* has been presented, although roughly half the taxa were included in a molecular phylogeny (Ramírez et al., 2010a). Morphological attributes suggest *M. insularis* sp. nov., *M. ambigua* sp. nov. and *M. salti* constitute a subgroup or monophyletic branch. The three species share: 1) paraocular markings gradually widened below (Figs. 1a,b,c); 2) fimbria on TIII very straight and of only simple hairs (Figs. 1e,f,g), and 3) fimbria on TIV-V straight and of simple and branched hairs. *Melipona insularis* sp. nov. and *M. ambigua* sp. nov. have wide and complete metasomal bands, like *M. triplaridis* and unlike *M. salti*. The remaining species of *Melikerria* have different combinations of these and other characters. We may thus
propose a phylogenetic hypothesis (((\textit{M. insularis sp. nov.} – \textit{M. ambigua sp. nov.}) \textit{M. salti}) \textit{M. triplaridis}). In this context, \textit{M. triplaridis}, a species of eastern continental Panama, is basal, perhaps sister to the other three species, but not a direct relative of \textit{M. insularis sp. nov.}. Ancestral populations of \textit{Melikerria} are implied as candidate direct ancestors of \textit{M. beecheii}.

A Bayesian molecular clock estimate for \textit{Melikerria} dates the group as mid-Miocene, and the Mexican and Central American endemic, \textit{M. beecheii}, possibly diverged from congener by late Miocene (Ramírez \textit{et al.}, 2010a). However, the branch support for estimated divergence time, based on four genes, is weak. The distinctive Mexican and Central America \textit{M. beecheii} is apparently a sister to other extant \textit{Melikerria}, thus its ancestral population was already in place well before the Panama isthmus arose. Because \textit{Melipona}, determined by analysis of nine nuclear genes (Rasmussen & Cameron, 2010), is sister to almost all extant Neotropical stingless bee genera, an Eocene dispersal to Mexico from South America cannot be discounted, yet \textit{Melikerria} is unlikely to be the oldest lineage among several \textit{Melipona} endemic to Mexico (Ayala, 1999). Nonetheless, ancestral \textit{Melikerria} may have been the only meliponine both present and able to disperse to and maintain a population on Coiba Island in the Miocene, when Coiba was more isolated from the mainland than at present.

\textit{Geological events in the isthmian region.} Interesting details on terranes comprising southeastern Costa Rica, Panama and the Colombian Chocó (Tremkamp \textit{et al.}, 2002; Coates \textit{et al.}, 2004; Kerr & Tarney, 2005, Montes \textit{et al.}, in press, see Fig. 2a) provide a possible explanation for disjunct distributions of closely related taxa or single species. In the late Eocene and Miocene, Central America was a peninsula (not a series of islands)
that extended 300 km farther east than the Canal area of Panama. South America was separated from Central America by approximately 150 km of deep ocean (Montes et al., in press). In addition, combined exotic terranes from an ancient volcanic arc (a microplate), which included the Nicoya and Osa peninsulas in Costa Rica, the Soná highlands and Azuero peninsula in the Gulf of Chiriquí, Coiba Island, and perhaps extending south to the Sapo and Baudó Massif to Gorgona Island (Gansser et al., 1979), could have been emergent from the mid Miocene (12.8-9.5 Ma) until the late Miocene (7-6.3 Ma) (Duque-Caro, 1990). That block of exotic terranes and land now subducted may have been available for colonization during a limited period in the mid to late Miocene, and also separated from newer terranes at the Caribbean margin. Thus we believe Melikerria may have crossed the South America-Central America gap in Miocene times, occupying both the San Blas massif and the Pacific side of the forming isthmus. An effective interruption in contact between Panama and South America then lasted until the Plio-Pleistocene at 3.7-3.1 Ma.

**Comparative biogeography.** Chronostragraphic studies of sufficient detail to allow correctly dated fossil evidence of ancient dispersal events between North and South America are few, but it is now clear that large land animals dispersed from Central America to Peru before closing of the isthmus of Panama (Campbell et al., 2010; Kirby et al., 2008; Retellack & Kirby, 2006). Cody et al., (2010) point out that no geological evidence proves that a land connection dated earlier than approximately 3 Ma. However, recent advances in geological sediment characterization indicate a relatively narrow gap between continents during as early as Eocene times (Montes et al., in press). Furthermore, beginning about 2.5 Ma, glaciation events were relatively longer and sea
levels were reduced by over 100 m. At 2.4 Ma there is a telltale rush of species crossing the isthmus (Coates, 1997; Webb, 1997). However, according to molecular divergence time estimates, the earliest freshwater fish dispersal occurred 7 Ma (Bermingham & Martin, 1998). Cody et al. (2010) summarize dozens of molecular dating and phylogeny studies, finding that all mammals crossed the isthmian area ≤ 10 Ma, and plants ≤ 50 Ma, but only 24% of those plants and 39% of the animals did so ≤ 3 Ma. Birds clearly dispersed more often north than south (Weir et al., 2009), and their forest lineages increased dramatically in dispersal rate after the Panamanian isthmus was complete. Thus, there has been a steady flow of biota across the isthmian region since the Eocene. That largely oceanic region was a very leaky barrier.

Xeric conditions have been related to low species diversity, local extinction, and failure by amphibians to recolonize Coiba Island, where eight bird families went extinct (Castroviejo, 1997). Only four plants species may be endemic on Coiba Island (Velayos, et al., 1997) but the putative endemics are not trees and perhaps less easily documented, thus potentially extant elsewhere. In contrast, a valued timber species endemic to rain forests is found on Coiba Island, Pelogyne purpurea “purpleheart” (Fig. 2d), and its distributional pattern is fragmented (Silva, 1976; Salinas & Cárdenas, 2006), in areas that correspond strikingly well to those of the species we describe of Melikerria. Disjunct distributions among Neotropical meliponines occur both at the species level (Ptilotrigona occidentalis, Meliponini, Fig. 2c, Appendix S2) and at genus level. Proplebeia (as an amber fossil of Miocene age) is found in Chiapas, Mexico and Dominica/Hispaniola (Camargo et al., 2000). Meliwillla is an apparent relict, confined to cloud forest of Costa Rica and Panama (Roubik et al., 1997) and putatively sister to a
widespread genus, *Scaptotrigona*, with endemic species throughout its range. Either extinction or more complex historical biogeography (Ricklefs & Bermingham, 2001; Camargo & Pedro, 2003; Morrone, 2006) may explain such patterns. It is likely that wet conditions prevailed on the higher elevations of Coiba Island since the Pliocene, while xeric or non-forest conditions prevented colonization between island wet forest biota and continental lowlands.

**Conclusions.** A mixture of northern and southern biogeographic elements composes the flora of Panama, which on the mainland is primarily N. American (Graham, 1990; MacFadden, 2009), northwest of the limit of endemic Darién *Melipona triplaridis* at the lowland division that contains the Panama Canal. In contrast, the Coiba Island flora is predominantly like the Darién and South America, rather than like that of Caribbean wet forests (Velayos et al., 1997), and contains our new Panamanian *Melikerria*.

While acknowledging a Laurasian presence of stingless bees in North America, Mexico and Greater Antilles, an older Gondwanan origin and vicariant dispersal with the rifting of South America from Africa places meliponines in South America in the mid Cretaceous (Camargo et al., 1988; Ramirez et al., 2010a; Rasmussen & Cameron, 2010). The island-endemic, *M. (Melipona) variegatipes* of Lesser Antilles, arrived from South America across land (Camargo et al., 1988), but general dispersal of meliponines farther northward was precluded until the Neogene Panama connection. Dispersal avenues in the Caribbean, to Central America or Mexico, may exist for non-colonial female euglossine and other bees (Ramírez et al., 2010b) but are unlikely for colony-dispersing meliponines. We see a pattern for multiple meliponine lineages, and their
vicariance, as the result of an early Neogene filter bridge between North and South America.

A final note on the conservation of a large, endemic stingless bee on the largest island on the Pacific coast of Central America is appropriate (see Kier et al., 2009). The Africanized honey bee colonized western Panama in 1985 (Roubik, 2002) and its foragers are now common on flowers, many visited by *M. insularis* on Coiba, and by the honey bees on most of the other islands (DWR, *pers. obs.*). The two comparably large honey-making bees, from the New and Old World tropics, have coexisted on Coiba for over 25 years. At present, Coiba is protected wildland and no beekeeping ventures are allowed. An escalation in the honey bee colonization process or diminution of the flora (Roubik & Villanueva, 2009) could lead to untimely demise of an ancient bee species.

**Acknowledgments**

We thank Drs. Silvia Menezes Pedro and Claus Rasmussen for their help, anonymous reivewers for comments, marine and field assistants in Panama for aid, and the Panamanian authorities of ANAM and Futuroforestal (Cébaco Island) for support.

**References**


Table 1. COI mitochondrial DNA similarity matrix for *Melipona* subgenus *Melikerria*,

‘*’ = Average from multiple collection localities

*(Melikerria):*

<table>
<thead>
<tr>
<th></th>
<th>beechii</th>
<th>beec</th>
<th>comp</th>
<th>gran</th>
<th>insul</th>
<th>inter</th>
<th>quin</th>
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<td>98.37</td>
<td>97.15</td>
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FIGURES:


Scales = 1 mm.

(Appendix S1). Measurements (mm) of holotype *Melipona (Melikerria) insularis* sp. n. and *M. (Melikerria) ambiguа* sp. nov.

<table>
<thead>
<tr>
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<td>Value 2</td>
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<td>-----------------------------------------</td>
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<tr>
<td>Length of mandible</td>
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<tr>
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<tr>
<td>(to apex of costal sclerite)</td>
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<tr>
<td>Width of tergum III</td>
<td>4.18</td>
<td>4.56</td>
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</table>
(Appendix S2). Our hypothesis is that current bee fauna on Coiba, the largest island off the Pacific coast of Central America, contains ancient endemic species, now relictual, and more recent immigrants from the Holocene.

Panama is very biogeographically complex. In the stingless bees (Meliponini) the discussion by Camargo et al. (1988) outlines the basic condition that various groups were already present in Central America and Mexico in the mid Miocene. Biogeographic relationships that concern glacial periods and reduces sea levels are applicable to Gorgona Island of Pacific Colombia (Fig. 2a), the extreme end of the exotic terranes that we argue may have provided a dry land link between Colombia and Central America in the Miocene. Gorgona is 26 km² in area (9 km long, 2.5 km wide). Its closest point to the continent is 30 km in distance from Ponta Reyes and ocean depth is no greater than 50 m. It has at least nine species of Meliponini in the genera *Melipona, Partamona, Plebeia, Tetragonisca, Trigonisca, Trigona, Nogueirapis, Nannotrigona* and *Scaptotrigona* (Cheesman, 1929, www.uky.edu/cgi-bin/cgiwrap/mjfharo/spgorgona.cgi and museum specimens), including two that make subterranean nests *Trigona fulviventris* Guérin, 1835 and *Nogueirapis mirandula* (Cockerell, 1917).

On Coiba island, our recent studies confirm earlier reports of Cheesman (1929) with one important exception—there is no *Trigona* species on the island and the earlier report was likely a mistake, probably referring to *Frieseomelitta pauper*, which occurs from Costa Rica and Panama to northern Colombia, Venezuela and Trinidad, and in the Coiba Island group, found also at least on Rancheria, Canal de Afuera, and Santa Catalina. DWR collections on Coiba also include *Scaptotrigona* sp. (unpublished, by H. F. Schwarz, manuscript name), as on Rancheria Island, and in the Gulf of Panamá (= *T. pectoralis*
*panamensis* in Cheesman, o.c.), on Taboga Island and in Veraguas Province, on the mainland, with a distribution from Pacific Panama to northern Colombia; *Trigonisca* sp. (unpublished, J. S. Moure) of Coiba and Rancheria Islands, and Taboga Island, corresponds to a continental population (Chepo and Balboa —Panama Prov., Portobelo— Colon and San Blas Prov., DWR & JMC, pers. obs.). On Coiba Island, *Nannotrigona perilampoides* (Cresson, 1878) also occurs, and is found nesting in some of the former prison buildings [also on Taboga Island, Bay of Panama, as *N. testaceicornis* (Lep.) in Cheesman, 1929]. In addition, Cébaco Island (DWR pers. obs.) has many *Trigona fulviventris* and *Partamona peckolti* Friese, 1901, and *Plebeia* sp., *N. perilampoides, Tetragonisca angustula* Latreille, 1911, *Scaptotrigona* sp. nov., and very likely *Trigonisca* sp. nov. *Trigona* and *Partamona*, present nowhere in the Coiba Island group, nest in the ground or exposed, or on epiphytes (Roubik, 2006). They are unable to colonize across oceanic gaps.

Notably, species richness on Coiba island, despite large size, is far less than the 20-47 meliponine species found in forest areas of equal size on Panamanian mainland (Roubik, 1992, 1993). The island Cébaco lies 7 km from the mainland and is separated by relatively shallow water of 10 m depth. It has the richest island meliponine fauna of Pacific Panama, but included only seven meliponines. Because only *Geotrigona, Nogueirapis* and some *Trigona* nest exclusively in the ground (Roubik, 2006), we suggest extinction and dispersal limitation have reduced higher taxa of island stingless bees that are separated by oceanic cepths >50 m and not close to the mainland, among them *Tetragonisca, Tetragona, Scaura, Nogueirapis, Plebeia, Trigona, Partamona, Cephalotrigona, Paratrigona, Oxytrigona, Geotrigona* and *Lestrimelitta* (Roubik, 1992). Extinction has far outpaced re-colonization in general. However, the first Pliocene glaciations and sea levels lowered by 100 m, which
coincided with a general breach of the isthmian barrier (2.4 Ma), could certainly have allowed various taxa to re-colonize Coiba, including *M. insularis* sp. nov.