Sexual dimorphism and phylogenetic position of *Chilodendron* (Coleoptera: Curculionidae: Scolytinae) – a long isolated lineage endemic to Madagascar

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Abstract. *Chilodendron* Schedl, 1953 is resurrected as a valid genus based on *Chilodendron planicolle* Schedl, 1953. This is the only representative of the tribe Hylesinini Erichson, 1836 found on Madagascar and a molecular phylogenetic analysis of several genes supports a position separate from *Hylesinopsis* Eggers, 1920 and other putatively close relatives. It is likely that *Chilodendron* is the oldest living lineage of bark beetles on Madagascar and possibly originated in the late Cretaceous not long after the separation of Madagascar from the Indian subcontinent.

INTRODUCTION

Madagascar is famous for its high degree of endemism of both flora and fauna (Yoder & Nowak, 2006). Although patterns vary among different insect groups (e.g. Monaghan et al., 2005; Vuataz et al., 2013; Bukontaite et al., 2015; Toussaint et al., 2016), a strong connection with the African mainland is by far the most common. Bark beetles are no exception, with either sister groups in the two regions, or most often a recolonization of the African mainland explaining the distribution of groups found in both regions (Jordal, 2013, 2017, 2021b, c; Jordal & Tischer, 2020, Eliassen & Jordal, 2021). The origin on Madagascar is often quite ancient, dating back to Eocene, Oligocene or Miocene in the majority of cases.

Many bark and ambrosia beetle lineages diversified substantially on Madagascar once they arrived on this island. However, a few peculiar lineages are extremely depauperate. One of these is the genus *Chilodendron* Schedl, 1953, with a single species on Madagascar, *C. planicolle* Schedl, 1953. This species is one of only two species described originally in this genus. They are now both placed in the genus *Hylesinopsis* Eggers, 1920, as tentatively suggested by Beaver (2010). Wood (in Wood & Bright, 1992) on the other hand placed the type species *C. planicolle* in *Xylechinus* Chapuis, 1969, which illustrates some of the taxonomic uncertainties associated with this species.

Based on its isolated geographical position and characteristic morphology, one should investigate more closely the relationship of *C. planicolle* to other genera in Hyle-sinini. Recent collecting from the type locality in Montagne d'Ambre on the northern tip of Madagascar provided material for DNA analyses and dissections of internal anatomical structures. It was also clear from field observations that sexual dimorphism is evident and is together with its biology described here for the first time.

MATERIALS AND METHODS

Specimens of *Chilodendron* were collected in November 2019 in Montagne d'Ambre. Five gene fragments of its DNA were amplified and sequenced, including Cytochrome Oxidase I (COI, 690 bp), Elongation Factor 1a (EF1a, 833 bp), carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD, 581 bp), Arginine Kinase (ArgK, 801 bp), and the large ribosomal subunit 28S (GBlock pruned, 701 bp). New sequence accession numbers are: ON110290, ON117772, ON125371, ON125372, ON125373. Details of the other specimens included in the phylogenetic analyses and their GenBank accession numbers are in Pistone et al. (2018). 28S was aligned using the software Muscle available at https://www.ebi.ac.uk/Tools/msa/muscle/. Settings in GBlock (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) included options for less stringent pruning and 229 sites were removed from the alignment. Pruning ambiguous alignment regions based on less stringent parameters maintains most of the informative part of the gene while simultaneously reducing artificially long branches due to extended loop regions that characterize ribosomal genes (see also Jordal et al., 2008).

The data were analysed in MrBayes (Ronquist & Huelsenbeck, 2003) and partitioned into genomes and positions (if coding) using a GTR+I+G model for each of the seven partitions

(nuclear protein coding positions, mitochondrial COI positions and 28S), with gamma shape, proportions of invariable sites, and substitution matrix, free to vary across partitions [unlink shape=(all) pinvar=(all) statefreq=(all) revmat=(all)]. Runs stabilized early, obtaining PSRF < 1.01 and SDSF < 0.01. The run included 20 million generations, with 2000 saved trees, removing 1000 as burnin. Sensitivity to partition exclusion was examined in PAUP (Swofford, 2002) using parsimony and Neighbour Joining and found to not affect the topology with respect to the focus taxa. The influence from inclusion (full alignment) and exclusion (gblock) of gapped regions in the 28S partition was also examined, with only a marginal difference in the position of *Phloeosinus* Chapuis, 1869.

Age estimates of clades were calculated in Beast using a Yule model of speciation, and a GTR model each for 28S, COI positions and nuclear protein coding gene positions. The time tree was calibrated with an approximate age of 100 Ma for Scolytinae as estimated by Pistone et al. (2018) for the taxa included here. The Beast tree was used to optimise areas in the software RASP using a Bayesian Binary MCMC (BBM) approach (Yu et al., 2020).

Specimens were photographed using a Leica 205C stereomicroscope and Leica LAS software and multiple photographs were aligned and stacked in ZereneStacker (Zerene Systems). All measurements were taken as previously explained in Jordal (1998). Proventriculus and male genitalia were macerated in 8% KOH and mounted on slides in Euparal. Photographs of slide preparations were stacked and aligned using the Leica LAS software connected to a Leica microscope.

Types and other material are stored in Musee National d'Histoire et Naturelle, Paris (MNHN) and University Museum of Bergen (ZMUB).

RESULTS

Bayesian analysis of the molecular data resulted in a well resolved and highly supported tree topology (Fig. 1). Three species of *Hylesinopsis* Eggers, 1920 were monophyletic and grouped as sister to *Hapalogenius* Hagedorn, 1912, *Rhopalopselion* Hagedorn, 1909 and *Strombophorus* Hagedorn, 1909. *Chilodendron planicolle* grouped with *Alniphagus* Swaine, 1918 and *Hylastinus* Bedel, 1888, near *Xylechinus* and was separated several nodes away from the Afrotropical Hylesinini. The Bayesian reconstruction of ancestral geographical areas clearly excluded the Afrotropical region and supported a Holarctic distribution for the ancestor of *Chilodendron* (Fig. 18).

Testing further the phylogeny for putatively close relatives, including using similar data for Australasian species of *Ficicis* Lea, 1910 and *Chaetoptelius* Fuchs, 1913, did not provide better clues of potential sister group relationships (as indicated by Pistone et al., 2018). *Chilodendron* was also genetically very different from its nearest neighbours, supporting a separate standing as a monotypic genus.

Chilodendron Schedl, 1953, stat. rev.

Type species: *Chilodendron planicolle* Schedl, 1953: 74 (monotypic).

Chilodendron planicolle Schedl, 1953

*Chilodendron planicolle* Schedl, 1953: 74.
Xylechinus planicolle (Schedl, 1953), combination by Wood & Bright, 1992.

*Hylesinopsis planicolle* (Schedl, 1953), combination by Beaver, 2010.

Figs 2, 3, 5, 6, 8, 10, 12, 14, 16

Diagnosis. Stout species, twice as long as broad; head large, epistomal lobe impressed, lower frons bulbous; eyes small, entire, bulbous; antennal funicle 6-segmented; club subconical with two transverse sutures; scutellum visible, elongated; elytral interstriae in females with small granules, in males with longer sharp spines; elytral ground vestiture of very fine hair-like setae; body fluffily hairy below, strongly plumose laterally from prothorax to first abdominal ventrite; protibia with three unsocketed teeth on distal fourth, meso- and metatibiae with two smaller apical and two larger unsocketed lateral teeth.

Description, male. Body length 3.3–3.8 mm, 2.0–2.1× as long as wide, colour dark brown. Head substrostrate, very finely and densely punctured, vertex with a shiny longitudinal line; frons transversely impressed at upper level of eyes, bulbous below, epistomal lobe and lower median part of epistoma strongly impressed; vestiture consisting of fine and densely set hair-like setae. Antennal scapus elongated, longer than club; funiculus 6-segmented; club conical, 3-segmented, densely setose, sutures transverse. Eyes small, bulbous, entire, separated above by 3.5 × their width. Pronotum in lateral view almost flat, in dorsal view the anterior third is constricted laterally, anterior margin with a fine and partly crenulated rim; surface densely, irregularly punctured; vestiture of very fine, short, hair-like setae. Scutellum visible, elongated, at level of elytra but slightly dome-shaped. Elytral base with a transverse and finely crenulated rim, slightly notched near scutellum. Striae slightly impressed, punctures small, deep, separated by less than their diameter. Interstriae broad, 3× wider than striae, on anterior disc smooth and shiny, then gradually
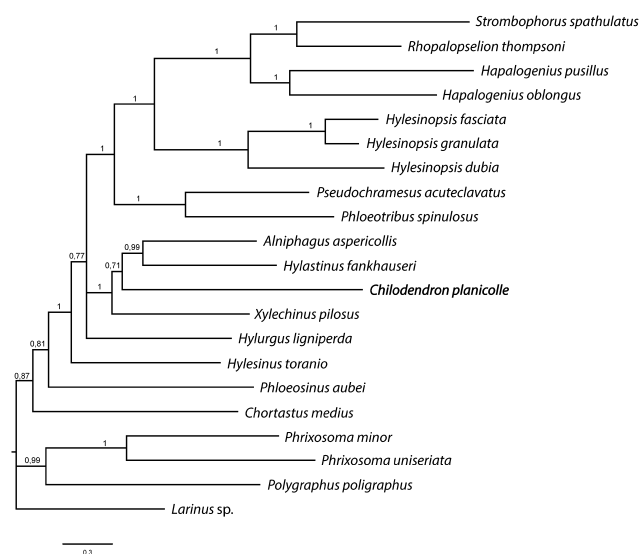
Fig. 1. Phylogenetic tree based on the Bayesian analysis of DNA nucleotide data from five gene fragments. Numbers on nodes are posterior probabilities. The species of Scolytinae were selected based on a high degree of morphological similarity with *Chilodendron planicolle*.
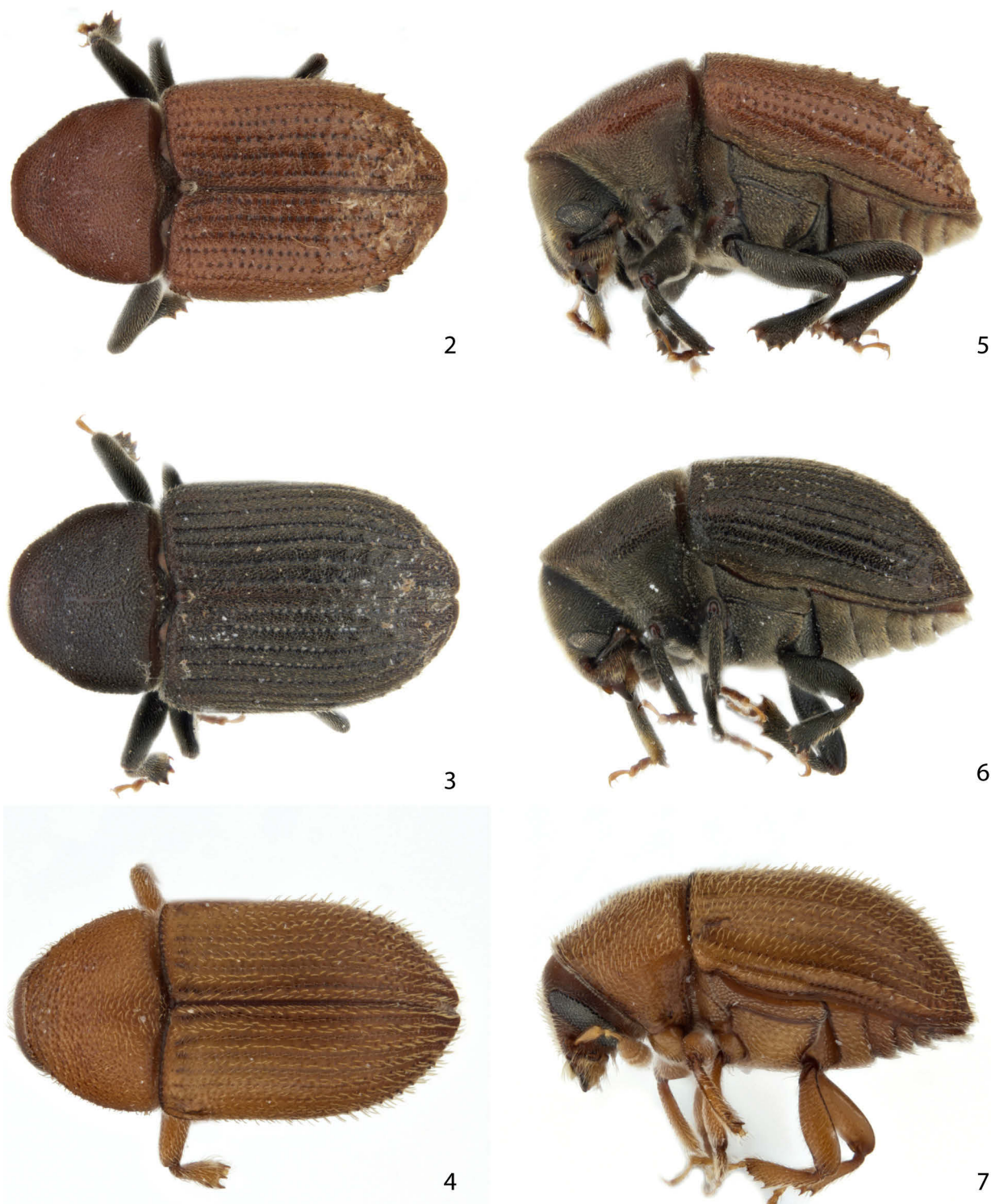
Figs 2–7. Dorsal and lateral view of *Chilodendron* and *Hylesinopsis*. 2, 5 — *C. planicolle* male; 3, 6 — *C. planicolle* female; 4, 7 — *Hylesinopsis fasciata*.

more granulated on posterior disc, with spines longer than wide irregularly placed on declivity. Vestiture of few erect thin bristles, and densely set ground vestiture of very fine short setae. Ventral side densely setose with fine light hair-like setae, on lateral sclerites from prothorax to abdominal ventrite 1 or 2 the setae are strongly plumose, each with a scale-like base and 3–6 terminal filaments. Protibiae with apical third twice as broad as proximal third, with three strong unsocketed lateral teeth. Inner mucro thin, curved posterolaterally. Meso and metatibia with two large lateral and two slightly smaller apical teeth. Procoxae separated by half the width of one coxa, mesocoxae slightly wider apart, mesoventral process expanded, declining. Proventriculus with apical plate half as long as posterior plate, with smooth transverse ridges becoming toothed near the closing teeth; apical and femoral teeth absent. Aedeagus

Figs 8–17. External and internal anatomical structures of *Chilodendron planicolle* (8, 10, 12, 14, 16) and *Hylesinopsis fasciata* (9, 11, 13, 15, 17) showing front (8, 9), protibiae (10, 11), male genitalia (12, 13), metatibiae (14, 15) and proventriculus (16, 17).

with large and strongly sclerotised basal sclerites; apophyses narrowly attached and about the same length as the aedeagal body; tegmen a closed ring without manubrium. Spiculum gastrale thick, as long as aedeagus, forked at base.

Female externally similar to male except for the spines on declivity that are reduced to small granules.

Type material. Syntypes (2 females): Madagascar, Mt. d'Ambre, 1952, K.E. Schedl, leg. [MNHN].

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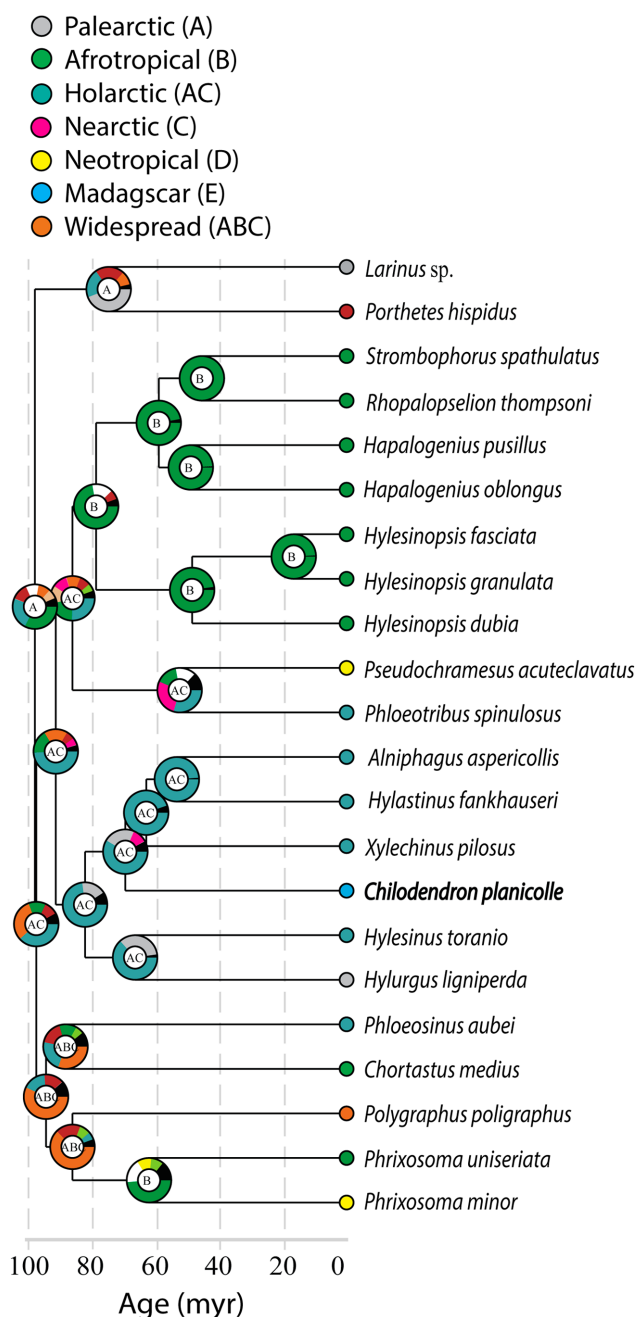
Fig. 18. Beast time tree with ancestral areas estimated using the Bayesian Binary MCMC method in RASP. The most likely area is denoted by a letter (A–E).

Other material. Madagascar, Diana region, Montagne d'Ambre National Park [GIS: -12.54, 49.17], 2 Nov. 2019, ex dead *Ficus* branch, 10 ♂ and 10 ♀, B. Jordal, leg. [ZMUB].

Biology and distribution. This species is only known from the type locality. It was dissected from dead aerial roots and stems of a strangler fig (Moraceae), which was densely colonized. Pairs of male and female were found and both were present until at least late larval stages or later. Brood production was high with 30–50 tenerals per family in thicker branches, and smaller broods in branches less than 5 cm thick. Maternal egg tunnels were cut transversely to the grain, in the phloem just under a thin layer of bark. Larval tunnels were rather irregular but generally perpendicular to the maternal tunnel.

DISCUSSION

Morphologically this species is most similar to species of *Hylesinopsis* (Figs 2–17). The club in *Chilodendron* is more conical with straight sutures whereas in *Hylesinopsis* the club is more flattened and the two sutures are slightly procurved. Only *Chilodendron* has bulging eyes of such comparatively small size, and no other genera have an impressed epistomal lobe extending into the middle of a bulbous epistomal area. In *Hylesinopsis* the entire epistoma is instead impressed from base to base of mandibles. The latter genus also has on the distal end of its metatibiae a well-developed inner flange separating the tarsal attachment from an apical transverse row of four small socketed denticles ('false corbel' sensu Thompson, 1992), a feature only seen in Hylastini and Phrixosomatini (see Fig. 2i in Jordal, 2012).

Molecular data clearly document a lack of connection to species in *Hylesinopsis* and other genera of typical Hylesinini and Hylurgini affinity (Fig. 1). Although most morphological characters are not particularly unique to *Chilodendron*, the general habitus of this species is somewhat unusual. Many genera in Hylesinini and Hylurgini (sensu Wood, 1986) have relatively similar body shapes, which is also reflected in comparable internal anatomical structures. Diverse genera such as *Hylastes* Erichson, 1836, *Hylesinus* Fabricius, 1801, *Xylechinus*, *Alniphagus* and *Hylesinopsis* have similar proventriculi with a ridged apical plate without apical teeth (Nobuchi, 1969, Lopez-Buenfil et al., 2001). The male aedeagus has narrowly attached apophyses, huge basal sclerites, and a thick and forked spiculum gastrale in all of these genera, but a simple ring-shaped tegmen is present only in *Chilodendron* and some of these genera (e.g. *Alniphagus*, *Hylastinus*), whereas a dorsally open tegmen is found in *Xylechinus*, *Hylastes*, *Tomicus* and related genera in Hylurgini (Yin et al., 1984; Petrov, 2018) and *Hylesinopsis* (Fig. 13). Most of the compared genera have a 7-segmented funicle, except for being 5-segmented in *Xylechinus*, whereas the 6-segmented funicle observed in *Chilodendron* is typical for *Hylesinopsis* and other Afrotropical Hylesinini.

The biology and sexual dimorphism in *Chilodendron planicolle* are described for the first time. Only two syntypes were present before this study, both were females as indicated by the small granules present on the elytral interstriae. The much more pronounced spines and sharp granules observed in some of the individuals reported here are the sole external characteristic of males. These morphological differences are of such a magnitude that different sexes could potentially be identified as different species unless identified as pairs in the field. It is not uncommon to observe sexual dimorphism in bark beetles although its functional basis is very uncertain (Kirkendall et al., 2015). When dimorphism does occur, males are generally spinier (e.g. Browne, 1970; Pfeffer, 1995; Jordal, 2021c). After mating just inside the entrance hole, the male in many spe-

cies of Scolytinae may continue to guard the entrance of the tunnel and sharp spines could therefore facilitate this type of defence. Alternatively, the spines may be part of the sexual recognition system, especially if pheromones are inefficient signal providers (Kirkendall et al., 2015). The reproductive behaviour observed in *C. planicolle* is otherwise typical for normally outbreeding bark beetles (Kirkendall et al., 2015) where both males and females remained together at least until the larvae were nearly fully developed.

Fig trees are commonly reported as host plants for bark beetles and are possibly the most prevalent host plant genus among the angiosperms (Wood & Bright, 1992). Host specialization can be high in many bark beetles, including species that utilize fig trees. The common occurrence of such trees in Madagascar, and the high density of reproducing families in a single branch of a strangler fig, suggest that fig trees are likely to be important host plants for these beetles.

Biogeography

Based on the distant genetic relationship to the morphologically most similar genera, it is evident that *Chilodendron* has been isolated on Madagascar for a very long time. Additional genera from the African and Indian regions are not closely reminiscent of *Chilodendron* morphology. The most likely candidates for a sister relationship are included here and none of these were able to shorten the long branch leading to *Chilodendron*. Cursory analyses of much larger data sets of individual genes (used in Jordal & Cognato, 2012; Pistone et al., 2018) did not indicate other potential close relatives. Hence, we assume that *Chilodendron* belongs in a clade somewhere near *Xylechinus*, *Alniphagus* and related taxa, which had maximum posterior probability (see Fig. 1). The split between each of these taxa was previously estimated to occur close to 70 Ma (Pistone et al., 2018). A cursory re-analysis of some of these taxa together with *Chilodendron* indicated exactly the same (crown) age (Fig. 18). This estimate is older than the oldest Micracidini on Madagascar (see Jordal, 2021c) and is within the older range for the origin of the platypodine genus *Mitosoma* on this island (see Jordal, 2015). Thus, the origin is potentially from the time of connection to, or just after the split from the Indian subcontinent drifting north from Gondwana (Yoder & Nowak, 2006). Still, there are no obvious close relatives in Asia based on the very different morphology of the Oriental taxa. During the late Cretaceous the distance to the African mainland was also shorter than today and does not exclude an Afrotropical ancestor as documented for the few known Cretaceous origins on Madagascar, including examples of plants, frogs, lemurs and butterflies (Braby et al., 2005).

Without further clues to the origin of this unique taxon, it is almost certainly the oldest bark beetle lineage on Madagascar. It is notable that there is only a single species and it is unknown whether this is due to a lack of diversification or excessive extinction. Colonisations without further diversification on the island is nevertheless a fairly common phenomenon in insects (summarized in Bukontaite et al.,

2015). But the great majority of insect lineages, diverse or not, are much younger, with an origin in the Oligocene or later, particularly the Miocene (Jordal, 2013, 2021a, b, c; Bukontaite et al., 2015 and references within; Eliassen & Jordal, 2021). In terms of evolutionary value, *Chilodendron* is an important taxon of high conservation priority.

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