Things are not always as they seem: High-resolution X-ray CT scanning reveals the first resin-embedded miniature gecko of the genus *Ebenavia*

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Abstract. We identify a presumed specimen of *Sphaerodactylus* in amber from the Zoological Research Museum Alexander Koenig as being embedded in copal, rather than amber. Further, the specimen matches the morphology not of a Hispaniolan gecko, but of the extant Madagascan species *Ebenavia boettgeri*, which occurs in a known area of copal deposits.

Key words. *Sphaerodactylus*, *Ebenavia*, CT scan, Madagascar, Osteology.

Fossil lizards embedded in amber are frequently spectacular since they preserve, in high definition, the three dimensionality of ancient organisms. To date, fossil remains of squamates have been found in six amber deposits around the world (Daza et al., 2016). Examples include the oldest reptile in amber (*Baabdasaurus xenurus*) from the Early Cretaceous of Lebanon (Arnold et al., 2002); some scales attributable to a squamate from the Albian of France (Perrichot and Néraudeau, 2005); 14 fossil squamates from the mid-Cretaceous of Myanmar, including members with affinities to Iguania, Gekkota, Scincoidea, Anguimorpha, and Ophidia (Arnold & Poinar, 2008; Daza et al., 2016; Fontanarrosa et al., 2018; Daza et al., in press; Xing et al., 2018); a gekkotan and numerous lacertids in Baltic amber (*Succinilacerta succinea*, Böhme & Weitschat, 1998; Borsuk-Białynicka et al., 1999; *Yantarogekko balticus*, Bauer et al., 2005; see also Čerňanský & Augé, 2013); and many lizards from the Miocene deposits of Mexico and Hispaniola classified in the genus *Anolis* (*A. electrum*, Lazell, 1965; Rieppel, 1980; de Queiroz et al., 1998; Polcyn et al., 2002; Castañeda et al., 2014; Sherratt et al., 2015) and *Sphaerodactylus* (*S. dommeli*, Böhme, 1984; *S. ciguapa*, Daza & Bauer, 2012). Copal specimens have received relatively less attention and were reviewed in Broschinski & Kohring (1998). The genera *Phelsuma*, *Lygodactylus*, and *Geckolepis* have been preserved in Madagascan copal.

The study of lizards in amber has been facilitated by the use of X-rays and High-Resolution X-ray computed tomography (HRCT; Polcyn et al., 2002; Daza et al., 2013; Castañeda et al., 2014; Sherratt et al., 2015; Daza et al., 2016), allowing the rendering of the skeleton without distortion, in addition to providing incredible integumentary detail. As part of an ongoing research project, we examined all available gecko specimens in Miocene amber from Hispaniola preserved in amber using HRCT. One specimen was revealed to be neither a *Sphaerodactylus* nor embedded in Miocene amber. The specimen was scanned at the Center for Nanoscale Systems, Harvard University using a Nikon (Metris) X-Tek HMXST 225 scanner with a molybdenum target at 70kV, 135 µA, 1000 ms exposure, 3143 projections, 0.1° rotation step, and no filter. The reconstructed voxel size for the particular specimen was 14.251 µm. The original data set has been archived and is available to the public at Morphosource (https://www.morphosource.org/Detail/ProjectDetail/Show/project_id/545). Additional specimens for comparison were scanned at UTCT | The University of Texas High-Resolution CT Facility in a Xradia – Zeiss machine. The specimens were scanned with a 4X objective, 70kV/10W using variable parameters. These specimens are part of a large data base of skull Micro-CT that includes nearly all gekkotan genera (Aaron M. Bauer digital collection). All post- processing of the scan data was performed using Avizo Lite 9.5.0 (©FEI SAS, Thermo Fisher Scientific, 2018).

The specimen in question, from the Alexander Koenig Research Museum (ZFMK 94000, Fig. 1), had been obtained by the museum sponsoring society (Alexander-Koenig-Gesellschaft) in November 2012 under the assumption that it was a piece of Dominican amber with a *Sphaerodactylus* (Gekkota: Sphaerodactylidae) inclusion. Although the size and overall appearance is consistent with that of these miniaturized geckos (Daza et al. 2008), a more thorough analysis of this material and comparison with the Micro-CT data base of gekkotans indicated that this specimen is a Madagascan clawless gecko of the genus *Ebenavia* in the family Gekkonidae.

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Fig. 1. Specimen ZFMK 94000, in dorsal view. Scale bar equals 5 mm.

Morphological data indicates that the specimen is a subfossil, and that the resin is copal, not amber.

Copal from Madagascar is botanically assigned to the fabacean species *Hymenaea verrucosa* (Penney et al., 2005). Copal can be differentiated from mature resins, such as amber, with Raman spectroscopic analyses, showing more intense bands at around 1640 cm^{-1} due to more stretching vibrations of the v(C=C) attributed to the olefinic group (C=CH2; Winkler et al., 2001). Thermal analyses have also been used to characterize resins; copal from Madagascar may be differentiated from amber and other copal resins in reaching a peak in differential thermogravimetric analysis at 384 °C, while Colombian copal and amber from other localities peaks at 400 °C or more (Ragazzi et al., 2003). The age of copal resins may be only a few hundred to up to four million years old. Some resins from Madagascar have been dated using carbon dating analyses to be as young as just a few decades (Poinar, 1999; Bosselaers et al., 2010). Other estimates for the age of Madagascan copal include a range from Holocene to Recent (10,000–100 y; Schlüter & Gnielinski, 1987; Lourenço, 1996; Winkler et al., 2001). In this study, we confirmed that the specimen is embedded in copal based on the morphological similarities with modern species, and some simple tests on the resin: 1) A hot needle was pushed into the piece, causing rapid melting at the point of insertion (rather than slow melting expected in amber); the melting resin released a mild fragrance (amber yields a sooty odor). 2) Under a UV lamp the piece did not show any color change (rather than emitting a bluish glow, as does amber).

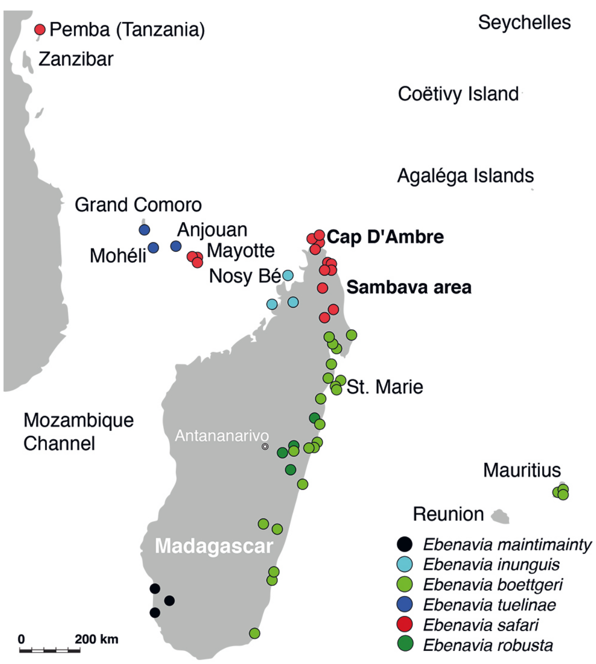

Fig. 2. Distributional map of *Ebenavia* species. Colors follow Hawlitschek et al. (2018). Additional localities for *E. maintimainty* taken from Nussbaum and Raxworthy (1998).

Morphological comparisons considering members of nearly all described gekkotan genera indicate that *Ebenavia* shares most morphological characters with ZFMK 94000. The genus occurs on Madagascar and satellite islands, Pemba Island, Grand Comoro, Mohéli, Anjouan, Mayote, Nosy Bé, Nosy Komba, Île Sainte-Marie (Nosy Boraha), Nosy Mangabe, and Île aux Prunes

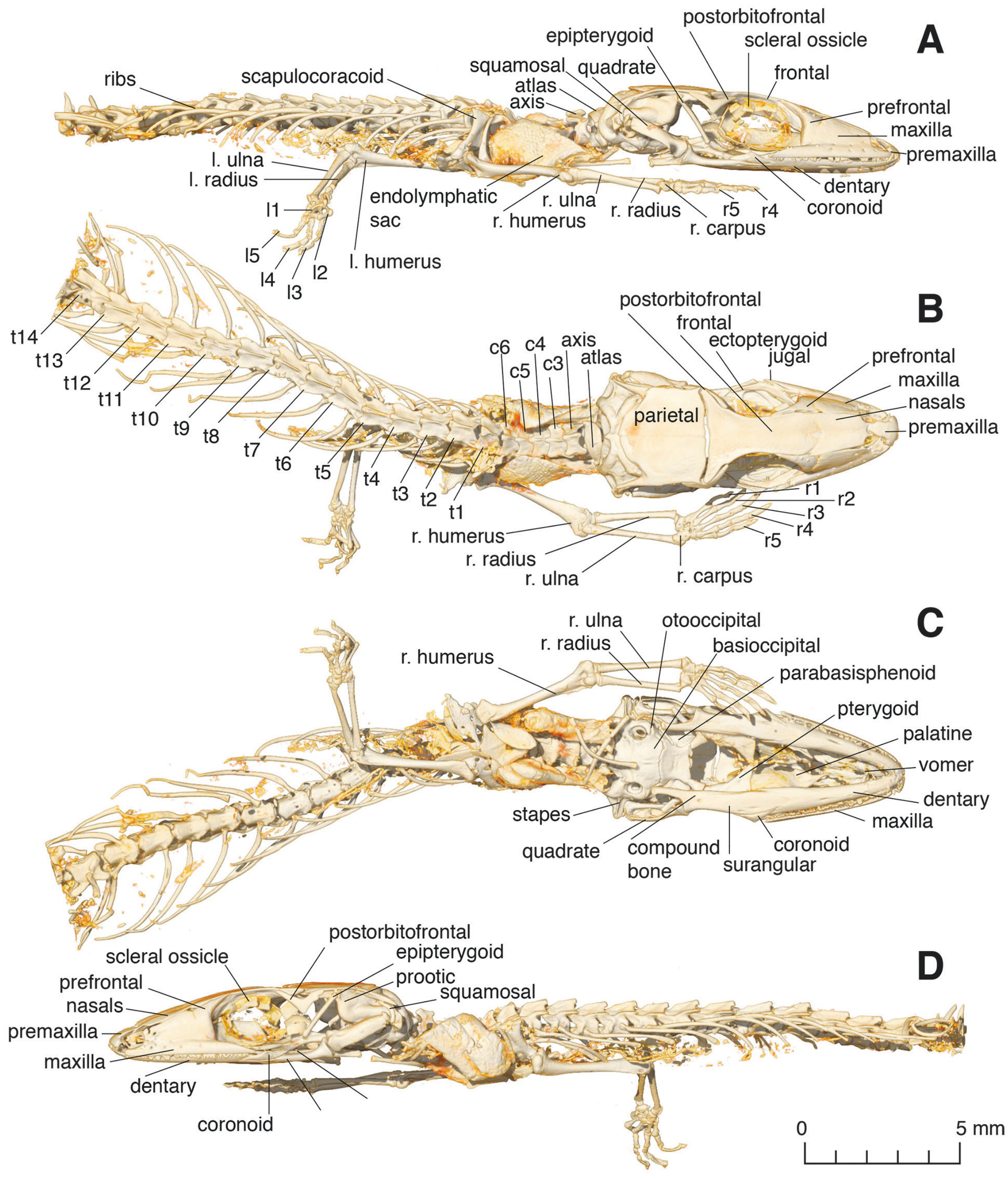

Fig. 3. Gecko in copal (*Ebenavia boettgeri*, ZFMK 94000) **A.** right lateral view, **B.** dorsal view; **C.** ventral view; **D.** left lateral view indicating the major bones. Abbreviations c#, cervical vertebrae #, l#, left toe #, r#, right toe #, t#, thoracic vertebrae #.

(Nosy Alañaña), as well as Mauritius (Ramanamanjato et al., 2002; Hawlitschek et al., 2017, 2018; Uetz et al., 2018). Until recently *Ebenavia* included only two species (*E. maintimainty* and *E. inunguis*). *Ebenavia maintimainty* has a restricted range, being found in Toliara Province in southwestern Madagascar (Nussbaum & Raxworthy, 1998), while the more widespread *E. inunguis* was recently split into four new species (viz., *E. boettgeri*, *E. robusta, E. safari, E. tuelinae*; Hawlitschek et al., 2018, Fig. 2). Copal deposits in Madagascar are concentrated in the northern part of the island (i.e., Cap D'Ambre and the Sava Region, Geirnaert 2002), which is compatible with the distribution of *E. safari* and *E. boettgeri* (Hawlitschek et al., 2018).

The specimen is embedded in a cone-shaped piece of orange resin (Fig. 1). The preservation is exceptional, conserving the complete anterior half of the body. It appears desiccated, but the skeleton is in perfect condition,

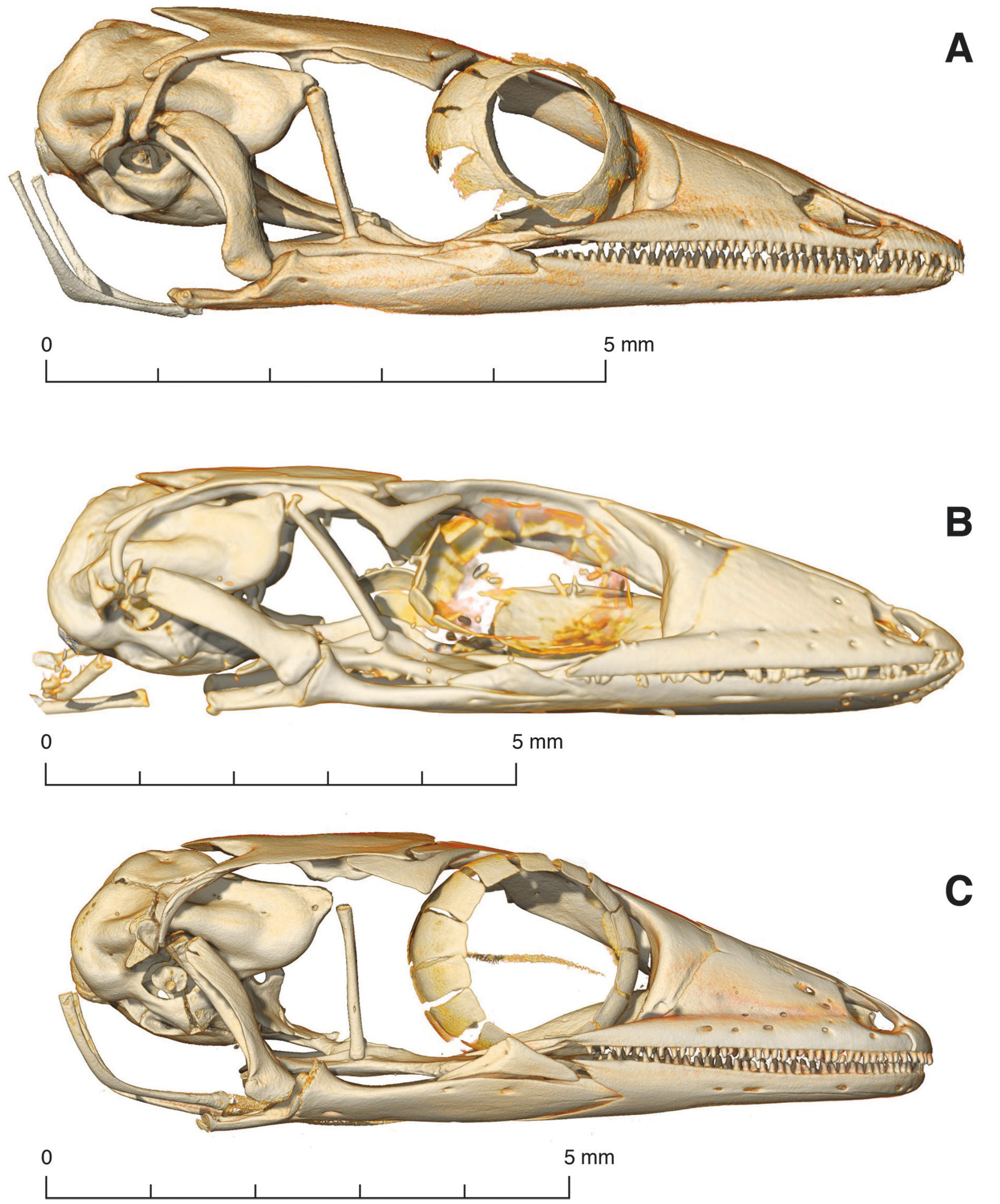

Fig. 4. HRCT of the skulls of three geckos. Sphaerodactylidae: **A.** *Sphaerodactylus semasiops* (MCZ R-55766); Gekkonidae: **B.** *Ebenavia boettgeri* (ZFMK 94000), and **C.** *Ebenavia boettgeri* (CAS 66195).

including the skull, vertebral column (all six cervical and 14 thoracic vertebrae), ribs, pectoral girdle and forelimbs (Figs 1, 3). The skull is intact, with the exception of the anterior portion of the left maxillary bone, which appears damaged, and both sclerotic rings, which are collapsed. Even fine details of the skeleton are visible (e.g., small sesamoids in the elbow; Fig. 3). Although having an intact skeleton is not necessarily an indication of its young geological age (for example, a Mesozoic gecko in amber exhibits a near pristine skeleton, Daza et al., 2016), the skeleton typically exhibits multiple fractures in the majority of Miocene *Sphaerodactylus* from Hispaniola. Using two X-rays from three ethanol preserved specimens of *Ebenavia boettgeri* (CAS 66195 [male, based on the presence of cloacal bones], CAS 66196 [gravid female with 2 eggs], 16º 54' 37.08" S, 49º 54' 40.716" E, St. Marie, and USNM 495825 25° 01' 12.0" S, 46° 58' 48.0" E [gravid female with 1 egg]) we were able to determine

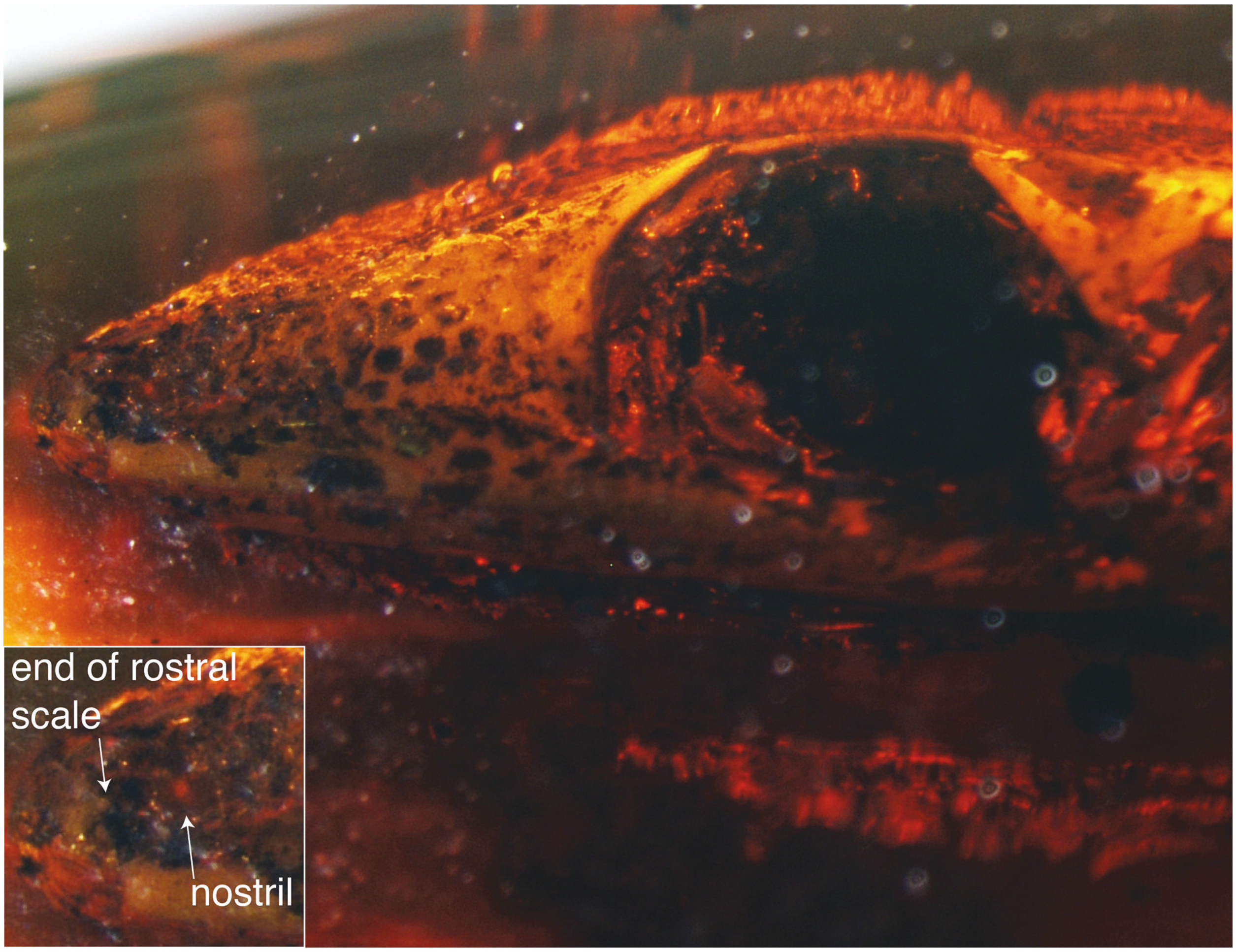

Fig. 5. Lateral view of the snout of ZFMK 94000, inset shows the separation between the rostral scale and the nostril.

that the missing portion of the precloacal region (SVL) is between 18 and 25% of the SVL. Using these values, the estimated SVL of the copal specimen is 34.8–37.8 mm. The estimated size matches several species of *Ebenavia,* although it greatly exceeds the adult size range of *E. maintimainty* (21–24 mm; Nussbaum & Raxworthy, 1998).

The specimen in copal was compared to similarly sized, formalin-fixed, ethanol preserved specimens of *E. boettgeri* from St. Marie (Fig. 4) and *E. robusta* (ZSM296/2010; Hawlitschek et al., 2018). Shared traits with *Ebenavia* include a small premaxilla with a short ascending nasal process (long in *Sphaerodactylus*); fused nasals (unfused in *Sphaerodactylus*); frontal broad with flat dorsal surface (narrow and convex in *Sphaerodactylus*); quadrate more or less straight with a slightly convex conch (curved and extremely convex conch in *Sphaerodactylus*); high number of foramina in the maxillary facial process (fewer foramina in *Sphaerodactylus*); high, discrete splenial (fused to coronoid in *Sphaerodactylus*); stapedial foramen absent (present in *Sphaerodactylus*);dentary ending at the level of the coronoid eminence (extending beyond the coronoid in *Sphaerodactylus*); and retroarticular process narrow (broad in *Sphaerodactylus*). Morphology of the manus is very similar between the two genera, both *Ebenavia* and *Sphaerodactylus* having the same (plesiomorphic) phalangeal formula (2-3-4-5-3) and similar relative length of toes, from largest to smallest III>IV>II>V>I.

The copal gecko is clearly differentiated from *E. maintimainty*. Head length in the *E. inunguis* group is 9.2–9.5 mm (~9.5 in the copal gecko and between 5.4–5.9 in *E. maintimainty*), dorsal scales are partially keeled in the *E. inunguis* group and the copal gecko (vs. fully keeled in *E. maintimainty*), and the rostral scale is broad in the *E. inunguis* group and the copal gecko (vs. narrow in *E. maintimainty*). Using the key from Hawlitschek et al. (2018), we were able to confirm that ZFMK 94000 has the rostral scale separated from the nostril (Fig. 5), which is a character that defines *Ebenavia boettgeri*. This identification is compatible with the fact that Madagascan copal mines are only found in the distribution range of *E. safari* and *E. boettgeri*.

Even if the gecko in copal is potentially less than several hundred years old, the material provides an historical record of a living species of *Ebenavia* in the northeast of Madagascar based on the known location of copal deposits (Geirnaert, 2002). The North of Madagascar is a critical area for understanding the current distribution of *Ebenavia* in Madagascar, as ancestral area reconstructions may indicate a colonization of northern Madagascar from the Comoros Islands (Hawlitschek et al., 2017).

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