First record and probable reproductive mode of a true Stumpffia from Ambohitantely Special Reserve in the central highlands of Madagascar

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The genus *Stumpffia* Boettger, 1881 includes some of the smallest vertebrates in the world, and it is the most diverse genus among the cophyline microhylid frogs of Madagascar (Vieites et al., 2009, Köhler et al., 2010, Klages et al., 2013, Perl et al., 2014, Scherz et al., 2016). The taxonomic status of the genus was recently the subject of a major revision that led to the description of 26 new species in addition to 15 previously recognized ones (Rakotoarison et al., 2017). With the recent description of *S. froschaueri* Crottini et al., 2020 the genus comprises 42 described species (AmphibiaWeb, 2020). With the current pace of species discovery in this group still high, many new species are likely still awaiting scientific recognition and description.

The mode of reproduction of *Stumpffia* is known only for a few members of the genus. *Stumpffia psologlossa* Boettger, 1881, *S. pygmaea* Vences & Glaw, 1991, and *S. analamaina* Klages et al., 2013 lay their eggs in foam nests in the leaf litter (Glaw and Vences, 1994; Klages et al., 2013). *Stumpffia davidattenboroughi* Rakotoarison et al., 2017 lays its eggs in a jelly nest in the leaf litter (Rakotoarison et al., 2017), and *S. achillei* Rakotoarison et al., 2017 was found to lay its eggs in a jelly nest inside water-filled snail shells (Rakotoarison et al., 2017). As far as known, all of these species have nidicolous,

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non-feeding tadpoles (Glaw and Vences, 1994; Klages et al., 2013; Rakotoarison et al., 2017). Reproductive modes are unknown for the other 37 members of this genus, severely limiting conservation efforts due to this missing life history information.

The Ambohitantely Special Reserve is one of the few remaining forest fragments in the central highlands of Madagascar. For several years, *Stumpffia helenae* (Vallan, 2000) was the only species of the genus considered to be present in this reserve. However, Scherz et al. (2016) concluded that this species is not, in fact, a *Stumpffia* but rather belongs to its own genus, *Anilany* Scherz et al., 2016. After that study, which was based on a nearly complete molecular phylogeny of cophyline microhylids, Ambohitantely remained without any known species of *Stumpffia*.

While carrying out a systematic transect in the northern part of the Ambohitantely Special Reserve between 08:30-11:00 h during the rainy season in December 2018, at GPS coordinates 18.1755°S, 47.2841°E (elevation 1560 m), we encountered for the first time an individual of a true Stumpffia species in this reserve (Fig. 1). As can be concluded from the approximate body size and body proportions, the frog was an adult, found in a bamboo node of 2.5 cm diameter cut off at 2 cm from the ground, filled with rainwater (Fig. 1b). The specimen was guarding a clutch containing 15 eggs. No tadpoles had yet developed. Eggs were unpigmented, as is typical for cophyline microhylids. Their diameter was not measured, but in the photographs the eggs appear to be relatively large. Disturbed by the process of egg counting, the frog emerged from the bamboo hole, but stayed nearby (Fig. 1).

The specimen had a snout–vent length of approximately 15–20 mm, with a distinct tympanum. The coloration of the dorsal side was reddish-brown with a dark brown triangle-shaped patch between the eyes and dark brown patches of varying sizes along the dorsum and sides. The ventral coloration was reddish, including the lower limbs. These features agree perfectly with the

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Figure 1. Photographs of *Stumpffia* cf. *kibomena* from Ambohitantely Special Reserve. (a) shows the frog emerging from the bamboo hole, (b) shows it submerged in the water-filled bamboo hole, along with a few eggs, and in (c) the specimen is outside of the tree-hole, and several of the rather large, unpigmented eggs can be seen. Photos by Jary Razafindraibe.

description of Stumpffia kibomena Glaw et al., 2015, as given in the original description (Glaw et al., 2015), and the specimen very likely belonged to this species. However, given the high number of cryptic species within Stumpffia, some of which are still unnamed, we cannot be certain of this identification without voucher specimens or DNA sequences (collection was not possible due to the lack of permits). Still, due to its extreme similarity in colour pattern with S. kibomena, the species can almost certainly be assigned to a clade of moderately-sized to large species of Stumpffia named Clade C by Rakotoarison et al. (2017), but this needs to be clarified by future studies. The current observation represents the first record of a Stumpffia laying eggs in a tree (bamboo) hole. This observation, together with the record of eggs in a water-filled snail shell for S. achillei, another representative of Clade C (Rakotoarison et al., 2017), suggests that species of this clade may reproduce opportunistically in various kinds of water-filled cavities near the ground. Although only eggs and early-stage embryos have been observed both in our study, and in S. achillei by Rakotoarison et al. (2017), the large size and lack of pigmentation of these suggests they will develop into non-feeding tadpoles, as in all species of cophylines for which the reproductive mode is known to date.

Previous reliable records of S. kibomena are restricted to the area around Andasibe, at elevations of 850-900 m in humid rainforest (Glaw et al., 2015; Rakotoarison et al., 2017). In comparison, S. cf. kibomena in Ambohitantely occurs at a higher elevation of 1560 m in partly subhumid forest in the central highland of Madagascar (Schatz, 2000; Glaw and Vences, 2007). There are numerous species of amphibians shared between Ambohitantely and Andasibe, but Ambohitantely also harbours several unique regional endemics not known from Andasibe and there is therefore reason to suspect that the Stumpffia population from the reserve may be genetically divergent, possibly constituting a distinct species. Further fieldwork must now be undertaken to collect voucher material of the population in Ambohitantely, which, if confirmed to be a new species, is doubtlessly critically Threatened with extinction.

Acknowledgments. We are grateful to our field assistants Rojo N. Ravelojaona and Etienne Ralovarisoa and to our guide Tovo Raditra for their help during fieldwork. We thank the Durrell Wildlife Conservation Trust's Madagascar Programme staff for logistical support to enable our work to take place. Funding was provided through a Mohammed bin Zayed Species Conservation Fund grant to Durrell Wildlife Conservation Trust. We also acknowledge the Malagasy authorities for providing permits to conduct this research, which was carried out under permit no.259/19/MEEF/SG/DGF/DSAP/SCB.Re.

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