Introduction

Gephyromantis is a monophyletic genus within the family Mantellidae that contains frogs of largely brownish coloration, which primarily inhabit the leaf litter in the rain forests of Madagascar (Glaw and Vences, 2006). Five subgenera are currently recognized (Duboimantis, Phylacomantis, Laurentomantis, Vatomantis, and Gephyromantis). However, a recent molecular study using both mitochondrial and nuclear genes revealed that the subgenus Gephyromantis is monophyletic only after exclusion of G. klemmeri Guibé, 1974, and that Duboimantis Glaw and Vences, 2006 includes two strongly supported clades of uncertain relationships (Kaffenberger et al., 2012). The focal group of the present study, the subgenus Vatomantis, is monophyletic and is the sister group to G. klemmeri + Laurentomantis Dubois, 1980.

The subgenus Vatomantis contains three species of small-sized frogs from north-eastern Madagascar (Vences, Glaw and Andreone, 1997; Vences, Glaw and Marquez, 2006; Glaw and Vences, 2006). Gephyromantis webbi and G. silvanus were originally described from the island of Nosy Mangabe, with G. webbi being additionally found on the adjacent mainland (Grandison, 1953; Vences, Glaw and Andreone, 1997; Glaw and Vences, 2007). According to current knowledge (Glaw and Vences, 2007; Gehring, Ratsoavina and Vences, 2010), G. rivicola has a distribution from Marojejy south to the Masoala Peninsula at elevations from 0-700 m, while Gephyromantis webbi and G. silvanus live in coastal lowland forests between Andranofotsy and Ambodiriana below 100 m elevation.

The three frog species in the subgenus Vatomantis are typically 22–33 mm in size (snout-vent length) and can be distinguished from other species of the genus due to their olive green coloration (Glaw and Vences, 2006). Vatomantis frogs are usually found within 20 m from streams containing large mossy stones and boulders. Species of the subgenus Vatomantis call during the day (G. webbi) and during the evening and night (G. silvanus), with G. rivicola being known to show both diurnal and nocturnal calling behaviour (Vences, Glaw and Andreone, 1997; Glaw and Vences, 2006). The calls of G. webbi from its type locality, Nosy Mangabe,
as well as call recordings of all three species from Andranofotsy, have been documented on an audio-CD (Vences, Glaw and Marquez, 2006). However, the bioacoustic analysis and description so far refers to the \textit{G. webbi} recordings from Nosy Mangabe only (Glaw and Vences, 1992; Vences, Glaw and Andreone, 1997) and is not very detailed.

It is known that calls in anurans play an important role in attracting females, advertising the position of an individual to conspecific males, defending calling sites and announcing a more aggressive behaviour (Wells, 1977, 2007; Duellman and Trueb, 1994; Ryan, 2001). Advertisement calls of anuran males are species-specific, and comparative bioacoustic analyses of frog vocalizations are valuable tools in the discovery of new taxa, assessment of taxonomic rank and species identification (Kok and Kalamandeen, 2008). The application of bioacoustics in an integrative taxonomic approach is especially important in tropical areas, as it opens the door to new frontiers of data exploration that may increase the rate of species discovery (Padial and De la Riva, 2009). The sometimes drastic, bioacoustic differences between similar species may act as an efficient premating isolation mechanism (Vieites et al., 2012). As body temperature has an impact on the call of anurans, this should also be considered in taxonomic comparisons among closely related species (Schneider and Sinsch, 2006). In mantellids, as well as in other anurans, temperature mainly influences inter-note intervals and note repetition rate but affects note length only weakly (Glaw et al., 2010; Vieites et al., 2012).

Here we describe and compare the calls of the three closely related \textit{Gephyromantis} species, all classified in the subgenus \textit{Vatomantis} (\textit{G. webbi}, \textit{G. rivcola} and \textit{G. silvanus})

\textbf{Materials and Methods}

All advertisement calls of the three species analysed here were recorded in close syntopy along a stream near Andranofotsy (-15.4353, 49.8439, 85 m a.s.l.), a coastal site near the small town of Maroantsetra, on 17 December 2001 during the afternoon and in the early evening (15–19 h), at a temperature of 25.4°C, by M. Vences. Calls were recorded in the field using a Sony WM-D6C tape recorder with an external microphone (Vivanco EM 238). Recordings of the three males were made at a distance of about 100-150 cm from the frogs. For \textit{G. silvanus} and \textit{G. webbi}, recordings of 10 s and 30 s were selected for analysis whereas for \textit{G. rivcola}, 60 s of continuous calling was selected, each recording probably representing one male. Recordings were digitized at sampling rate 22.05 kHz and 16-bit resolution and computer-analysed using the software CoolEdit Pro v. 2.0 (Syntrillium Software Corporation). Frequency information was obtained through Fast

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{oscillograms.png}
\caption{Oscillograms of \textit{G. silvanus} (left) and \textit{G. webbi} (right). Red-brown denotes one call, green represents one pulse, A is the inter-call interval, and B is the pulse interval. Each picture is a zoomed in view of the one above it and graphs are not to scale.}
\end{figure}
Fourier Transformation (FFT; width 1024 points). Spectograms were obtained at Hanning window function with 512 bands resolution. The temporal parameters “calls” and “pulses” are used according to Fig. 1. Call descriptions follow Vences et al. (2002) in structure and terminology. The analysed call recordings are deposited in full length in the Fonoteca Zoologica (www.fonozoo.com) of the Museo Nacional de Ciencias Naturales of Madrid, Spain (CD Code: MV07B-CD68; recording numbers: *G. rivicola*: 3016, 3030; *G. silvanus*: 3017-3025; *G. webbi*, 3026-3029). Those recordings used for analysis herein are the ones included in the CD of Vences, Glaw and Marquez (2006), and deposited in the Fonoteca under numbers 3016 (*G. rivicola*), 3025 (*G. silvanus*), and 3026 (*G. webbi*).

Dorsolateral and ventral views of representative frogs from the three species were photographed shortly after capture to ensure optimal representation of their natural coloration (Figs. 2-3). These specimens were euthanized with chlorobutanol, and subsequently fixed in 7% formalin solution and preserved in 70% ethanol. Voucher specimens were catalogued in the Zoologische
Staatssammlung München (ZSM), Germany for permanent storage. MV refers to field numbers of Miguel Vences. All specimens listed were collected at the same time and site as call recordings were made, but recordings cannot be assigned reliably to any specific voucher because the recorded individuals, although observed calling, could not be captured immediately upon calling.

Results

Call descriptions of *G. webbi*, *G. rivicola*, and *G. silvanus*

Calls of *G. webbi* were emitted during the day from mossy stones along a stream in rainforest, close to the recording site of *G. rivicola* and *G. silvanus*. Calls from *G. rivicola* were emitted during the day as well from rocks and the ground at hidden positions in an area where large boulders formed caves near a small stream in rainforest. Finally, the calls of *G. silvanus* were emitted in the late afternoon from within cave-like formations between large boulders, close to a small rainforest stream. Calling specimens of this latter species were very secretive but could be observed at one occasion. All males were calling more or less continuously.

*Gephyromantis webbi* (Grandison, 1953)


The call is reminiscent of the sound made by two stones smacking together in quick succession, generally in triplets, with the middle pulse having the highest energy. Calls were recorded from one of the four collected males and consisted of three pulses (n=3 calls) (Fig. 4). Call duration was 496-571 ms (523±42 ms, n=3 calls), duration of intervals between calls was 1697-2155 ms (1926±324 ms, n=2 calls). Pulse duration was 55-86 ms (73±12 ms, n=9 pulses), duration of intervals between pulses was 124-132 ms (129±3 ms, n=6 intervals). Frequency range was 1470-8879 Hz, with a dominant frequency range of 2756-4478 Hz (3968±341 Hz, n=3 calls).

*Gephyromantis rivicola* (Vences, Glaw and Andreone, 1997)


The frog call could be perceived as marbles rapidly clicking together. Calls of one male have 16-19 pulses (17±1 pulses, n=5 calls) (Fig. 5). Call duration was 1113-1543 ms (1257±169 ms, n=5 calls), duration of intervals between calls was 79-109 ms (97±11 ms, n=5 intervals). Pulse duration was 31-63 ms (46±6 ms, n=50 pulses), duration of intervals between pulses was 18-56 ms (34±9 ms, n=50 intervals). Frequency range was 1970-8263 Hz, with a dominant frequency range of 3229-4995 Hz (4689±341 Hz, n=5 calls). The pulses have descendant modulation, with the highest sound energy concentrated at the start of a pulse, in a short period with duration of 12 ms. Immediately after this condensed burst of sound, the pulse energy decreases rapidly.
**Gephyromantis silvanus**

(Vences, Glaw and Andreone, 1997)


The call (Fig. 6) sounds like an old door being closed, or a spoon being dragged along a washtub. The two recordings are analysed separately because of their marked difference. There is however no indication that the two calls were of fundamentally different types with different functions, such as territorial vs. mating. Instead, we hypothesize that the second recording includes simultaneously emitted calls of two males and therefore is difficult to analyze. We consider the first recording most reliable and informative for taxonomic purposes, hence our focus on the call parameters derived from it. Since calls were emitted from within a cave it was not possible to ascertain which individual has been recorded. We succeeded only in one single case to directly observe call emission and thereby assign this vocalization to *G. silvanus*, but the observed male could be neither recorded nor collected as it was calling from a deep fissure within the cave.

Recording 1 (single calling male): Call consisted of 49-57 pulses (53±4 pulses, n=3 calls). Call duration was 2143-2257 ms (2217±64 ms, n=3 calls), duration of intervals between calls was 1088-2243 ms (1666±817 ms, n=2 intervals). Pulse duration was 31-49 ms (41±2 ms, n=10 pulses), duration of intervals between pulses was 14-33 ms (24±6 ms, n=10 intervals). Frequency range was 1492-3776 Hz, with a dominant frequency range of 2067-3100 Hz (2526±526 Hz, n=3 calls).

Recording 2 (antiphonal call): Calls consisted of 99-117 pulses (107±6 pulses, n=7 calls). Call duration was 3152-4575 ms (4048±492 ms, n=7 calls), duration of intervals between calls was 154-733 ms (381±207 ms, n=6 intervals). Pulse duration was 31-49 ms (41±2 ms, n=10 pulses), duration of intervals between pulses was 12-26 ms (19±4 ms, n=10 intervals). Frequency range was 1311-4847 Hz, with a dominant frequency range of 1550-3445 Hz (2583±879 Hz, n=7 calls).
Discussion

Despite their importance in the biology of anurans, there is no standard methodology for describing calls. The definitions used herein were suggested by Duellman and Trueb (1994), and have been widely accepted and used by many others (e.g., Kok, 2010; Lehtinen et al., 2012; Kok et al., 2013). In this system, a call is defined as the assemblage of acoustic signals produced in a given sequence, a note is an individual unit of sound contained within a call, and a pulse is an energetic impulse in the temporal spectrum of a note. Due to their species specificity, knowledge on the characteristics of advertisement calls is integral in performing integrative taxonomic work, such as in the identification and discovery of species (Padial et al., 2010; Vences et al., 2012). Furthermore, once the call of a species is known, the information can be used in a variety of biological and ecological studies such as measuring species diversity by call analyses or determining a species reproductive habitat by determining call locations (Funk et al., 2011).

The sister subgenus to Vatomantis is Laurentomantis (Kafflenberger et al., 2012). This latter subgenus inhabits eastern and northern Madagascar and is composed of small frogs, with single subgular vocal sacs, mainly nocturnal activity, and in many species a remarkably broad head and warty dorsal surface (Glaw and Vences, 2006, 2007). Six Laurentomantis species are currently recognized, all of which have calls differing from the species analyzed here. The call of G. malagasius (Methuen and Hewitt, 1913) is composed of a single, long, pulsed note with a low frequency, which can last for as long as a 1.5 seconds (Vences et al., 2002). On the other hand, the call of G. horridus (Boettger, 1880) is composed of a fast sequence of unharmonious pulses with 16 to 33 widely spaced pulses (Vences et al., 2002). The calls of G. klemmeri and G. striatus (Vences, Glaw, Andreone, Jesu, and Schimmenti, 2002) are similar to the one of G. horridus although the former is faster (Vences et al., 2006) and the latter has a maximum of 6 pulses per note (Vences et al., 2002). The call of G. ventrimaculatus (Angel, 1935) is similar to the one of G. striatus, but is slower and has a lower intensity (Vences et al., 2002). Lastly, the call of G. ranjomavo Glaw and Vences, 2011 is not yet recorded, but appears to consist of a sequence of unharmonious, distinctly pulsed notes (Glaw and Vences, 2011). The calls of G. webbi and G. rivicola can be differentiated from all the calls associated with the sister subgenus by being slower and by each note being composed of less pulses in the former and more pulses in the latter. The call of G. silvanus can be differentiated by being longer and lasting for more than two seconds. Given that most Laurentomantis calls are composed by long series of pulses, the low number of pulses and the slow repetition rate found in G. webbi can be hypothesized to constitute a derived condition within the (Laurentomantis, Vatomantis) clade.

In comparison with the calls of G. webbi from the island Nosy Mangabe (described by Glaw and Vences 1992), which were recorded at similar temperature (23°C) it is remarkable that these calls were composed of up to 10 pulses whereas those from Andronofotsy contained only three pulses. Remarkable but poorly understood variation in call parameters is also evident between the two recordings of G. silvanus suggesting that further research is necessary to understand the acoustic repertoire of Vatomantis species.

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References

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