First assessment of the male territorial vocal behaviour
of a Malagasy leaf litter frog (*Gephyromantis thelenae*)

Katharina C. Wollenberg1* and James Harvey2

Abstract. We infer the role of vocalization in male territorial behaviour in an inconspicuous frog species (*Gephyromantis thelenae*). We test for differences in the vocal response of males to playback of conspecific calls versus calls of the syntopic sister species (*G. eiselti*). In addition, we describe a novel “aggressive” call type, produced to challenge territorial intruders. We found *G. thelenae* males to significantly increase their vocal activity towards conspecific playback, sometimes accompanied by positive phonotactic behaviour. A few males reduced their vocal response relative to control recordings, and also tended to show negative phonotaxis. These differences in territorial behaviour may be related to differences in call parameters affected by male quality. This indicates that male *G. thelenae* probably do not perceive calls of *G. eiselti* as conspecific competitors. Such acoustic niche partitioning might be the result of divergent sexual selection on advertisement calls that could have promoted speciation in these two closely related, syntopic species, but alternate explanations cannot be ruled out at this point until preferences of females are assessed and more data on population genetics are available.

Key words. Amphibia, Mantellidae; acoustic niche partitioning; bioacoustics; playback; phonotaxis; territoriality.

Introduction

Mantellid frogs of the *Gephyromantis boulenegeri* species group (subgenus *Gephyromantis*, Methuen, 1920; sensu Glaw and Vences, 2006) are small, brown, diurnal anurans that can be found in the leaf litter. Their distinctive loud calls make up a large proportion of the acoustic environment of the rainforests and secondary vegetation in eastern Madagascar. Described species belonging to this monophyletic group are *Gephyromantis blanci* Guibé, 1974, *G. boulengeri* Methuen, 1920, *G. decaryi* Angel, 1930, *G. eiselti* Guibé, 1975, *G. enki* (Glaw and Vences, 2002), *G. leuocephalus* Angel, 1930, *G. rueunewsweeki* Vences and De la Riva, 2007, and *G. thelenae* (Glaw and Vences, 1994). Due to significant genetic differences, we do not include *G. klemmeri* Guibé, 1974 in this subgenus (cf. Vieites et al., 2009). All species in this group are suspected to have endotrophic development (lacking a feeding tadpole stage), as they occur exclusively within forest leaf litter far from aquatic habitats. Endotrophic (nidicolous or direct) development has been confirmed in two of the eight species (*G. eiselti* and *G. enki*; Glaw and Vences, 2007), and an undescribed candidate species (*G. cf. blanci*, KCW own data). Despite their interesting life histories, information about the biology, mating behaviour and geographical modes of speciation in this group are very scarce, as these frogs are extremely secretive and difficult to observe. (I) The first objective of this paper is to assess the role of vocalization with respect to male territorial behaviour in one species of *Gephyromantis (G. thelenae)*.

Many lineages of frogs display high levels of morphological homoplasy, which often makes it virtually impossible to distinguish between closely related species by using external characters only (e.g., Emerson, 1986). In contrast, advertisement calls of frogs are directly linked to their reproductive behaviour, highly species-specific and potentially under sexual selection (e.g., Glaw and Vences, 1994). The call of a male frog is considered to serve as a quality trait, with different call parameters being linked to morphological and physiological properties, such as body size. One presumed mode of speciation, particularly in sister species with overlapping geographical ranges, is divergent sexual selection on advertisement calls due to changes in female preferences. This hypothesis has recently been confirmed in Neotropical frogs of the genus *Physalaemus*, using comparative methods (Boul et al., 2007).

In order to demonstrate that sexual selection drives speciation, the following criteria must be met: male signals must differ significantly among populations or recent sister species; females must prefer local male signals to foreign ones; divergence in male signals must result from selection and not genetic drift; and finally,
divergent signals and preferences must be correlated with restricted gene flow at nuclear loci, to show that populations are diverging towards distinct species, i.e. incipient species (Boul et al., 2007). Both ecological speciation to exploit different niches and differences in breeding phenology are also possible, but would not necessarily result in calling signal divergence. Gephyromantis thelenae bears high morphological resemblance to its sympatric and, in some localities, syntopically occurring sister species, G. eiselti, but can be distinguished from it by differences in male advertisement calls.

The study of female preferences in these frogs is very difficult, as females are even harder to find than males (as they do not vocalize, they might only occasionally be caught in pitfall traps). We therefore argue that male-male interactions also are a good proxy to delimit Boul et al.’s (2007) first criterion of significant call divergence, and test for differences in the behavioural response of male G. thelenae to conspecific versus interspecific calls (broadcast as playback). (II) We assume that a difference in behavioural response towards the two playback categories provides observational evidence for acoustic niche partitioning, which would make them candidates for divergent sexual selection that could have promoted speciation in these closely related sister species, in which case further effort to assess female preferences would be warranted.

The most common call type produced by frogs is the male advertisement call to attract females and to deter rival males, which can also have the additional function of delimiting territories (but not coercively, see Ryan, 1985). Fights can often be observed, when physical territories are violated by a conspecific intruder (Gerhardt and Huber, 2002). This call type is much more frequently produced than the response call of a female towards a male (reciprocation call, e.g., Shen et al., 2008), and the the release call during amplexus and the distress call (emitted towards a predator), respectively (for Malagasy frogs described in Glaw and Vences, 2007). Male phonotactic reactions towards the three different types of stimulus.

Materials and Methods

The vocal behaviour of male Gephyromantis thelenae was observed during the rainy season in January – February 2008, at two sites in the area of Andasibe – Mantadia National Park in central-eastern Madagascar: i) in the vicinities of the village of Andasibe, on a hilltop adjacent to a track, in secondary vegetation (dense ferns with interspersed Eucalyptus trees; 973 m asl; 18°55.069’S; 48°25.019’E), ii) at a site between Torotorofotsy swamp and Park Mantadia, known locally as Camp Prolenum; 986 m asl; 18°46.254’S; 48°25.917’E) in secondary vegetation (dense ferns with interspersed Eucalyptus trees). Calls of individual frogs of both G. thelenae and G. eiselti (sister species relationship confirmed in Vieites et al., 2009) were taken from a CD of frog calls of Madagascar (Vences et al., 2006) and repeated automatically, and then broadcast as conspecific or heterospecific playback to individuals of G. thelenae using a Sandisk Sansa Shaker mp3-player with a built-in speaker in close proximity to the frog. Signal amplitude was comparable to the sound pressure emitted from frogs encountered at the site (initially 30cm distance, amplitude not measured, but equal for all stimuli). We know that the used equipment (speakers) was not up to standard as compared to other bioacoustic behaviour studies in frogs, however we think that this was sufficient for a first bioacoustic assessment in this sympatric frog sister species pair.

Playback recordings were made in sets of 10 minutes. As a control, the individuals were first recorded for 10 minutes prior to conspecific experiments, and optionally followed by heterospecific and aggressive call playback (that were only tested on males that decided to stick around and be tested again). We collected supporting information on habitat, weather conditions and movement of the calling individuals relative to the speaker (phonotaxis) for each of those sets, and frogs were occasionally also videotaped. In total, the observations were made on seven individuals over seven days (these were identified by their localized home ranges and by comparison with reference pictures that were taken after initial visualization). Under those field conditions, the individual frogs were tested for different playback categories and varying numbers of replications. For instance, some frogs were not encountered again after one set of playback, while another male showed site fidelity and could be found and tested over several days. Because of this small sample size, it made sense to pool the data of individuals in order to have enough datapoints for preliminary tests – but this procedure did not allow to take
Figure 1. Sonagrams (upper) and oscillograms (lower) showing 10 seconds of A - advertisement call of *G. thelenae* B - aggressive call of *G. thelenae* (cf. Table 1, call No. 5) C – advertisement call of sister species *G. eiselti*. 
Table 1. Bioacoustic characters of the aggressive call of *Gephyromantis thelenae*. Eight recorded calls of the call voucher ZCMV 8131 were analyzed. For each call, pulse- and interpulse duration of all pulses were measured. In the summary row, temporal variables are given as mean ± standard deviation (minimum – maximum). ND = not defined.

<table>
<thead>
<tr>
<th>Call no.</th>
<th>Note duration in ms</th>
<th>Number of pulses per note</th>
<th>Pulse duration in ms</th>
<th>Inter-pulse interval duration in ms</th>
<th>Mean pulse rate per second</th>
<th>Dominant frequency in Hz</th>
<th>Fundamental frequency in Hz</th>
<th>Frequency range in Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75.0</td>
<td>5</td>
<td>2.6 ± 0.46</td>
<td>15.3 ± 4.75</td>
<td>66.6</td>
<td>7844</td>
<td>2000</td>
<td>18000</td>
</tr>
<tr>
<td>2</td>
<td>278.8</td>
<td>14</td>
<td>3.2 ± 0.59</td>
<td>17.7 ± 2.29</td>
<td>50.21</td>
<td>3551</td>
<td>2000</td>
<td>16000</td>
</tr>
<tr>
<td>3</td>
<td>169.4</td>
<td>9</td>
<td>1.9 ± 0.46</td>
<td>18.6 ± 1.59</td>
<td>53.13</td>
<td>4346</td>
<td>1000</td>
<td>16000</td>
</tr>
<tr>
<td>4</td>
<td>63.8</td>
<td>4</td>
<td>3.8 ± 1.86</td>
<td>16.6 ± 2.09</td>
<td>62.69</td>
<td>7738</td>
<td>2000</td>
<td>18000</td>
</tr>
<tr>
<td>5</td>
<td>307.7</td>
<td>15</td>
<td>2.6 ± 0.47</td>
<td>19.1 ± 3.23</td>
<td>48.75</td>
<td>7049</td>
<td>1000</td>
<td>17000</td>
</tr>
<tr>
<td>6</td>
<td>125.3</td>
<td>7</td>
<td>2.6 ± 0.42</td>
<td>17.7 ± 2.13</td>
<td>55.87</td>
<td>3975</td>
<td>2000</td>
<td>18000</td>
</tr>
<tr>
<td>7</td>
<td>108.5</td>
<td>6</td>
<td>2.6 ± 0.36</td>
<td>18.4 ± 1.39</td>
<td>55.3</td>
<td>8003</td>
<td>2000</td>
<td>18000</td>
</tr>
<tr>
<td>8</td>
<td>29.3</td>
<td>2</td>
<td>2.7 ± 1.98</td>
<td>23.7 ± ND (ND – ND)</td>
<td>68.26</td>
<td>6678</td>
<td>2000</td>
<td>18000</td>
</tr>
</tbody>
</table>

Summary: 144.7 ± 101.11 (29.3 – 397.7), 7.8 ± 4.65 (2.0 – 15.0), 2.7 ± 0.80 (1.1 – 5.1), 18.12 ± 2.55 (8.3 – 23.7), 57.6 ± 6.91 (48.8 – 66.6), 6148 ± 1876.6 (3551 – 8003), 1750 ± 462.9 (1000 – 2000), 1000 – 20000 (19000).

### Results and Discussion

Many observed male *G. thelenae* maintained their respective calling positions within the forest over the entire observation period. Males were always found calling near logs, piles of leaf litter or similar microhabitats that can support high local humidity, probably being related to their presumed endotrophic mode of development.

In most cases, individuals called from elevated positions (e.g. on ferns or branches), 20 to 100 cm above the ground. In contrast to Glaw and Vences (2007), who reported species of the subgenus *Gephyromantis* as calling solitarily, we found *G. thelenae* males calling in loose choruses with members of these choruses calling approximately 100 to 300 cm apart from each other in close temporal succession, and subsequently followed by other, more distant conspecific choruses.
Description of the aggressive call

Calls of the subgenus *Gephyromantis* have so far been thought to consist of only one call type (i.e., the advertisement call, Figure 1A). We observed an additional call type that was emitted by two out of the seven males as response to broadcasting of conspecific advertisement calls in humid weather conditions (Figure 1B). These males were also highly motivated, as they showed a higher vocal response (number of notes per minute) than males who did not emit the novel call type (call voucher ZCMV 8131 and ZCMV 8144, Figure 2). These "aggressive" calls were always emitted in close proximity to the speaker (~30 – 0 cm distance as the frogs were moving phonotactically), and can therefore be interpreted as male-male aggression vocalization to challenge rival males, which is the first observational confirmation of such a vocalization for Malagasy frogs. The total number of observed aggressive calls during the observation period was \( n = 20 \), but only eight calls could be analyzed with CoolEdit, as the others were not recorded but were videotaped instead and were not available in .wav format (Table 1). The aggressive call of *G. thelenae* consists of a number of single unharmonious pulsed notes, with high variation in both note duration and the number of pulses emitted per note. In addition, the dominant frequency varied considerably between calls. The notes are clearly pulsed and resemble a succession of “click” sounds. Parameters that were found to be relatively constant among the analyzed calls were pulse- and interpulse duration, and the frequency parameters, fundamental frequency and frequency range. We did not evaluate the duration of inter-note intervals, as the aggressive call was irregularly emitted.

Response to conspecific playback and phonotaxis

Male *Gephyromantis thelenae* were found to significantly increase the number of notes per playback minute relative to the control recordings when conspecific advertisement calls were broadcast (i.e., the calls were longer \( p < 0.01 \); Table 2, Figure 3, Figure 4A), whereas no difference was found in the number of calls emitted per playback minute (Table 3). Broadcasting of the conspecific aggressive call type also resulted in a significant increase in the number of notes, as well as in the number of emitted calls (\( p < 0.01 \), Table 2; \( p < 0.001 \), Table 3; Figure 3, Figure 4B; although with low effect sizes). Important for the interpretation of these results is, that the responses to conspecific playback almost exclusively were calls overlaying the stimuli (as shown in Figure 4A, 4C). As an observation we might state here that the males really seemed to try very hard to be louder than the playback in their vocal response, and to call at the same time. On the other hand, responses to heterospecific playback never overlaid the playback signal.

Phonotactic behaviour of both males and females towards broadcast conspecific calls has been well documented for many frog species since the 1980’s (e.g., Hödl, 1982; Hödl, 1987; Passmore et al., 1984; Narins et al., 2003). We can confirm phonotactic behaviour as a response towards both conspecific advertisement and aggressive calls in mantellid frogs, which provides additional evidence of territoriality in *G. thelenae*. When the playback was broadcast, males showing positive pho-
notaxis called during the stimulus while moving closer
to the speaker with a hopping motion. The maximal ex-
tent of such behaviour was sitting on top of the speaker
or the observer holding the speaker, while screaming
advertisement calls very loudly. But we also observed
that some males responded to conspecific playback less
than they emitted in the control recording. These males
also tended to show negative phonotactic behaviour (i.e.
moving away from the speakers and eventually out of
sight), which might partly account for the high variance
observed in the response to conspecific playback (to-
gether with varying weather conditions, Figure 3).

Although we could not find significant differences
(Kruskal-Wallis ANOVA, results not shown), there
seems to be a relationship between the number of no-
tes and calls emitted per minute, and the direction of
phonotactic behaviour. Frogs that responded least to
playback tended to show negative phonotaxis, where-
as positive phonotaxis was observed in individuals that
emitted both a high number of notes and calls per minu-
te (Figure 5A, B). One of the males was tested on four
different days (24., 25., 26., and 28. January 2009, and
exhibited calling site fidelity during that time).

Furthermore, we found the calls of males that showed
negative phonotactic behaviour to have higher dominant
and fundamental frequencies than the playback, whereas
males that showed positive phonotactic behaviour were
more similar in frequency parameters to the playback
calls (Figure 5C, D). Call frequency parameters have
been found to be correlated with body size in frogs, and
are used as assessment cues for fighting ability of a terri-
torial intruder (e.g., Davies and Halliday, 1978). The ob-

![Figure 4](Image)

**Figure 4.** Typical temporal successions of playback and reactions A – reaction (larger peaks, asterisks) towards conspecific advertisement call playback (smaller peaks) B – aggressive call (asterisks) emitted towards conspecific advertisement call C – advertisement calls (larger peaks, asterisks) emitted in the time intervals between broadcast advertisement calls of the sister species *G. eiselti* (smaller peaks). Axes not to scale - grey bars below oscillograms indicate 1 second.
served negative phonotaxis combined with site fidelity of males showing positive phonotaxis can therefore be interpreted as territorial behaviour (although further data and tests are needed to assess the physical properties of these territories). Territories could harbour suitable egg deposition sites, as males were calling from the top of elevated structures like logs and tree stumps surrounded by leaf litter. As negative phonotaxis was observed in three males only, we did not apply any statistical tests, so these results should be considered tentative.

Male *G. thelenae* are dorsally camouflaged, but have a conspicuous yellow colouration on the jaw and axils, together with black spots that are characteristic of all species in the subgenus *Gephyromantis*. The inner thighs have a reddish colouration. We found the intensity of this yellow-reddish colouration to vary significantly among the observed males. The specimen that emitted this yellow-reddish colouration to vary significantly so these results should be considered tentative.

**Response to playback of sister species**

*G. thelenae* males reacted towards broadcast calls of the sister species *G. eiselti* by significantly reducing the overall number of calls and notes relative to the response when confronted with conspecific advertisement calls ($p < 0.0001$, Table 2, $p < 0.001$ Table 3). Furthermore, the number of calls and notes per minute was even significantly lower than in the control recordings ($p < 0.0001$, Table 2, $p < 0.01$ Table 3). The call of *G. eiselti* consists of a long series of rather short, melodious notes, and all observed *G. thelenae* males avoided signal overlays with these signals (in contrast to the conspecific stimuli) by calling in the short intervals between the notes (Figure 4C). We did not observe phonotaxis (positive or negative) as behavioural response towards this playback category.

The uncorrected genetic p-distance between *G. eiselti* and *G. thelenae* for a fragment of the mitochondrial 16S rRNA gene that has been considered suitable for DNA barcoding in amphibians is 2.23% (falling below a proposed threshold of 3% for distinct anuran species, Fouquet et al., 2007; Vieites et al., 2009) and morphological differences between the two species are low (Glaw and Vences, 2002). Both species have single subgular vocal sacs, in contrast to all other species in the subgenus *Gephyromantis* that have bilobate vocal sacs when inflated. Nevertheless, their advertisement calls are strikingly different from each other (long series of short, melodious notes in *G. thelenae*, versus short series of long, unmelodical and pulsed notes in *G. eiselti*, Grazi and Vences, 2002, Vences et al., 2006).

Furthermore, the two species occur sympatrically (and in some cases, syntopically) between Andasibe and Mantadia National Park (own observations). Therefore, we consider the fact that male *G. thelenae* did not react to calls of *G. eiselti* (i.e., reducing their vocal response) as evidence for acoustic niche partitioning (cf. Martins et al., 2006), and as a possible signature of disruptive sexual selection promoting divergence in advertisement calls that possibly led to speciation in sympatry in these two recently diverged frog lineages – however, these conclusions remain speculative until further testing.

**Table 2.** Results of bivariate Kruskal-Wallis ANOVA (test statistics = KW-$H$) for differences in response between different playback categories. Response is measured as the number of calls per minute. Significant reactions are in bold. As the significance level for $n = 4$ non-independent tests is 0.0125 after Bonferroni correction, all differences found are significant.

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>KW-$H$</th>
<th>$p$ - level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><em>G. thelenae</em> advertisement call</td>
<td>8.90</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Control</td>
<td><em>G. thelenae</em> aggressive call</td>
<td>7.17</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Control</td>
<td><em>G. eiselti</em> advertisement call</td>
<td>23.73</td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td><em>G. thelenae</em> advertisement call</td>
<td><em>G. eiselti</em> advertisement call</td>
<td>31.82</td>
<td>$p &lt; 0.0001$</td>
</tr>
</tbody>
</table>

**Table 3.** Results of bivariate Kruskal-Wallis ANOVA (test statistics = KW-$H$) for differences in response between different playback categories. Response is measured as the number of calls per minute. Significant reactions are in bold. As the significance level for $n = 4$ non-independent tests is 0.0125 after Bonferroni correction, all differences found are significant.

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>KW-$H$</th>
<th>$p$ - level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><em>G. thelenae</em> advertisement call</td>
<td>1.32</td>
<td>$p = 0.251$</td>
</tr>
<tr>
<td>Control</td>
<td><em>G. thelenae</em> aggressive call</td>
<td>10.98</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>Control</td>
<td><em>G. eiselti</em> advertisement call</td>
<td>6.19</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td><em>G. thelenae</em> advertisement call</td>
<td><em>G. eiselti</em> advertisement call</td>
<td>10.51</td>
<td>$p &lt; 0.001$</td>
</tr>
</tbody>
</table>
Figure 5. Box plots of the responses under different types of phonotaxis; A - number of calls per minute B - number of notes per minute and call; C – dominant frequency compared to conspecific playback (highlighted in gray) D – fundamental frequency compared to conspecific playback (highlighted in gray). Square = Median; box = 25-75% interval of data, circle = outlier, filled square = extreme value.
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References


BHWK.


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