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The advertisement calls of *Theloderma corticale* (Boulenger, 1903), *T. albopunctatum* (Liu & Hu, 1962) and *T. licin* McLeod & Ahmad, 2007 (Anura: Rhacophoridae)

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Abstract. Based on the species specificity of anuran vocalization, bioacoustics can be utilized in terms of species identification and species delimitation. The genus *Theloderma* comprises 23 to 29 species, depending on inclusion of the (sub)genera *Nyctixalus* and *Stelladerma*, from which the majority of 14 species was described in this century. In spite of numerous publications about species descriptions and phylogenetics, studies about life history traits, particularly about advertisement calls, are lacking for the most species. In this study, acoustic signals of the mossy or bug-eyed frogs *Theloderma corticale*, *T. albopunctatum* and *T. licin* were recorded, and detailed temporal and spectral advertisement call properties are presented and compared to other congenerics (*T. auratum*, *T. stellatum*, *T. vietnamense*). We found that the advertisement calls of the six herein compared species are species-specific and are significantly distinguishable from each other. While the temporal features (i.e. arrangement in call groups, note repetition rate) are species-specific call properties, the spectral features (i.e. dominant frequency) can partially overlap among the small-sized species.

Key words: Amphibians, bioacoustics, bug-eyed frogs, mossy frogs, species delimitation, species identification

Introduction

Acoustic signals produced by animals primarily serve as a method of advertising the presence of one individual to others of the same species, and many studies suggested that anuran acoustic properties are species-specific since they act as powerful premating isolation mechanisms (cf. Duellmann & Trueb 1994, Marquez & Bosch 1995, Gingras et al. 2013, Röhr et al. 2020). Based on these species-specific acoustic signals in anurans, bioacoustics is an established tool providing informative features for identifying and delimiting species (Köhler et al. 2017, Röhr et al. 2020). Next to taxonomic applications (Padial et al. 2008, Glaw et al. 2010, Jansen et al. 2011, Röhr et al. 2020), bioacoustics can be used in environmental monitoring programs including approaches for estimating the size of anuran populations (Duellmann & Trueb 1994, Nelson & Graves 2004, Dorcas et al. 2009, Crovetto et al. 2019), or as indicator of environmental health (Lebboroni et al. 2006, Price et al. 2007, Alonso et al. 2017).

Anurans can provide a complex vocal repertoire with spectral and temporal properties that are important for species recognition (Schwartz 1987, Chuang et al. 2016, Röhr et al. 2020). Based on their function anuran vocalizations can be classified as one of the three main call types (Toledo et al. 2015): reproductive, aggressive, or defensive call. These categories can be subdivided in 13 sub-categories, in which the mating (Bogert 1960) or advertisement calls (Wells 1977) represent the primary vocalization (Duellmann & Trueb 1994, Ryan 2001, Narins et al. 2007, Wells 2007, Chuang et al. 2016, Emmrich et al. 2020).

The genus *Theloderma* Tschudi, 1838 (Anura: Rhacophoridae) currently comprises 23 to 29 small- to large-sized arboreal frogs depending on the inclusion of the three members of the (sub)genus *Nyctixalus* Boulenger, 1882 and three members of the (sub)genus *Stelladerma* Poyarkov et al. 2015 (Frost 2020). The majority of 14 species were described in this century (Stuart & Heatwole 2004, Orlov & Ho 2005, Orlov et al. 2006, McLeod & Ahmad 2007, Bain et al. 2009, Fe et al. 2009, Rowley et al. 2011, Poyarkov et al. 2015, Nguyen et al. 2016, Sivongxay et al. 2016, Dever 2017, Poyarkov et al. 2018). The genus *Theloderma* sensu stricto (later abbreviated with s.str.) is distributed in northeastern India to Myanmar and southern China through Indochina to Malaysia, Sumatra and Borneo (Frost 2020). However, the taxonomical status of some members, especially *T. moloch* (Annandale, 1912) (Li et al. 2009, Pyron & Wiens 2011, Li et al. 2013, Nguyen et al. 2015, Lalronunga & Lalhinchrana 2017, Mian et al. 2017), and the relationship to the genera *Nyctixalus* (Rowley et al. 2011, Li et al. 2013, Sivongxay et al. 2016) and *Philautus* Gistel, 1848 is strongly debated. While Frost et al. (2006) considered *Theloderma* to be the sister taxon of *Philautus,* other authors (Yu et al. 2009, Li et al. 2013) considered *Theloderma* as sister taxon of *Nyctixalus* or consider *Nyctixalus* as subgenus of *Theloderma* (for a review see Poyarkov et al. 2015). *Theloderma* comprises several cryptic taxa, which complicates species delimitation. For example the *T. asperum group* or the subgenus *Stelladerma*, which are morphologically nearly identical but genetically distant, or the *T. truongsonense clade*, which has distinct morphological characteristics but small genetic distances (Poyarkov et al. 2015, Dever 2017).

In spite of the high number of species descriptions and phylogenetic publications, studies about life history traits are lacking for the most species. In view of their vocalizations, *Theloderma* species are highly diverse. *Theloderma corticale* and *T. asperum* (sensu lato, later abbreviated with s.l.) have a variety of calls, performed by both females and males, for yet unknown purposes (Kunz et al. 2010, Mattison 2011), although descriptions of advertisement calls or other call types are lacking for the most species. In this study, we analyze and describe temporal and spectral call features of *T. corticale* (Boulenger, 1903), *T. albopunctatum* (Liu & Hu, 1962) and *T. licin* McLeod & Ahmad, 2007. Further, we compare these calls with already described advertisement calls of *T. auratum*, *T. stellatum* and *T. vietnamense* (Poyarkov et al. 2015, Poyarkov et al. 2018).

Material and Methods

Species selection

We recorded advertisement calls of three *Theloderma* species. Study populations comprise the specimens of *T. corticale* (n=5), *T. albopunctatum* (n=10) and *T. licin* (n=4; Figure 1). *Theloderma corticale*, colloquially known as Mossy frog because of its illusive appearance (Kunz et al. 2010, Mattison 2011), is a large-sized member of the *T. leporosum group* (Poyarkov et al. 2015). The species is distributed in China, northern Vietnam and Laos (Frost 2020). *Theloderma albopunctatum* and *T. licin*, both belong to the *T. asperum group* (Poyarkov et al. 2015). This species group contains several small to large-sized taxa characterized by a uniform reddish-brown iris and large white blotches on the rear part of their dorsum (except for *T. petilum*, which does not have white blotches) (Nguyen et al. 2015, Poyarkov et al. 2015). The species *T. asperum* s. l. was suggested to encompass at least three genetically distant but morphologically almost identical taxa. For certainty, *T. asperum* s. str. is only known from its type locality in Perak, Malayan Peninsula but probably all populations south of the Isthmus of Kra across southern Thailand and the Malayan Peninsula can be assigned to *T. asperum* s.str.. At least populations from China, central and northern Vietnam, and Laos can be assigned to *T. albopunctatum* (Poyarkov et al. 2015). Recently, Dever (2017) suggested that the populations from northern and western Myanmar represent another cryptic species, *T. pyaukkya*. *Theloderma licin* is closely related to *T. asperum* s.str. but can morphologically easily be distinguished from it by having a smooth skin, specific coloration and other species-specific characteristics (for a detailed description see McLeod & Ahmad 2007). *T. licin* occurs on the Malayan Peninsula possibly as far north as the Isthmus of Kra, and western Sarawak, western Malaysia (Borneo) (Frost 2020).

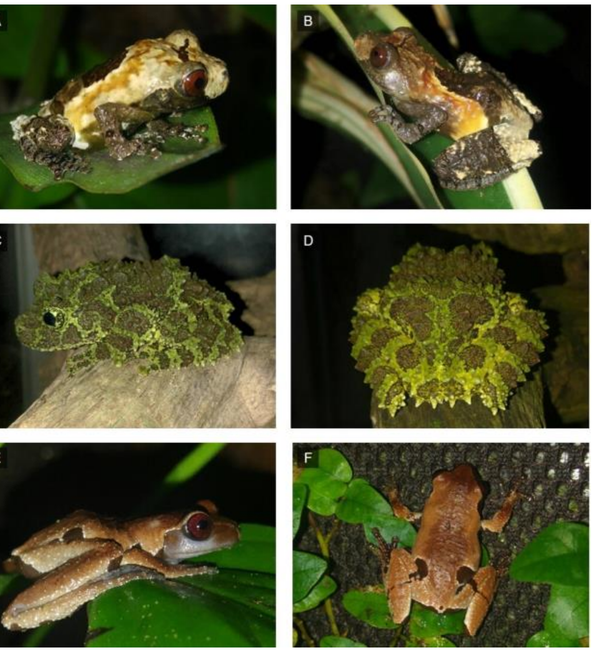

Figure 1. Photographs of selected specimens of all studied populations. A, B: *T. albopunctatum*. C: *T. corticale.* D: *T. corticale* with large conical warts near the vent. E: *T. licin*. F: *T. licin* with two visible continuous lines formed by brown coloration of the dorsum and the adpressed hind legs.

Species identification

The frogs were obtained as captive bred animals from zoo stock and since some species of *Theloderma* are hard to distinguish from each other, species identification was confirmed by DNA barcoding using a fragment of the mitochondrial 16S rRNA. Sequences were generated by following the procedure described in Koch et al. (2013) and subsequently compared with newly generated sequences of specimens from the ZFMK collection and 167 sequences of *Theloderma* spp. obtained from GenBank. We used Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) via the web version (https://bioinfo.mnhn.fr/abi/public/abgd/) to assign sequences to species. Furthermore, uncorrected p-distances were calculated using the ape package for R to investigate sequence divergences. All sequences produced by this study were uploaded to GenBank (SUB9831879 DR01 MZ376706 to SUB9831879 DR01 MZ376722).

Animal husbandry

The population of *T. corticale* was kept in an enclosure measuring 80 cm x 60 cm x 60 cm (H x W x D). The walls and the bottom of the enclosure were uncovered, the bottom was filled with water, and the terrarium was equipped with branches and bark. Rain was simulated by using an irrigation system running at 0800 and 1500 h for two minutes. The ambient temperature was between 18 and 28 °C due to season and daytime.

The population of *T. albopunctatum* was kept in an enclosure measuring 50 cm x 120 cm x 50 cm (H x W x D). The walls were covered with Hygrolon®, and the bottom of the enclosure was filled with water and equipped with branches. Vegetation of the terrarium comprised tropical plants such as the bromeliad *Vriesea era,* some mosses and tendrils. Rain was simulated by using an irrigation system running three times per day for two minutes. The ambient temperature was between 18 and 25 °C due to season and daytime.

The population of *T. licin* was held in an enclosure measuring 70 cm x 60 cm x 50 cm. The walls were covered with Hygrolon®, and the bottom was filled with water and equipped with pieces of bark. Vegetation of the terrarium comprised tendrils and some mosses. Rain was simulated by using an irrigation system running three times per day for two minutes. The ambient temperature was between 18 and 25 °C due to season and daytime.

Audio recording and subsequent analyses

Other than the enclosure of *T. corticale*, the enclosures of *T. albopunctatum* and *T. licin* were both submitted to a higher level of background noise, since they were placed in a room containing a higher number of ambient terrariums and aquariums connected to water pumps. The acoustic signals were recorded in May 2019 between 2100 and 2300 h by holding a Handheld Solid State Recorder (Marantz PRO Model PMD520MKII) near the sound-permeable grit at either the enclosure's top or front side. The sampling frequency was 44.1 kHz. For inducing vocalization rain was also simulated using a water spray bottle before each recording sequence. Additionally, for inducing vocalization in *T. corticale,* the record of a frog chorus at a pond in Borneo (Anderson 2014) was played. After each recording the air temperature was measured immediately using KlimaLogg Pro Thermo-Hygro-Logger (Kat.Nr. 30.3039.IT) and a Non Contact Infrared Body Thermometer (Model UV-8810). For purposes of taking photographs and filming, a smartphone LG G4 (Model H815) was used. All calls were cut and processed using Audacity V. 2.3.1 (Audacity Development Team 2019, available at http://audacity.sourceforge.net). Noise reduction was utilized before measuring *T. albopunctatum* and *T. licin* calls to reduce the above-mentioned background noise. Measurements of note duration, inter-note length, total number of notes per call and note repetition rate were taken using oscillograms; Spectrograms were used to measure dominant frequency and harmonics based on plots with a Hanning Window with a size of 512 and a logarithmic representation. For visualization of oscillograms and spectrograms the seewave package for R 3.3.2 (Sueur et al. 2008, R Core Team 2019) was used. In addition, mean values, maxima and minima of call features were computed. We compared the calls with already existing call descriptions of congenerics (Poyarkov et al. 2015, Poyarkov et al. 2018). For this, we adapted the terminology used by the latter authors and unified the following termini: Call duration = note duration, inter-call interval duration = inter-note length, calling rate = note repetition rate, and amplitude frequency = dominant frequency.

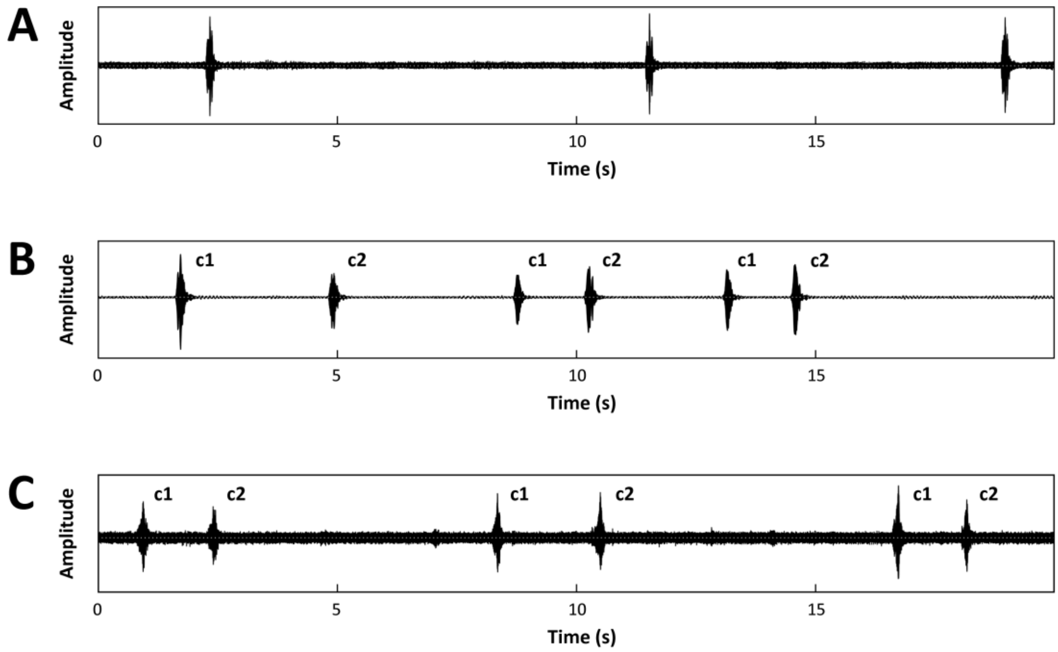

Figure 2. Oscillograms of a call series of (A) *Theloderma albopunctatum*, (B) *Theloderma corticale*, and (C) *Theloderma licin*.

Results

DNA barcoding by ABGD assigned all our *Theloderma* samples to the respective species. Observed p-distances of our samples of *T. albopunctatum* to other *T. albopunctatum* range from 0.00 % to 5.46 %, with the total intraspecific variance of p-distances ranging from 0.00 % to 6.81 % (n=34). The samples most similar to ours were from Sa Pa, northern Vietnam (p-distance 0.00 % to 0.33 %). All other species of *Theloderma* were divergent by 6.77 % to 20.06 %. In *T. corticale*, our sample has p-distances between 0.00 % and 2.27 % to other *T. corticale* (including *T. kwangsiense*), with the total intraspecific variance of p-distances ranging from 0.00 % to 2.89 % (n=21). Our sample of *T. corticale* is identical to several samples from mainland China and northern Vietnam, while all other species of *Theloderma* are divergent by at least 6.61 %. Our sample of *T. licin* differs from other *T. licin* by 0.00 % to 3.53 %, with the total intraspecific variance of p-distances ranging from 2.90 % to 3.55 % (n=3). The sample is identical to a sample from Kuala Lumpur, Malayan Peninsula. All other species of *Theloderma* are divergent by at least 8.96 %.

For *T. corticale,* 20 calls were analyzed, for *T. albopunctatum,* 26 calls were analyzed and for *T. licin,* 20 calls were analyzed. The calls of *T. albopunctatum* and *T. licin* were high-pitched, whereas the calls of *T. corticale* were at lower-frequencies. Also, the calls of *T. corticale* and *T. licin* were found to be organized in call groups (call 1: c1, call 2: c2; Figs. 2, 3). The acoustic signals of all three species each consist of a dominant frequency with numerous harmonics located above (Fig. 3; Table 1). In general, calls could not be related to specific individuals within the monitored tanks, since individuals stopped calling, when being interrupted. Nevertheless, one specimen of the species *T. albopunctatum* was audio-visually recorded. Furthermore, one specimen of *T. corticale* was visually observed calling while it was sitting in water. For *T. licin,* the call record represents alternating calls of more than one *T. licin* specimen, and it is suggested that these include calls of one or more females.

The calls of *T. corticale* are organized in call groups of two calls (c1, c2) (Figs. 2, 3). C1 and c2 were composed of 1 note ± 0 (range: 1–1 note; *n*=20) and had a note repetition rate of 28 notes/min ± 0 (range: 28–28 notes/min; n=20); notes were unpulsed. Notes of c1 feature an average duration of 0.26 s ± 0.04 (range: 0.21–0.38 s; n=20), and the average length of intervals between notes was 1.27 s ± 0.26 (range: 0.18–1.44 s; n=20). The dominant frequency possessed an average value of 0.830 kHz ± 0.022 (range: 0.794–0.881 kHz; n=20) with up to seven harmonics showing a frequency band of 1.583–6.985 kHz. The first harmonic averaged at a frequency of 1.662 kHz ± 0.041 (range: 1.583–1.737 kHz; n=20), and the last harmonic was observable at a frequency of 6.617 kHz ± 0.256 (range: 6.152–6.985 kHz; n=9). Detailed harmonic frequency values are shown in Table 1. Notes of c2 feature an average duration of 0.24 s ± 0.03 (range: 0.19–0.32 s; n=20), and the average length of intervals between notes was 2.64 s ± 0.23 (range: 2.40–3.36 s; n=20). The dominant frequency possessed an average value of 0.809 kHz ± 0.011 (range: 0.795–0.835 kHz; n=20) with up to seven harmonics showing a frequency band of 1.366–6.970 kHz. The first harmonic averaged at a frequency of 1.615 kHz ± 0.064 (range: 1.366–1.665 kHz; n=20), and the last harmonic was observable at a frequency of 6.724 kHz ± 0.131 (range: 6.601–6.970 kHz; n=5). Detailed harmonic frequency values are shown in Table 1. Average ambient temperature was 24.5 °C ± 0.2 (range: 24.3–24.6 °C; n=20).

The calls of *T. albopunctatum* were high-pitched with one unpulsed note per call and four harmonics located above the dominant frequency (Fig. 3). The temporal sequence of calls is illustrated in Fig. 2. The call of *T. albopunctatum* was composed of 1 note ± 0 (range: 1–1 note; n=26) and had a note repetition rate of 7.4 notes/min ± 2.1 (range: 4–10 notes/min; n=26). Every note features an average duration of 0.30 s ± 0.03 (range: 0.22–0.36 s; n=26), and the average length of intervals between notes was 10.21 s ± 5.17 (range: 6.38–26.82 s;

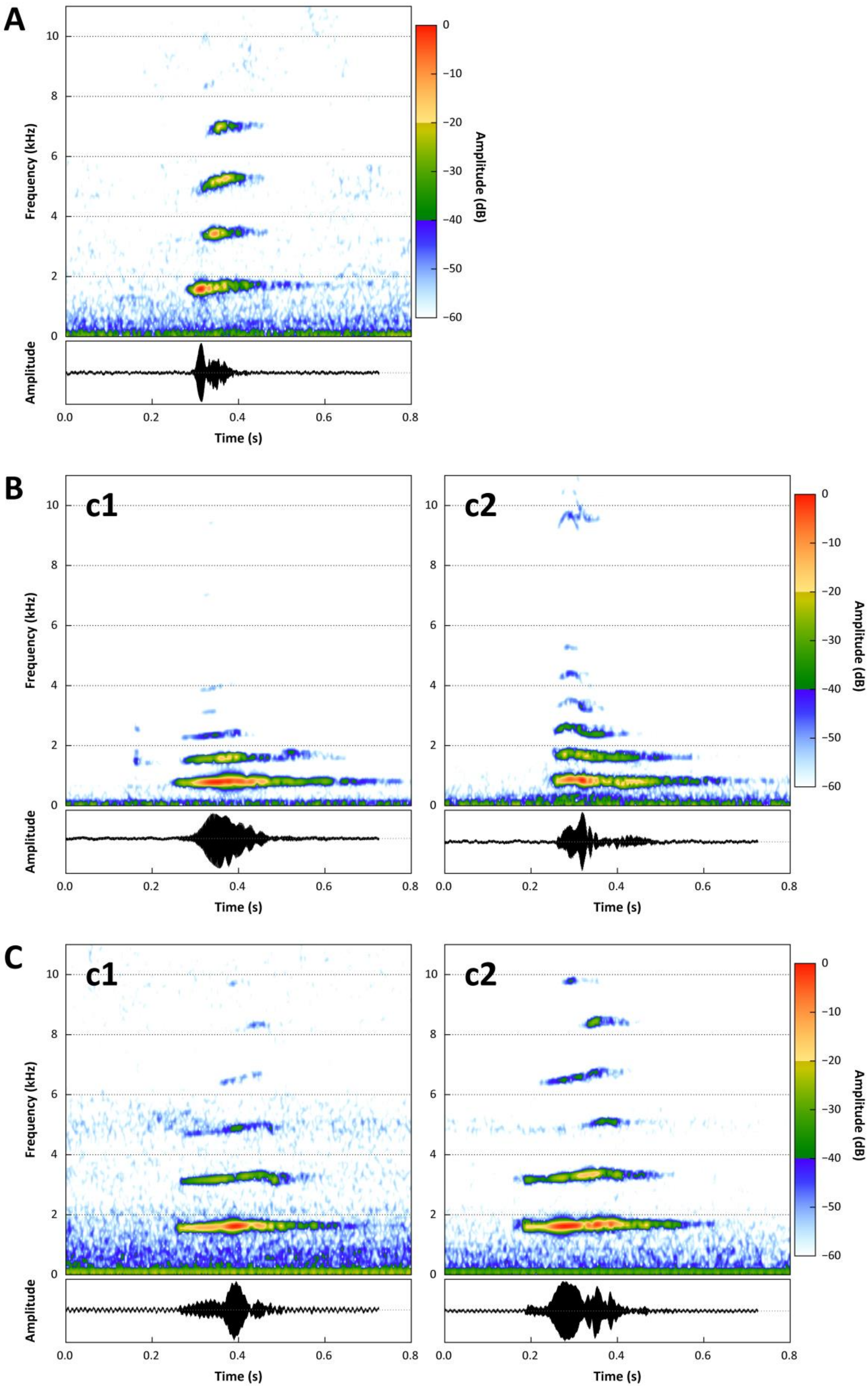

Figure 3. Spectro- and Oscillograms of the single notes in the advertisement calls of (A) *Theloderma albopunctatum*, (B) *Theloderma corticale*, and (C) *Theloderma licin*. The advertisement calls of *T. corticale* and *T. licin* are arranged in call groups of two calls (c1, c2)

Table 1. Properties of the calls of *Theloderma albopunctatum*, *T. corticale* and *T. licin*. Calls of *T. corticale* and *T. licin* are organized in call groups with each two calls (c1, c2). Advertisement calls of *T. auratum* (Poyarkov et al. 2018), *T. stellatum* and *T. vietnamense* (Poyarkov et al. 2015) were used for comparison. Values are given as mean ± standard deviation (SD).

Species	*T. albopunctatum*	*T. corticale*		*T. licin*		*T. auratum*	*T. stellatum*	*T. vietnamense*
Feature		c1	c2	c1	c2			
Note duration (s)	0.30 ± 0.03	0.26 ± 0.04	0.24 ± 0.03	0.27 ± 0.05	0.24 ± 0.03	0.06 ± 0.00	0.09 ± 0.00	0.16 ± 0.00
	(0.22–0.36) *n*=26	(0.21–0.38) *n*=20	(0.19–0.32) *n*=20	(0.20–0.39) *n*=20	(0.20–0.30) *n*=20	(0,03–0.08) *n*=214	(0,07–0.11) *n*=32	(0,13–0.18) *n*=16
Total number of notes per call	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	14.27 ± 1.31	1 ± 0	1 ± 0
	(1–1) *n*=26	(1–1) *n*=20	(1–1) *n*=20	(1–1) *n*=20	(1–1) *n*=20	(7–21) *n*=15	(1–1) *n*=32	(1–1) *n*=16
Note repetition rate (notes/min)	7.4 ± 2.1	28 ± 0	28 ± 0	16 ± 0	16 ± 0.6	101 ± 0.2	57 ± 0	28 ± 0
	(4–10) *n*=26	(28–28) *n*=20	(28–28) *n*=20	(16–16) *n*=20	(14–18) *n*=20	(92–119) *n*=15	(57–57) *n*=32	(28–28) *n*=16
Inter-note length (s)	10.21 ± 5.17	1.27 ± 0.26	2.64 ± 0.23	1.40 ± 0.28	5.87 ± 0.70	0.53 ± 0.01	0.88 ± 0.08	2 ± 0.02
	(6.38–26.82) *n*=26	(0.18–1.44) *n*=20	(2.40–3.36) n=20	(1.06–1.86) n=20	(3.06–6.58) *n*=20	(0.41–0.84) *n*=199	(0.38–2.39) *n*=32	(1.91–2.2) *n*=16
Dominant frequency (kHz)	1.676 ± 0.031	0.830 ± 0.022	0.809 ± 0.011	1.625 ± 0.015	1.628 ± 0.018	2.829 ± 0.002	1.530 ± 0.030	1.300 ± 0.010
	(1.635–1.764) *n*=26	(0.794–0.881) *n*=20	(0.795–0.835) *n*=20	(1.611–1.662) *n*=20	(1.612–1.672) *n*=20	(2.760–2.950) *n*=214	(1.370–1.720) *n*=32	(1.270–1.330) *n*=16
1. Harmony (kHz)	3.388 ± 0.114	1.662 ± 0.041	1.615 ± 0.063	3.236 ± 0.064	3.256 ± 0.054		2.910 ± 0.010	2.550 ± 0.010
	(3.224–3.645) *n*=26	(1.583–1.737) *n*=20	(1.366–1.665) *n*=20	(3.134–3.327) *n*=20	(3.160–3.313) *n*=20		(2.840–2.970) *n*=32	(2.540–2.560) *n*=16
2. Harmony (kHz)	5.203 ± 0.198	2.449 ± 0.061	2.389 ± 0.024	4.869 ± 0.092	4.859 ± 0.135			
	(4.934–5.655) *n*=26	(2.329–2.574) *n*=20	(2.355–2.432) *n*=20	(4.739–5.039) *n*=20	(4.521–5.111) *n*=20			
3. Harmony (kHz)	6.770 ± 0.130	3.302 ± 0.097	3.221 ± 0.061	6.555 ± 0.120	6.570 ± 0.151			
	(6.434–7.112) *n*=26	(3.102–3.531) *n*=20	(3.146–3.360) *n*=20	(6.348–6.739) *n*=16	(6.356–6.799) *n*=15			
4. Harmony (kHz)	8.047 ± 0.099	4.234 ± 0.190	4.053 ± 0.127	8.345 ± 0.052	8.401 ± 0.068			
	(7.949–8.201) *n*=6	(3.868–4.679) *n*=20	(3.836–4.269) *n*=20	(8.268–8.456) *n*=15	(8.201–8.514) *n*=16			
5. Harmony (kHz)		4.984 ± 0.254	4.778 ± 0.086	9.457 ± 0.573	9.752 ± 0.089			
		(4.450–5.608) *n*=20	(4.570–4.974) *n*=20	(8.318–9.796) *n*=5	(9.602–9.857) *n*=9			
6. Harmony (kHz)		5.740 ± 0.161	5.581 ± 0.124					
		(5.375–6.105) *n*=20	(5.412–5.962) *n*=20					
7. Harmony (kHz)		6.617 ± 0.256	6.724 ± 0.131					
		(6.152–6.985) *n*=9	(6.601–6.970) *n*=5					

n=26). The dominant frequency possessed an average value of 1.676 kHz ± 0.031 (range: 1.635–1.764 kHz; n=26) with up to four harmonics showing a frequency band of 3.224–8.201 kHz. The first harmonic averaged at a frequency of 1.676 kHz ± 0.031 (range: 3.224–3.645 kHz; n=26), and the last harmonic was observable at a frequency of 8.047 kHz ± 0.099 (range: 7.949–8.201 kHz; n=6). Detailed harmonic frequency values are shown in Table 1. Average ambient temperature was 20.9 °C ± 0.9 (range: 18.6–21.2 °C; n=26).

The calls of *T. licin* were high-pitched, and they are also organized in call groups of two calls (c1, c2) (Figs. 2, 3). C1 and c2 were composed of 1 note ± 0 (range: 1–1 note; n=20); notes were unpulsed. C1 had a note repetition rate of 16 notes/min ± 0 (range: 16–16 notes/min; n=20), and notes feature an average duration of 0.27 s ± 0.05 (range: 0.20–0.39 s; n=20), and the average length of intervals between notes was 1.40 s ± 0.28 (range: 1.06–1.86 s; n=20). The dominant frequency possessed an average value of 1.625 kHz ± 0.015 (range: 1.611–1.662 kHz; n=20) with up to five harmonics showing a frequency band of 3.134–9.796 kHz. The first harmonic averaged at a frequency of 3.236 kHz ± 0.064 (range: 3.134–3.327 kHz; n=20), and the last harmonic was observable at a frequency of 9.457 kHz ± 0.572 (range: 8.318–9.796 kHz; n=5). Detailed harmonic frequency values are shown in Table 1. Notes of c2 had a note repetition rate of 16 notes/min ± 0.6 (range: 14–18 notes/min; n=20), and notes feature an average duration of 0.24 s ± 0.03 (range: 0.20–0.30 s; n=20), and the average length of intervals between notes was 5.87 s ± 0.70 (range: 3.06–6.58 s; n=20). The dominant frequency possessed an average value of 1.628 kHz ± 0.018 (range: 1.612–1.672 kHz; n=20) with up to five harmonics showing a frequency band of 3.160–9.857 kHz. The first harmonic averaged at a frequency of 3.256 kHz ± 0.054 (range: 3.160–3.313 kHz; n=20), and the last harmonic was observable at a frequency of 9.759 kHz ± 0.089 (range: 9.602–9.857 kHz; n=9). Detailed harmonic frequency values are shown in Table 1. Average ambient temperature was 22.8 °C ± 0.0 (range: 22.8–22.8 °C; n=20).

Comparing the call characteristics of *T. corticale, T. albopunctatum,* and *T. licin*, they all feature 1 ± 0 note per call, and note durations were found to be similar (*T. corticale* c1: 0.26 s ± 0.04, c2: 0.24 s ± 0.03; *T. albopunctatum*: 0.30 s ± 0.03; *T. licin* c1: 0.27 s ± 0.05, c2: 0.24 s ± 0.03). Dominant frequencies (*T. albopunctatum*: 1.676 kHz ± 0.031; *T. licin* c1: 1.625 kHz ± 0.015, c2: 1.628 kHz ± 0.018) and harmonic frequencies (Table 1) are similar in the high-pitched call of *T. albopunctatum* and *T. licin*. In *T. corticale* the dominant frequencies (c1: 0.830 kHz ± 0.022, c2: 0.809 kHz ± 0.011) and frequencies of harmonics (Table 1) are lower than those of *T. albopunctatum* and *T. licin*. All three species feature different note repetition rates (*T. corticale c1:* 28 notes/min ± 0, c2: 28 notes/min ± 0; *T. albopunctatum*: 7.4 notes/min ± 2.1; *T. licin* c1: 16 notes/min ± 0, c2: 16 notes/min ± 0.6). Also, *T. albopunctatum* and *T. licin* calls differ regarding inter-note length (*T. albopunctatum*: 10.21 s ± 5.17; *T. licin* c1: 1.40 s ± 0.28, c2: 5.87 s ± 0.70), and only *T. corticale* and *T. licin* calls are grouped.

Discussion

Despite frogs of the genus *Theloderma* being highly vocal, according to our knowledge there are only three concrete call descriptions currently available (Poyarkov et al. 2015, Poyarkov et al. 2018). A comparison of advertisement calls of six *Theloderma* species showed that all calls are species-specific and are clearly distinguishable among each other. Particularly, the temporal features (i.e. arrangement in call groups, call repetition rate) are significant for each species, while the dominant frequencies, especially of *T. albopunctatum*, *T. licin*, *T. stellatum* and *T. vietnamense*, can overlap (for details see Table 1).

The advertisement call of *T. auratum* was described based on two males recorded in their natural habitat at 21.5 °C. The advertisement call comprises of a call group of 14.3 notes ± 1.3 (7-21; n=15) and sounds like an orthopteran call for the human ear. Among the six *Theloderma* species, which we here compare, *T. auratum* has the highest note repetition rate with 101 notes/min ± 0.2 (range: 92–119 notes/min; n=15). These two temporal features and the highest dominant frequency [2.829 ± 0.002 (range: 2.760 – 2.950; n=214)] make the species unique among the here compared taxa (compare Table 1) (for details see Poyarkov et al. 2018).

The advertisement calls of the two members of the (sub)genus *Stelladerma* (here called *Theloderma*) were recorded at 25 °C (*T. stellatum*) and 25.5 °C (*T. vietnamense*), both from one male specimen each (for details see Poyarkov et al. 2015). Advertisement calls of both species consist of 1 note ± 0 (range: 1-1 notes), but significantly differ in their note repetition rates [*T. stellatum*: 57 notes/min ± 0 (range: 57-57 notes/min; n=32); *T. vietnamense*: 28 notes/min ± 0 (range: 28-28 notes/min; n=16); for details of other call features see Table 1]. The advertisement call of *T. albopunctatum*, which is also not arranged in a call group, can easily be distinguished by a much lower note repetition rate [7.4 notes/min ± 2.1 (range: 4-10 notes/min; n=26)], while the dominant frequencies of the three species partially overlap (for details see Table 1).

The calls of *T. corticale* and *T. licin* are the only ones, which are organized in call groups consisting of two calls. Taking into consideration the similarities of *T. corticale* c1 and c2 in note duration, total number of notes, note repetition rate, dominant frequency and harmonic frequencies (Table 1), it can be stated that a *T. corticale* call group consists of two equal calls. This finding is valid for the same call features in *T. licin* c1 and c2 (Table 1). Consequently, the advertisement calls of both *T. corticale* and *T. licin* appear to be a regular sequence of call groups each consisting of two equal calls.

Taking the context into consideration in which the acoustic signals of *T. corticale*, *T. albopunctatum* and *T. licin* were recorded, we assume that these calls represent advertisement calls and that specimens were attempting to either attract conspecific females, or to segregate other calling males (Toledo et al. 2015, Emmrich et al. 2020). Since disturbance was avoided, and both *T. albopunctatum* and *T. corticale* were observed calling while sitting in water or on bark, we can exclude that these calls were performed in an aggressive or defensive context. Further, Kunz et al. (2010) also noted the aggressive calls of *T. asperum* s.l. during a fight of two males as a staccato-like sound.

The context of *T. licin* calls was unclear, since individuals have not been visually observed calling. Nevertheless, we assume that the herein analyzed vocalizations are advertisement calls. The additional calls occurring in the recording of *T. licin* might be courtship calls, since in some species a reciprocation call is given by a receptive female in response to advertisement calls of conspecific males (Duellman & Trueb 1994, Toledo et al. 2015, Mendoza-Henao et al. 2020). As mentioned above, vocalizing females are known in other *Theloderma* species (Mattison 2011). These facts lead to the assumption that alternating courtship calls and advertisement calls were recorded.

The differing numbers of harmonics in all three species (*T. corticale* c1, c2: up to 7 harmonics; *T. albopunctatum*: up to 4 harmonics; *T. licin* c1, c2: up to 5 harmonics) should be interpreted with caution, since they might result from a discrepancy in submission to background noise as the intensities of harmonics at higher frequencies decreases. Nevertheless, in four of five calls (*T. corticale* c1; *T. albopunctatum*; *T. licin* c1, c2), the spectrograms (Fig. 2) confirmed the number of previously computed harmonics, and in *T. corticale* c2 the spectrogram revealed a seventh harmonic compared to the previously computed six harmonics (Table 1). Hence, both *T. corticale* c1 and c2 feature seven harmonics.

Since frogs are ectotherms, the acoustic characteristics of frog calls will vary with change in ambient temperature (Duellman & Trueb 1994, Lingnau & Bastos 2007, Morais et al. 2012, Furtado et al. 2016, Röhr et al. 2020). Further, anuran vocalizations may be influenced by snout-vent-length, body mass and distance between calling males (Lingnau & Bastos 2007, Morais et al. 2012, Bee et al. 2013, Röhr et al. 2020). This might explain the range in note repetition rate in *T. albopunctatum* [7.4 notes/min ± 2.1 (4–10)] and respectively inter-note length [10.21 s ± 5.17 (6.38–26.82)]. Alternatively, the range might represent a characteristic call pattern and demands further investigation.

The taxonomic relationships within the genus *Theloderma* and its sub- or sister clades is far from well-studied and it is likely that these clades contain several cryptic species (see introduction for details). The investigation of animal vocalizations can help to delimit cryptic species (Forti et al. 2017, Hasiniaina et al. 2020) and using such data in terms of integrative species concepts can help to solve taxonomic issues (Köhler et al. 2017). For this reason, further knowledge about the remaining taxa assessed using standardized metrics of spectral and temporal properties for advertisement calls are necessary.

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