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„*Micrixalus saxicola* a foot-flagging frog from India: Acoustic and visual signaling behavior during male-male agonistic interactions“

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Micrixalus saxicola a foot-flagging frog from India:

Acoustic and visual signaling behavior during male-male agonistic interactions

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Introduction

Animals use a range of different signaling modalities to mediate social behavior. Several different sensory channels are employed during communication (Endler, 1992; Endler, 1993; Bradbury and Vehrencamp, 2011). Communication using signals or cues in more than one sensory channels (multimodal communication) could improve signal perception in complex environments (Partan and Marler, 1999; Rowe, 2002) and facilitate faster detection for receivers (Rowe, 1999).

Anuran amphibians use predominantly acoustic signals for communication. A long history of studies of social behavior in anuran amphibians has provided comprehensive information about acoustic signal properties (Duellmann and Trueb, 1986; Gerhardt and Huber, 2002). However, in recent years evidence is mounting that a growing number of anurans use additional visual cues for intraspecific communication (Hödl and Amézquita, 2001; Amézquita and Hödl, 2004; Hartmann et al., 2005; Hirschmann and Hödl, 2006).

While advertising, males inflate and deflate the vocal sac whose primary function is to recycle air during vocalizing. The vocal sac is inevitably moved while a male is calling. In Túngara frogs (*Physalaemus pustulosus*) the male's pulsating sac increases the attractiveness of advertisement calls towards females. The visual display of vocal sac might help females to find males by enhancing localization, detection and discrimination in dense noisy choruses (Rosenthal et al., 2004; Taylor et al., 2011). The importance of the visual display of vocal sacs was also shown in male-male aggressive behavior of *Allobates femoralis*. In *A. femoralis* only bimodal signals, male vocalizations in combination with the visual cue of a pulsating vocal sac, trigger an aggressive behavior in competitors (Narins et al., 2003; Narins et al., 2005).

Visual displays might represent an alternative or additional signaling strategy in species inhabiting noisy environments that potentially constrain anuran communication. Disturbances caused by environmental noise while breeding at fast-flowing streams or in dense aggregations could have promoted the use of visual signals (Hödl and Amézquita, 2001; Amézquita and Hödl, 2004; Boeckle et al., 2009; Preininger et al., 2009). Common visual signals such as upright posture, body raising and toe trembling function as alerting signals ensued by other visual and acoustic gestures; whereas hind-feet lifting and body lowering are performed mainly as passive defense or displacement movements during agonistic encounters (Hödl and Amézquita, 2001). Visual displays and movements can be further emphasized by coloration or strong contrasts as the white or blue interdigital webbings in the genus *Staurois* (Hödl and Amézquita, 2001; Preininger et al., 2009).

Some frog species use their arms and/or legs for visual displays independently of sound production (Lindquist and Hetherington, 1996; Hödl and Amézquita, 2001; Amézquita and Hödl, 2004). Foot-flagging behavior was observed in species from 5 anuran families on three continents (Hödl and Amézquita, 2001; Hartmann et al. 2005) and is associated with agonistic territorial male-male encounters and courtship behavior. Previous studies of frogs in the genus *Staurois* have demonstrated that males perform both visual and acoustic displays during agonistic interactions. Advertisement calls in *Staurois sp*. are displayed in short latencies to the foot flags and were suggested to act as an alert signal directing the attention of the receiver to the subsequent visual display (Grafe and Wanger, 2007;

Preininger et al., 2009; Grafe et al., 2012). The multimodal (acoustic and visual) signal might allow easier localization and detection of opponents in noisy environments.

The Small Torrent Frog (*Micrixalus saxicola)* is endemic to the Western Ghats of India and occurs exclusively along perennial streams (Chandran et al., 2010). Males use a diverse signal repertoire of calls, foot flagging and tapping (foot lifting) during agonistic interactions. Former investigations have shown that acoustic signals are not impaired by environmental stream noise but suggest that concurrently calling conspecifics could mask acoustic communication (Preininger et al., in press).

The aims of our study were: (1) to investigate the signaling behavior of *Micrixalus saxicola* during agonistic male-male encounters, (2) to determine activity patterns of signal production (3) to test the alerting signal hypothesis of multimodal signaling and compare our results with previous studies on foot-flagging frog species from 2 families (Ranidae, Hylidae).

Methods

Study species & area

The Small Torrent Frog (*Micrixalus saxicola*) is diurnal and inhabits streams within the evergreen forest characterized by low water, air and soil temperature (Reddy et al., 2002). Males produce advertisement calls from exposed sites on rocks. The study was undertaken with a population of *M. saxicola* males (mean snout-urostyle length: 23.8 mm, mean mass: 1.1g, N = 20; Preininger et al. in press) signaling in a stream at the Kathalekan *Myristica* swamp forest (14.27414°N, 74.74704°E) in the central Western Ghats, India at the end of the monsoon season from 12 September to 19 October 2010. The study site is considered to be a relict forest with evergreen vegetation and is exposed to the South-West monsoon with seasonal rainfall of 3000-5000mm (Chandran et al., 2010) and an average temperature and humitidy of 25 ± 1°C and 85% during the period of study, respectively.

Data collection

We recorded 10 male – male agonistic interactions between 2 individuals with a video camera (Sanyo Xacti WH1) on a tripod from a distance of approx. 1 - 2 m from the focal individuals. For video analysis we only used interactions that included a clear visible intruding male and ended with a winner and loser accordingly. An intruder was determined when one individual jumped to the resident individual at a distance closer than 30 cm and immediately started signaling. The individual that first left the area was considered the loser.

We analyzed frequencies and durations of the behaviors (call, kick, location change, turn, tap, foot flag) with the video coding software Solomon Coder (Péter, 2011). Two types of behavior can be considered as exclusive visual signals (tap and foot flag). A tap consisted of lifting of either right or left leg without extending it. During a foot-flagging display (Fig. 1), a male raised either one hind leg or both simultaneously and stretched it/them in an arc above the substrate level (sensu Hödl and Amézquita, 2001) and rested it on the ground before returning it/them to the body side. We documented foot flags carried out with closed and open interdigital webbing separately. To determine side preferences we documented foot flagging carried out with left or right leg in connection to the positions of interacting males. The behavior termed “kick” was a physical attack with one hind leg that usually resulted in pushing the interacting individual off a rock (Fig. 2). When individuals moved to, away from or to either side of the other male we recorded this behavior as location change and stationary movement as turns.

To determine the activity peaks of *M. saxicola*, we scan sampled 2-3 individuals for 5 minutes every half hour from 6am until 6pm for a period of 5 days and recorded 3 behavioral parameters (call, foot flag and tap). Behaviors reported determine means corrected for one individual.

Statistical analysis

To compare the frequency of acoustic and visual signals (calling, foot flagging and tapping) during morning and during afternoon hours the chi-squared test was used. Behavioral frequencies during male-male agonistic interactions were compared using Kruskal-Wallis-Test for non-normal data distribution.

To investigate differences in durations between foot-flagging behaviors performed with the interdigital webbing spread out or closed we chose a Linear Mixed Model (LMM). The LMM allows repeated measurements of the same individual to be fitted in the model as random variables, thus controlling for differing number of displays per male individual. The statistical assumptions for LMM analysis were met (Kolmogorov-Smirnov test). Foot-flagging duration was entered as the dependant variable with the relationship of open or closed web as predictor variables. The identity of individual (foot flag) was entered nested as random variable. The duration of the different stages of foot flagging and overall taping behavior were determined by calculating medians and ranges.

To test the hypothesis that foot flags are directional signals towards the interacting male we used chi-square tests to analyze possible side preferences. To analyze differences in behavioral frequencies between winners and losers and between residents and intruders we used Wilcoxon signed ranks test.

To analyze signals in relation to successive behaviors of interacting males, behavioral transitions were displayed in the form of a first-order 6x6 contingency table (Amézquita and Hödl, 2004), using a Monte Carlo Test. Behavioral transitions that suggested the largest differences between observed and expected were further tested using chi-square tests.

To test the alert signal hypothesis we compared the temporal interaction between the advertisement call and the foot-flagging behavior and vice versa. The latency times between behaviors were compared with Wilcoxon signed ranks test. All statistical tests were performed with SPSS version 19.

Results

Territorial *M. saxicola* males are highly active during the whole day (Fig. 3), with higher calling (χ^2 = 29.2; df = 1; P < 0.001) and tapping (χ^2 = 9.7; df = 1; P < 0.01) activity levels during the morning (06:00 - 12:00) compared to the afternoon (12:00 - 18:00). Foot-flagging behavior did not differ between morning and afternoon hours (χ^2 = 0.1; df = 1; P > 0.05).

We analyzed 10 male-male interactions with an average duration of 395.6 s (range: 35 – 1285 s). Comparison of behavioral frequencies during male-male agonistic interactions indicated differences between the number of displayed behaviors during male-male agonistic interactions (Kruskal-Wallis ANOVA: H = 15.375, df = 5, P < 0.01, N = 10; Fig. 4). Males performed more calls than foot-flagging behaviors (pair-wise comparison: T = 23.250, SE = 7.8, P < 0.05). Other behaviors did not show significant differences in signaling frequency.

Micrixalus saxicola males performed foot-flagging displays with the webbing closed or open. We found no difference in duration between foot flags performed with the web open or closed (GLMM: $F_{1/60}$ = 2.023, P = 0.16). The median duration of a foot flag was 4.38 s (range: 3.19 - 13.31 s), the interdigital webbing was spread for an average period of 1.08 s (range: 0.54 - 1.61 s) and the leg was rested behind the body for 2.67 s (range: 1.88 - 12.04 s). Median tap duration was 0.35 s (range: 0.29 - 0.38 s). There were no differences between foot-flagging behaviors conducted with the right or left leg (c*2* = 0.89, df = 1, p > 0.05; Tab. 1). Foot flags were performed significantly more often in the direction of the interacting male than to the opposite side (c*2* = 5.8, df = 1, p < 0.05; Tab. 1), whereas no differences in signaling frequency were observed between the opponent male sitting in front or behind of the displaying individual (c*2* = 2.6, df = 1, p > 0.05; Tab. 1).

Intruders changed their location more often than residents (Wilcoxon signed ranks test: Z = 36, SE = 7.124, P = 0.012, N = 10; Fig. 5 a), all other behaviors did not differ between the resident or intruding male.

We found no difference in behavioral frequencies between winners and losers e.g.: foot flags: (Wilcoxon signed ranks test: foot flag Z = 25.5, SE = 7.089, P = 0.29, N = 10; Fig. 5 b). Analyses of a dyadic matrix (Tab. 2) showed that a behavior performed by one individual was associated with the subsequent behavior performed by another individual significantly more often than random expectations (number of trials = 100000, $P < 0.001$, $n = 538$). Calling was preceded by calling, tapping and location change significantly more often than expected. Although calling was the primary response (35%) to all displays from an interacting male, the behavior did not occur more often than expected (e.g.: call - call: $\chi^2 = 1.135$, df = 20, $P > 0.05$; Tab. 2). Kicking was preceded by kicking significantly more often than expected ($\chi^2 = 42.131$, df = 20, $P < 0.01$).

To investigate if the call is functionally linked with the foot-flagging signal we compared the timing relationship between advertisement calls and foot flags for 19 males. The average delay between an advertisement call and a foot flag was 2.52 s (range 0.12–9.87 s, n = 19). The mean latency time between a foot flag and a subsequent advertisement call was 2.91 s (range 0.15–8.49 s, n = 19). We found no differences in latency times between the observed behaviors (Wilcoxon matched pairs, $Z = 104.5$, $P = 0.702$, $n = 19$; Fig. 6).

Discussion

The results of our study indicate that males of *M. saxicola* announce the readiness to defend their perching sites against conspecifics by using both acoustic and visual signals. Advertisement calls were the primary response to all signals displayed by an interacting male and appeared not to function as a unit with foot-flagging behaviors as observed in foot-flagging species from the genus *Staurois*.

Activity patterns showed that the most common signals (calls and taps) during agonistic interactions are displayed more frequently in the morning than in the afternoon. In a former study first amplectant pairs were observed at 11 am (Gururaja, 2010) hence it could be important to defend areas favorable for reproduction in early hours of the day to attract females. During the breeding season (July and October) concurrent with monsoon rains a high density of males accumulates in shallow water within the stream. The reproduction mode of *M. saxicola* relies chiefly on water in the Western Ghats frog population (Krishna and Krishna, 2006). The oviposition sites are near the surface of rocks where the flow of perennial stream is gentle (Gururaja, 2010).

In all agonistic encounters the resident male started calling and tapping as soon as an approaching male was detected, possibly to signal readiness to defend its advertising location. The calling activity of neighboring males might influence the calling behavior from conspecific individuals, isolated males rarely displayed calls (Krishna and Krishna, 2006). Calling (35%) was the most frequently observed response to signals from an opponent. Foot tapping (25%) occured more often than foot flagging (2%). The short foot-lifting behavior could represent a reduced execution of the longer foot-flagging display. Visual signal responses (foot flagging and tapping) were less abundant than acoustic displays.

In contrast, Preininger et al. (2009) demonstrated that in the Bornean foot-flagging species *Staurois latopalmatus* visual displays (foot flags 30.5 %) dominate over acoustic signals (20.2 %) during agonistic male-male interactions. Our results also support a recent across-species comparison between response frequency of *M. saxicola* and *Staurois parvus* to acoustic and visual playback presentation (Preininger et al., subm.) that demonstrated the predominant use of visual signals in *S. parvus* opposed to acoustic signals in *M. saxicola*. Further studies in the genus *Staurois* suggest that advertisement calls and foot flags form a temporally functional unit in a bimodal signal pattern (Grafe and Wanger, 2007; Preininger et al., 2009; Grafe et al., 2012). Short latency times between calls and following foot-flagging displays support the alert and attention altering hypothesis (sensu Hebets and Papaj, 2005) in the *Staurois* spp.. The advertisement call functions as an alert signal to receivers and draws the attention to the second signal component, the visual signal. In comparison, time-relationship analysis in *M. saxicola* corroborated the lack of functional coupling between calls and foot flagging, which suggests that the displays are uncoupled independent signaling modalities. The visual displays might represent flexible signals adaptive to environmental conditions not yet coupled to bimodal communication signals

Foot webbings of *S. parvus* are almost five times brighter than those of *M. saxicola* (Preininger et al., subm.). Bright web colorations might improve the contrast to environment shaped by altered light conditions (Inger and Stuebing, 1997; Endler et al., 2005; Preininger et al., 2009). Diurnal species might exploit colorations that pose a contrast to the environment as cue when for example acoustic communication is hampered by environmental noise. However, formers studies in genus *Staurois* have suggested frequency adaptations of the advertisement call and demonstrated that acoustic communication is not constrained by the ambient stream noise (Boeckle et al., 2009; Grafe et al., 2012). Similar results were reported for *M. saxicola*, although concurrently calling conspecifics potentionally mask advertising males (Preininger et al., in press). Nevertheless visual displays in both species might enhance detection and localization of conspecifics.

In the Amazonian tree frog *Hyla parviceps*, breeding is limited to periods after heavy rains when streamside ponds are formed. Males signal from perches in dense vegetation at night or early morning hours (Amézquita and Hödl, 2004). For reproductive success, it is important to detect and localize conspecifics during short mating incidents. Males of nocturnal hylid species also used a limited repertoire of visual displays such as foot flags and calls to defend perch sites (Hödl et al., 1997; Hödl and Amézquita, 2001; Amézquita and Hödl, 2004;

Hartmann et al., 2005). Visual signals in *H. parviceps* are given only after seeing opponent males. When approaching, intruders call and show visual displays, the initial display from the resident male is repeated or followed by fights. The combination of calls and visual displays in *H. parviceps* are used in an aggressive motivational context as a form of spacing mechanism (Amézquita and Hödl, 2004). In *M. saxicola*, frequencies of foot flagging were not associated with winning or losing. Intruding males exerted more location changes compared to residents. Results of a dyadic matrix analysis showed that a behavior performed by one individual was associated with subsequent behavior performed by another male. All behaviors, apart from kicks, of an opponent male in *M. saxicola* were responded with calls, indicating that the acoustic display is the predominant signal to announce signaling sites to conspecifics. Physical attacks were significantly followed by kicking behavior of the opponents. Physical attacks were observed in all recorded interactions, whereas this or a similar behavior has never been recorded in *Staurois sp*. Kicks represent the fighting strategy of *M. saxicola* to defend resources needed for survival, maintenance of perch sites and reproduction success. *Nyctibatrachus major* a frog residing in the same region in deeper water preys on *M. saxicola*, therefore being kicked of the perch site increasing the potential risk for attacks (personal observation Preininger and Hödl).

Interacting Individuals could reduce physical attacks by kicks and solve territorial fights if receivers are influenced by foot-flagging displays. Foot flagging was not associated with kicks or any behavior previously displayed by the interacting males. We suggest that the foot-flagging signal ritualized from the observed kicking behavior, the predominant fighting technique. Across-species comparisons of signaling behavior support the assumption that foot flagging in *M. saxicola* could represent a nascent state in visual signal evolution.

In summary, our data indicates that foot-flagging behaviors in *M. saxicola* are directional displays towards the opponent male and, similar to the advertisement call, signal the readiness to defend a perching site favorable for reproduction. However calls and foot flags, although sometimes displayed simultaneously, do not represent a functional unit and the acoustic signal is not used as an alert signal according the “alert and attention altering” hypothesis. Furthermore, we suggest that foot-flagging signals represent an agonistic behavior ritualized from the kicking behavior to minimize physical attacks.

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Table 1: Side preferences of foot-flagging behavior of the Small Torrent Frog (*Micrixalus saxicola)* collected from 20 individuals.

		Position of interacting individual				
		Right	Snout	Vent	Left	Total
Foot flags	Right	15	11	7	7	42
	Left	3	13	7	9	30
	Total	18	24	14	16	72

Table 2. Dyadic matrix of behavioral inter-individual transitions during 10 male-male agonistic interactions of *Micrixalus saxicola*. Asterisks show transitions that occurred at frequencies higher than expected (*P* < 0.01) according to chi-square tests.

		Successive behavioral unit						
		Call	Tap	Foot flagging	Kick	Location	Turn	Total
Call	Count	75	76	6	15	54	18	244
	Expected Count	85	62	6	31	47	14	244
Tap	Count	38	34	3	6	12	3	96
	Expected Count	33	24	2	12	18	5	96
Foot flagging	Count	12	8	0	3	4	0	27
	Expected Count	9	7	1	4	5	2	27
Kick	Count	13	2	1	27**	18	4	65
	Expected Count	23	16	2	8	12	4	65
Location	Count	30	8	3	16	12	4	73
	Expected Count	25	19	2	9	14	4	73
Turn	Count	19	8	0	2	3	1	33
	Expected Count	12	8	1	4	6	2	33
Total	Count	187	136	13	69	103	30	538
	%	35	25	2	13	19	6	100

Figure 1. Foot-flagging male of *Micrixalus saxicola* during an agonistic interaction. Photo by W. Hödl.

Figure 2. Kicking male of *Micrixalus saxicola* during an agonistic interaction. Photo by W. Hödl.

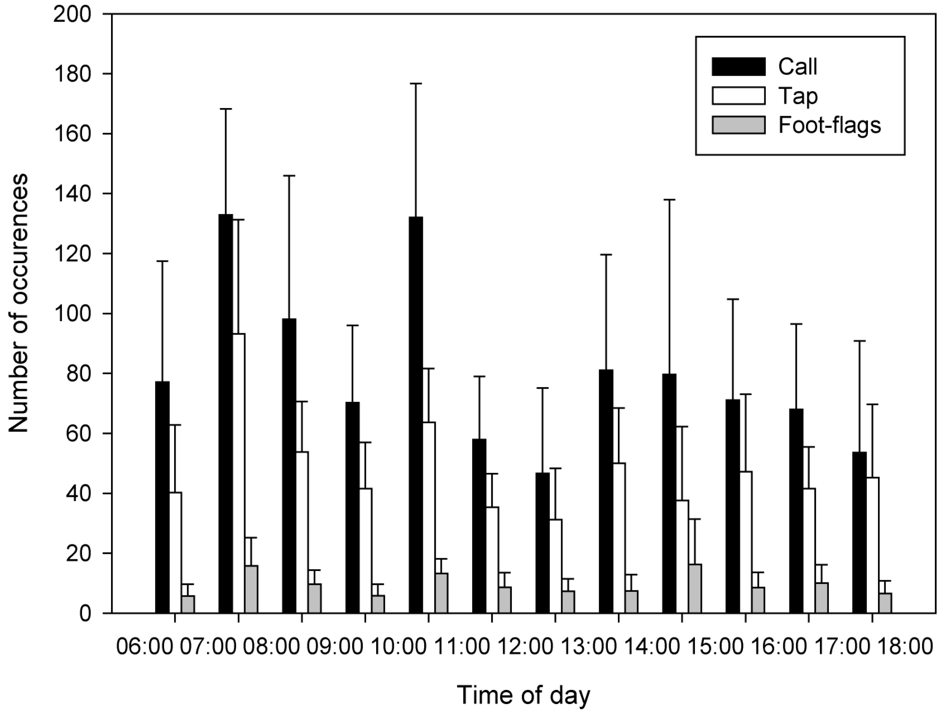

Figure 3. Daily individual signaling activity of *Micrixalus saxicola* of advertisement calls, taping (foot lifting) and foot-flagging displays. Bars show means + SE per individual and hour (n=4).

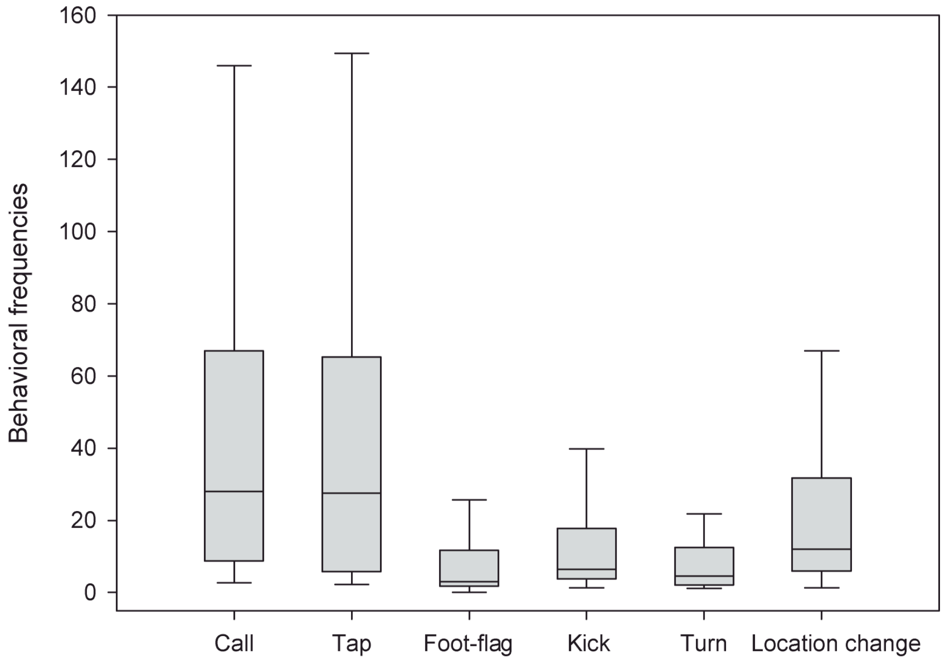

Figure 4. Frequency of behaviors displayed by opponent *Micrixalus saxicola* males during an agonistic interaction of an average duration of 395.6 s (n=10). Box plots show the median response with interquartile range and 10th and 90th percentile.

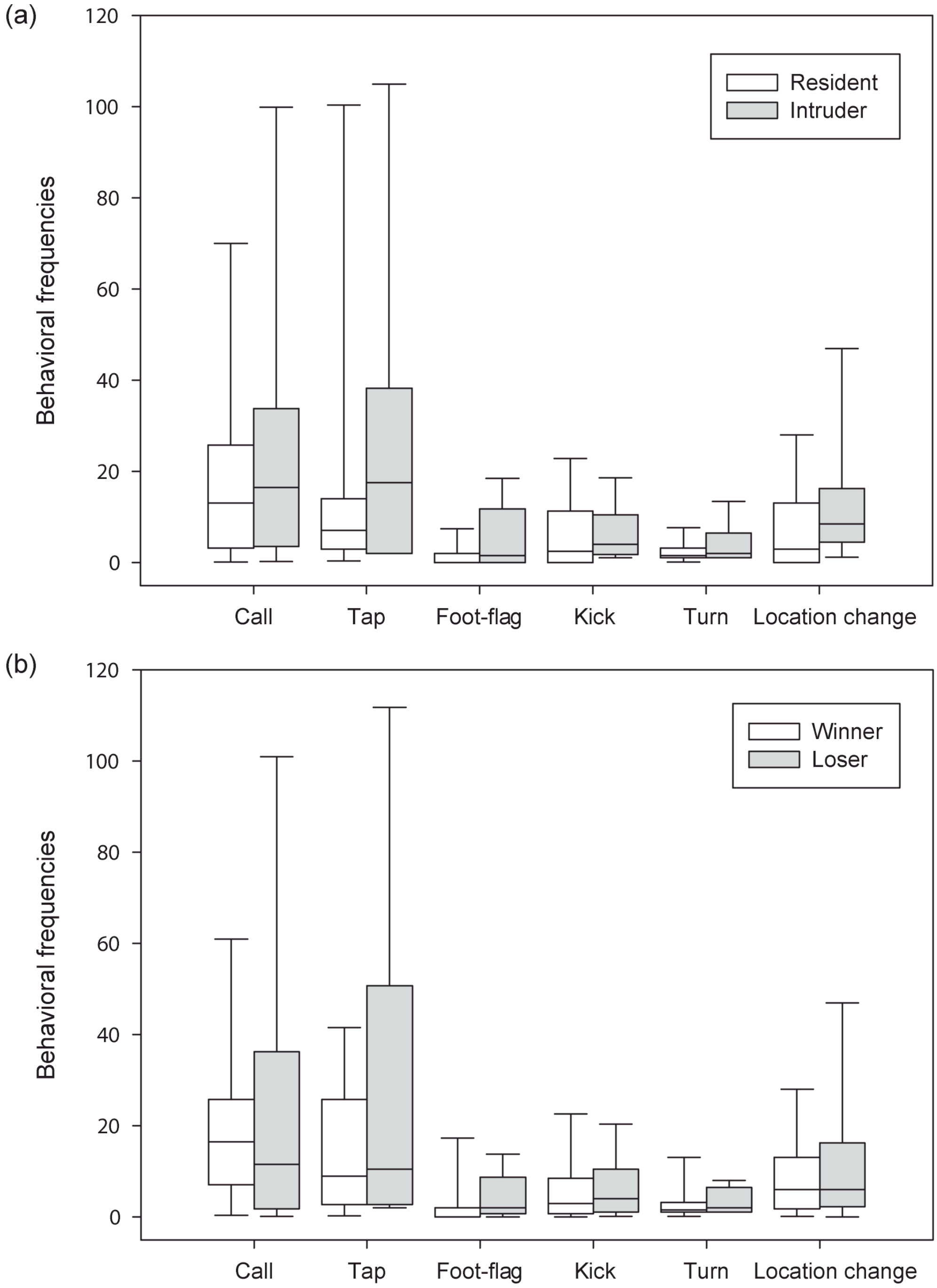

Figure 5: Frequency of behaviors displayed by two *Micrixalus saxicola* males during an agonistic interaction of an average duration of 395.6 s (n=10). Behavioral frequencies are separated according the status of the male individual at (a) the beginning (resident or intruder) and (b) end (winner and loser) of an agonistic interaction. Box plots show the median response with interquartile range and 10th and 90th percentile.

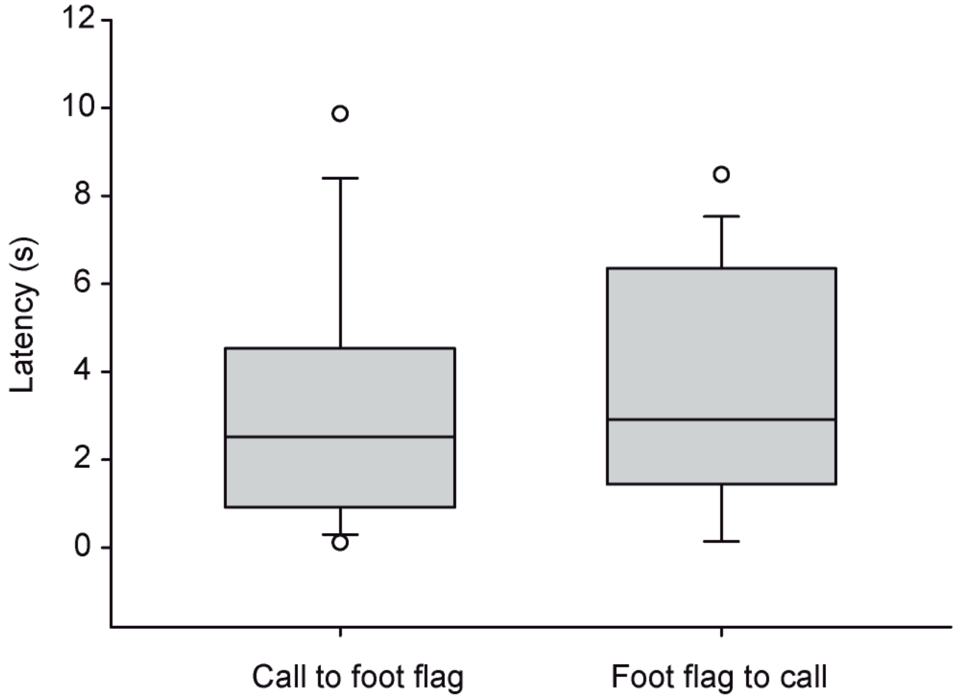

Figure 6. Comparison of timing relationships between advertisement call and foot-flagging display of 19 *Micrixalus saxicola* males during agonistic interactions with neighbouring males. Box plots show the median response with interquartile range and 10th and 90th percentile and minimum and maximum values.

Abstracts

English Abstract

Several anuran species use acoustic and visual signals for inter- and intraspecific communication in diverse social contexts. Our study describes acoustic and visual behaviors of the Small Torrent Frog (*Micrixalus saxicola*), a diurnal ranid frog endemic to the Western Ghats of India. During agonistic interactions males display advertisement calls, foot flagging and taping (foot lifting) behaviors to signal the readiness to defend perching sites in perennial streams. Our results from a quantitative video analysis of 10 male-male interactions indicate that foot-flagging displays were used as directional signals toward the opponent male, but were less abundant than calls. The acoustic and visual signals were not functionally linked as reported for foot-flagging frogs from the genus *Staurois*. Analysis of behavioral transitions revealed that kicking behaviors (physical attacks) significantly elicited kicks from interacting males. We suggest that foot-flagging displays ritualized from the frequently observed fighting technique (kicking) to reduce physical attacks. The results of this study support the assumption that foot-flagging behavior in *M. saxicola* represents a nascent state in the evolution of visual signaling frogs.

Deutsche Zusammenfassung

Manche Froscharten verwenden akustische und visuelle Signale für inter- und intraspezifische Kommunikation in unterschiedlich sozialem Kontext. Unsere Studie beschreibt das akustische und visuelle Verhalten eines in Indien endemischen Winkerfrosches (*Micrixalus saxicola*). Die tagaktiven Männchen zeigen während agonistischen Interaktionen Verhaltensweisen, wie Werberufe, Winken mit den Hinterbeinen und Fußheben, um die Bereitschaft zu signalisieren Standorte zu verteidigen. Unsere Ergebnisse aus einer quantitativen Videoanalyse von 10 Interaktionen zwischen jeweils zwei Männchen zeigen, dass Beinwinken als direktionales Signal in Richtung des Gegners verwendet wurde, aber seltener vorkam, als Rufen. Die akustischen und visuellen Signale waren nicht funktionell gekoppelt, wie es von Winkerfröschen der Gattung *Staurois* bereits bekannt ist. Die Analyse von Signalabläufen ergab, dass Verhaltensweisen, in denen der Gegner mit dem Bein gestoßen wurde (physische Angriffe), signifikant mehr Attacken von interagierenden Männchen auslösten. Wir behaupten, dass das Signal Beinwinken sich von der häufig zu beobachtenden Kampftechnik des Stoßens ritualisiert hat, um die Gefahr von physischen Angriffen zu reduzieren. Die Ergebnisse dieser Studie unterstützen die Annahme, dass das Verhalten des Beinwinkens bei *M. saxicola* einen naszierenden Status in der Evolution von visuell signalisierenden Fröschen darstellt.

Curriculum vitae

Persönliche Daten

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Ausbildung

1987 - 1990	**Bundeshandelsschule Schwaz/Tirol**
2000	**SAE international technology college Wien**
2006 - 2012	**Universität Wien, Biologiestudium/Evolutionsbiologie**

Beruflicher Werdegang

1990 - 1994	**Bankkaufmann Raiffeisenbank Zell am Ziller**
1995	**Zivildienst Rotes Kreuz**
1996	**Photograph**
1997 - 1999	**Typograph Tiroler Bezirksblätter**
2001 - 2012	**Selbständiger Multimediaproducer/Cutter**

Fähigkeiten und Kompetenzen

Muttersprache:	Deutsch
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Studienbezogene Erfahrung:	Auslandsaufenthalte: Freiwillige Mitarbeit bei einem Chamäleon-Monitoring-Projekt in Gialova/Griechenland 2007 Projektpraktikum „Ethologie und Diversität neotropischer Amphibien" in Französisch Guyana 2009 Leistungsstipendium der Universität Wien 2010