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Environmental constraints and call evolution in torrent-dwelling frogs

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Although acoustic signals are important for communication in many taxa, signal propagation is affected by environmental properties. Strong environmental constraints should drive call evolution, favoring signals with greater transmission distance and content integrity in a given calling habitat. Yet, few empirical studies have verified this prediction, possibly due to a shortcoming in habitat characterization, which is often too broad. Here we assess the potential impact of environmental constraints on the evolution of advertisement call in four groups of torrent-dwelling frogs in the family Ranidae. We reconstruct the evolution of calling site preferences, both broadly categorized and at a finer scale, onto a phylogenetic tree for 148 species with five markers (~3600 bp). We test models of evolution for six call traits for 79 species with regard to the reconstructed history of calling site preferences and estimate their ancestral states. We find that in spite of existing morphological constraints, vocalizations of torrent-dwelling species are most probably constrained by the acoustic specificities of torrent habitats and particularly their high level of ambient noise. We also show that a fine-scale characterization of calling sites allows a better perception of the impact of environmental constraints on call evolution.

KEY WORDS: Acoustic adaptation hypothesis, ancestral state reconstruction, Anura, noisy environments, phylogeny, Ranidae.

Acoustic signals are widely used in animals, particularly for long-distance communication. Many of these signals function primarily to attract mates, with males advertising their location and disposition to females. In frogs, these advertisement calls are most likely innate (Gerhardt 1974, 1994; Doherty and Gerhardt 1984; but see Narins et al. 2006) and play a pivotal role in reproduction, making them subject to sexual and natural selection pressures.

The evolution of amphibian vocal organs has been mentioned as early as in Darwin's "Descent of Man" (Darwin 1871) but the forces driving the evolution of vocalizations remain poorly understood. A multitude of factors could be shaping these acoustic signals, complicating the investigation of their evolution. Natural (environmental and predator-driven) and sexual (inter- and intra-) selection and phylogenetic inertia may act in opposite and

sometimes surprising directions. In addition, body size (e.g., Zimmerman 1983; Ryan and Kime 2003; Hoskin et al. 2009; Gingras et al. 2013) and physiological factors, such as body temperature (Gerhardt 1978; Gayou 1984; Marquez and Bosch 1995; Navas and Bevier 2001; Bevier et al. 2008) or hormonal level (Penna et al. 1992; Boyd 1994; Leary et al. 2004), affect some characteristics of the vocalizations and might induce analytical biases if they are not considered (Zimmerman 1983; Bevier et al. 2008; Erdtmann and Lima 2013). These challenges have resulted in relatively few papers on frog vocalizations within a phylogenetic context (Ryan and Rand 1993; Cocroft and Ryan 1995; Cannatella et al. 1998; Lodé 2001; Robillard et al. 2006; Wollenberg et al. 2007; Goicoechea et al. 2010; Gingras et al. 2013), where studies are often limited to few species and small phylogenetic groups.

Environmental pressure acts as one of the major selective forces on acoustic signals' evolution (Morton 1975; Marten and Marler 1977; Wiley and Richards 1978; Endler 1992; Schluter 2000; Ey and Fischer 2009; Weir et al. 2012). The physical features of the environment affect both sound transmission and its content integrity, favoring different calls in different environments. As a call that does not reach its intended receiver—or is too distorted to be efficiently recognizable or localizable—often reduces the fitness of the sender, the acoustic properties of a calling site are expected to constrain call evolution and might lead to convergent calls in similar environments. This idea is referred to as the Acoustic Adaptation Hypothesis or AAH (Morton 1975; Marten and Marler 1977; Wiley and Richards 1978; Endler 1992; Ey and Fischer 2009; Weir et al. 2012).

In the AAH framework, several environmental variables have been highlighted as relevant, for example, the vegetation density of a site or the ambient noise (Castellano et al. 2003; Ziegler et al. 2011). A high level of ambient noise can be considered a strong selective pressure, as ambient noise can mask the signal if their respective frequency ranges overlap (Brumm and Slabbekoorn 2005). In such situations, a frequency shift of the signal reducing the masking effect would be advantageous for the sender (Brumm and Slabbekoorn 2005; Nemeth and Brumm 2009; Cunningham and Fahrig 2010; Boonman and Kurniati 2011; Cardoso and Atwell 2011; Both and Grant 2012; Goodwin and Podos 2013). Other acoustic parameters of vocalizations have also been suggested as sensitive to environmental pressures such as rhythm, frequency, and amplitude modulation (AM; Dubois 1977a, 1977b; Dubois and Martens 1984; Grosjean and Dubois 2006; Penna and Meier 2011).

Despite several studies in birds, mammals, and anurans, no general adaptive pattern has been shown between vocalizations and environmental properties (Morton 1975; Dubois and Martens 1984; Nemeth and Brumm 2009; Peters and Peters 2010). This could be due to a shortcoming in environment characterization

(Bosh and De La Riva, 2004; Wells 2007; Ey and Fisher 2009). Calling sites are often simplified into broad habitat categories such as “forest,” “stream,” or “open habitat.” This simplification does not account for the variability within those categories (Morton 1975; Zimmerman 1983; Kime et al. 2000; Bosch and De la Riva 2004; Vargas-Salinas and Amézquita 2014), which might lead to misinterpretations or biased results as shown in other broad typologies (Grandcolas et al. 2011; Doody et al. 2013; Legendre et al. 2014). A few studies have attempted to address this issue but never at a large comparative scale (e.g., Castellano et al. 2003; Ziegler et al. 2011), taxonomic sample size being a major limitation.

Large and reliable phylogenetic hypotheses are not always available. Therefore, most studies testing environmental adaptation on acoustic signals either ignore phylogenetic relationships (e.g., Penna and Solis 1998; Kime et al. 2000) or integrate them by grouping species at different taxonomic levels (e.g., Zimmerman 1983; Bosch and De la Riva 2004; Bevier et al. 2008). Nevertheless, methods to account for phylogenetic nonindependence of species exist (Felsenstein 1985a; Grafen 1989) and have been used in a few studies on acoustic adaptations (e.g., Peters and Peters 2010). This approach allows testing for adaptations but ignores the temporal aspect of those adaptations as evolutionary novelties. To account for both phylogenetic relationships among species and the temporal pattern of lineage divergence, ancestral state reconstructions (ASRs) are needed (Grandcolas and D'Haese 2003; Glor 2010). ASR allows testing for the cooccurrence between an evolutionary novelty and a change in the environmental context, which complements comparative approaches considering phylogenetic relationships among species (Hansen 2014).

Herein we design a phylogenetic study, combined with two procedures to describe calling sites (“broad-type” vs. “fine-scale” codings), and assess how characterizing calling sites at a finer scale affects conclusions on both environmental constraints and call evolution. We examine the evolutionary pattern of several parameters of the advertisement call and, though we focus on torrent frogs (*sensu* Goutte et al. 2013), we undertake this study within a larger phylogenetic context. First, we build a molecular phylogeny including species for which we have acoustic and ecological data. We assess the relationship between body size and call characteristics, which could potentially alter our interpretation of the subsequent analyses. Then we estimate the ancestral values of calling site characteristics, using both broad-type and fine-scale coding methods, to delineate the environmental constraints. We test several models of evolution and evolutionary scenarios of calls based on these ASRs. Finally, we estimate the ancestral values of vocalization traits using the best-fitted model of evolution for these traits. We thus aimed at (1) evaluating the impact of a fine-scale coding of

habitats to characterize environmental constraints and (2) investigating the role of ecological constraints on call evolution in ranid frogs.

Materials and Methods

TAXON SAMPLE AND FIELD SITES

The family Ranidae (sensu Frost et al. 2006) is a large group of frogs (377 species, Frost 2015) showing high diversity in ecology and vocalizations (Wells 2007). Species of the family Ranidae call in ponds, lakes, waterfalls, rivulets, or large rivers. Species from four Ranidae clades call in or in the close vicinity of fast-flowing streams: the genera *Staurois*, *Amolops*, and *Odorrana*, and the group formed by three genera *Huia*, *Clinotarsus*, and *Meristogenys*. These six genera do not belong in a single monophyletic group and some are phylogenetically distant (Pyron and Wiens 2011), suggesting multiple, independent acquisitions of the torrent-dwelling lifestyle. The family Ranidae is thus an ideal group to test evolutionary questions on communication with regard to environmental constraints.

We focused on torrent frogs but also sampled species associated with other habitats. Data were collected at 11 localities within five regions of Southeast Asia: Preah Vihear province, Cambodia, December 11 to 20, 2010; West Kalimantan province, Indonesia, from June 9 to August 3, 2011; Sichuan, Hunan, and Hainan provinces, China, from June 7 to July 19, 2012; and Sabah province, Malaysia, August 8 to 24, 2012. Detailed locality data and permit numbers can be found in Goutte et al. (2013). No species currently considered as threatened were sampled (IUCN 2015).

We collected a total of 112 individuals of 37 species for which we recorded vocalizations and fine-scale measurements characterizing their calling sites. After recording, we captured specimens by hand and they were euthanized using a chlorobutanol solution. We collected and recorded 18 individuals of nine additional species, but without any precise data about their calling sites. We also collected individuals from 11 species, which were not calling during the period of our fieldwork, in order to complete our molecular dataset. As outgroups, we recorded and collected individuals from 23 species of the families Rhacophoridae, Dicroglossidae, and Microhylidae. This nonexhaustive sampling is unlikely to represent the diversity of these three families, and we will not discuss results for these groups (Grandcolas et al. 2004). We took tissue samples from liver or thigh muscle and stored them in 100% ethanol for subsequent sequencing. We fixed specimens with a 10% formalin solution for 12–24 h before storing in 70% ethanol solution for museum collection purposes. We measured them after fixation to the nearest 0.1 mm with a Swiss Precision Instrument caliper (model: 31-415-3). Specimens are deposited at the Museum Zoologicum Bogoriense (MZB), Indonesia; the

Chengdu Institute of Biology (CIB), China; and the Sabah Museum (SPM), Malaysia.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

DNA from the samples outlined above and additional samples (Table S1) was extracted using Qiagen DNeasy Tissue Kits. Five molecular markers for a total of approximately 3600 bp were selected according to previous works on ranids (e.g., Wiens et al. 2009) and sequence availability on GenBank. We used two mitochondrial markers 12S (810 bp) and 16S (580 bp) and three nuclear markers: tyrosinase (601 bp), rhodopsin (310 bp), and RAG-1 (1256 bp; Table S1). Amplified DNA fragments (methods detailed in Supporting Information) were sent to the Centre National de Séquençage (Génoscope, Evry, France) for sequencing.

ALIGNMENT AND PHYLOGENETIC ANALYSES

We obtained a total of 300 sequences and additional sequences were downloaded from GenBank (Table S2). We sampled 80.2% of the taxa for the 12S, 93.2% for the 16S, 74.7% for the tyrosinase, 77.8% for the rhodopsin, and 60.5% for RAG-1. Molecular sequences were aligned using MUSCLE version 3.8.31 (Edgar 2004). We selected partitioning scheme and substitution models using PartitionFinder (Lanfear et al. 2012). For each marker, the best model was a General Time Reversible model with a proportion of invariant sites (I) and a gamma distributed rate variation among sites (Γ). However, because I and Γ are strongly correlated (Sullivan et al. 1999) and using models mixing these parameters could bias their estimation, we used a GTR + Γ model instead. The best partitioning strategy involved two partitions (mitochondrial and nuclear genes separated).

Phylogenetic reconstructions were performed in maximum likelihood (ML) with RAxML version 7.0.4 (Stamatakis 2006; Stamatakis et al. 2008) and under Bayesian inference (BI) with MrBayes version 3.2.0 (Ronquist et al. 2012). We ran separate and combined analyses. In ML, we selected the best tree from 1000 runs computed from 1000 distinct randomized maximum parsimony starting trees. We calculated support values from 1000 bootstrap replicates using the standard bootstrapping algorithm. In BI, we launched two runs with four chains each for 100 million generations, with trees sampled every 10,000 generations. We assessed the burn-in with Tracer version 1.5 (Rambaut and Drummond 2009). The Potential Scale Reduction Factor (PSRF) values were between 0.999 and 1.014, except for the clock rate parameter (1.144), and effective sample sizes (ESS) were above 200 for all parameters, except the clock rate parameter, indicating a good sample from the posterior probability distribution.

We simultaneously estimated divergence times using an invariant gamma rate relaxed-clock analysis, following the method highlighted in Ronquist et al. (2012). We used two calibration

points: the root of our tree and the most recent common ancestor (MRCA) of our ingroup (i.e., Ranidae). Both values are secondary calibrations taken from dating estimates made in Bossuyt et al. (2006). Their accepted age intervals were 98.6–180.8 and 36.1–85.9 Mya for the root and the Ranidae MRCA, respectively. We used a uniform distribution for both calibration points because we did not have enough information to parameterize meaningfully nonuniform calibrations (Sauquet et al. 2012). We chose these two calibration points because they are outside of our focus group, for which we did not want to constrain dating. The biogeographic events and fossils used in Bossuyt et al. (2006) were inappropriate to our taxon sample and no other data were available, so we relied on secondary calibrations. To calculate the clock rate and branch length rate variance priors used in the relaxed-clock analysis, we first ran a nonclock analysis, thereby getting a starting topology. We then ran a nonclock and a noncalibrated strict-clock analysis on a fixed topology (obtained in the previous analysis), allowing the priors' calculations. All posttree reconstruction analyses were conducted on the combined phylogenetic hypothesis obtained in BI with a model with two partitions.

ACOUSTIC RECORDINGS AND SOUND ANALYSIS

Calling males were located acoustically and visually at night from 19:00 to 24:00 h or during the day from 7:00 to 17:00 h for the diurnal species of the genus *Staurois*. Advertisement calls were recorded from a distance between 0.5 and 2 m. During each recording, a dim-red headlamp was used, in order to minimize any disturbance by light (Pearse 1910; Buchanan 1993; Koskela-lainen et al. 1994). We used a Marantz PMD671 recorder with a Sennheiser ME64 microphone or an Avisoft CM16 microphone; sampling rate was 44.1 or 96 kHz at 16 bits. The call type most often heard from single males was considered as the advertisement call. Other call types produced by conspecific males, often heard when males were close to each other or physically engaged, were considered as aggressive or release calls, and they were disregarded. In order to complete our dataset, especially for taxa from nonsampled regions, recordings of 38 species were provided by collaborators or extracted from published compact discs (Table S3). Calls recorded for this study were deposited at the Fonoteca Zoológica in Madrid (Toledo et al. 2015).

Approximately 21,500 notes (sensu Robillard et al. 2006) from 225 individuals in 79 species (from one to 14 individuals per species) were analyzed (Table S4). The sound files (WAV format) were labeled and segmented with custom-made scripts in the software *Praat* (Boersma 2001). The segmented WAV files and the text file containing the labels and time information were then imported into the *R* environment (R Core Team 2013) and analyzed with the package *seewave* (Sueur et al. 2008).

We selected acoustic variables according to two criteria: their relevance to our question, based on existing literature, and their

availability for a large sample of species. Habitat acoustic properties are expected to impact the duration (Morton 1975; Dubois and Martens 1984; Ey and Fischer 2009), redundancy (Endler 1992; Ryan and Kime 2003), dominant frequency (DF; Morton 1975; Marten and Marler 1977; Arch and Narins 2008; Boonman and Kurniati 2011), bandwidth (BW; Dubois and Martens 1984), and amplitude and frequency modulation (FM; Wiley and Richards 1978; Richards and Wiley 1980) of the acoustic signal.

We therefore measured the following values from each note and averaged them for each individual and species for subsequent analyses: note duration, number of notes per call (defined here as an uninterrupted sequence of notes), DF, amplitude and FM within notes, and DF BW. For the amplitude and FM, the root-mean-square (RMS) sound pressure (in Pascal) and DF, respectively, were calculated for each 1-msec segment of the note. The slope of the resulting curve (RMS or DF values by time) was then calculated through linear model fitting. Each resulting curve was checked by eye. The values used for amplitude and DF modulations are thus representing slopes (without unit). This representation is more meaningful than amplitude or DF difference because it conveys information about the duration of the modulation: for example, a FM of 1 kHz would probably not have the same behavioral and evolutionary meaning over a 300-msec- or a 3-sec-long note. Dubois and Martens (1984) suggested that torrent-dwelling species produce short sequences of notes separated by long periods of silence and that the notes are rhythmically separated within the sequences. However, due to the high diversity in call structure in our dataset (including calls composed of a single note, one or several groups of notes, pulsed or nonpulsed notes), a comparative analysis of silence intervals based on the homology criterion was difficult to establish, and we limited our temporal analysis to the note duration and the number of notes per call.

CALLING SITE CHARACTERISTICS

In order to produce calling site categories, we measured habitat descriptors showed to be of importance in anuran habitat and calling site choice (Gillespie et al. 2005; Keller et al. 2009; Goutte et al. 2013). Once an individual had been captured, we measured five habitat descriptors at the exact individual calling location: depth, width, and average slope of the closest water body; air temperature; canopy coverage; and ambient sound pressure level (SPL). In brief, ambient air temperature was measured with a probe-K digital thermometer (Hanna) to the nearest 0.1°C. The canopy coverage was measured the day following the capture with a convex-A spherical densiometer. The ambient SPL, in decibels, was measured with a digital SPL meter (American Recorder Technologies), with A-weighting and slow capture (1-sec averaging) to the nearest 0.1 dB (precision: 1.5 dB at 1 kHz). The maximal value for a 30-sec time window was taken. For ponds, we

measured maximal depth and mean diameter. For streams, we measured the width of the water body, and the maximum depth across that width, at the focal male level. We also measured the maximal SPL in a 2-m radius around the frog calling post. A detailed description of habitat methods has been presented in an earlier paper (Goutte et al. 2013). We produced a broad-type coding for calling sites based on these precise measurements with multifactorial analysis and cluster analysis (Goutte et al. 2013): “torrent,” “pond,” and “river/lake.” In the present study, we added the category “forest,” as additional nonriparian species were included in this dataset.

For the fine-scale coding, we used the SPL and canopy coverage measures, two environmental variables predicted to impact call evolution (Morton 1975; Marten and Marler 1977; Wiley and Richards 1978; Brumm and Slabbekoorn 2005; Boonman and Kurniati 2011; Vargas-Salinas and Amézquita 2014). SPL was shown to be of the utmost importance to characterize frog habitats (Goutte et al. 2013), whereas canopy coverage measures the openness of a site more precisely than the typically used binary character (i.e., “open” vs. “closed”).

ANCESTRAL STATE ESTIMATION OF CALLING SITE TYPE AND CHARACTERISTICS

The choice of calling site may be subject to selection, as females may not mate with a male calling from a site unsuitable for egg laying, for example. Calling site preference can thus be considered as inheritable, even though, as any behavioral trait, it is also subjected to circumstantial factors (Losos 2011). We reconstructed ancestral preference of calling sites using two coding options: broad-type and fine-scale. We reconstructed ancestral states of broad-type using the function *rerootingMethod* in the *R* package *phytools* (Revell 2012), and estimated the likelihood of each ancestral state with a Markov model allowing every transition rate between two states to be different (Pagel 1994; Paradis et al. 2004). For the fine-scale coding method, we estimated ancestral values of canopy coverage and SPL using the function *fastAnc* in the *R* package *phytools*, assuming a Brownian motion (BM) process. Before the analysis, we log-transformed the continuous variables to satisfy a normal distribution of the data. For both coding methods, the analyses were done on 500 trees, randomly sampled from the best-fitting trees in the BI analysis, to account for phylogenetic uncertainty. For each internal node of interest, we checked the monophyly across all sampled trees and excluded the trees for which the corresponding clade was nonmonophyletic.

RELATIONSHIP BETWEEN BODY SIZE AND CALL CHARACTERISTICS

In order to assess whether morphological constraints on acoustic characters could impede our interpretation of the effect of environmental constraints on call evolution, we tested the relationship

between body size and acoustic traits using phylogenetic generalized least squares (pGLS), with a correlation structure of the residuals corresponding to the phylogeny topology and chosen model of evolution (BM or Ornstein–Uhlenbeck [OU], see “Models of evolution” methods section below) for the given variable.

EVOLUTION OF CALL CHARACTERISTICS

Models of evolution

In the case of strong environmental pressures, similar environments may lead to similar phenotypes (Williams 1972; Morton 1975; Rothstein and Fleischer 1987). Torrent frogs could thus display similar advertisement calls regardless of phylogenetic relatedness. To test this prediction, we fitted and compared models of evolution to our data. The BM model (Felsenstein 1985b), commonly used for trait evolution analyses, assumes that continuous characters can diverge indefinitely toward any value, which is generally not realistic for biological characters. The OU model corresponds to a BM process model with a stabilizing selection toward an optimum, denoted θ (Hansen and Martins 1996). The values of the traits are thus constrained around a given value and the strength of this constraint is controlled by another parameter, denoted α . An α -value of 0.001 reflects a very weak constraint of the trait toward the optimum θ , whereas an α of 100 corresponds to instantaneous transition to θ ; if α equals 0, the OU model is reduced to a BM model. The parameter θ can be estimated for the whole phylogeny, or different values of θ can be estimated with changes in θ at nodes corresponding to a change in selective regime (Hansen 1997; Butler and King 2004).

For each acoustic trait, we tested a series of models of evolution: a BM model assuming no trend or constraint (null model), an OU model assuming a single optimum θ , and five OU models assuming two to three optima in the phylogeny (Fig. 1). Models with multiple optima were defined according to biological hypotheses derived from the ancestral values estimation of calling site characteristics and previous hypotheses linking call features and environmental conditions (Morton 1975; Ryan and Kime 2003; Ey and Fischer 2009; Boonman and Kurniati 2011). They assume that extant taxa and ancestors belonging to the same “regime” are under the same evolutionary constraints. For each regime, a different θ was estimated. We also estimated α and the variance of the process (σ^2) for the whole phylogeny. As body size is often correlated to acoustic properties of vocalizations (e.g., Zimmerman 1983; Ryan and Kime 2003; Hoskin et al. 2009; Gingras et al. 2013), and in order to test whether calling sites might have constrained the evolution of body size, we also performed the same analyses with the species average body size (snout-vent length).

Model OU2 assumed two regimes based on the major changes in calling site category estimated by the ASR (Fig. 2), one for torrent dwelling frogs (*Staurois*, *CHM*, *Amolops* and *Odorrana*) and another one for the other frogs. Model OU2b assumed

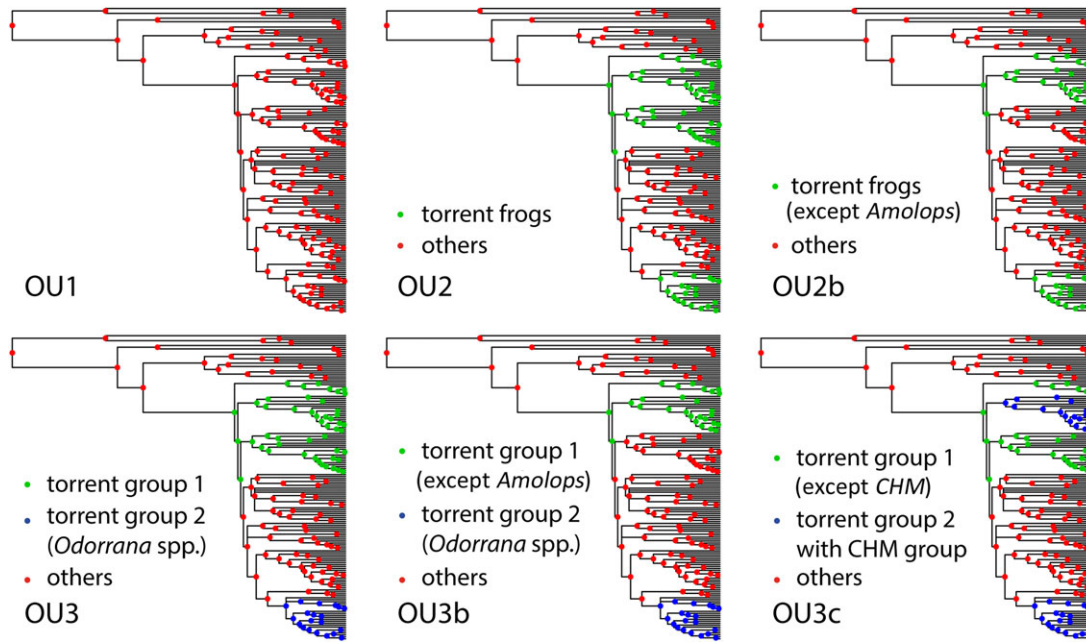


Figure 1. Tested OU models of evolution. Panels represent models from a single regime of selection (OU1) through multiple regimes derived from biological hypotheses (OU2-3c). Regimes are color-coded. θ is estimated for each regime while α and σ^2 are estimated for the whole phylogeny and kept constant.

the same groups, except that the lineage *Amolops* and non-torrent-dwelling species shared the same regime. This model was based on the ancestral values estimation of precise calling site characteristics (SPL and canopy coverage, Fig. 3), which showed similar values for the lineage *Amolops* and non-torrent-dweller groups. Model OU3 assumed three regimes and three major changes in calling site: a first change toward torrent life at node 5 (Fig. 2), placing the groups *Staurois*, *CHM*, and *Amolops* in the same regime; a second change after the divergence of *Amolops* (node 13, Fig. 2); and a third change before the divergence of *Odorrana* (node 21, Fig. 2). In this model, the lineage *Odorrana* is assumed to have a different regime than the other torrent-dwelling groups because it corresponds to a different evolutionary event, potentially involving different mechanisms. All non-torrent-dwelling groups shared a single regime. Model OU3b, as for model OU2b, only departed from the previous model regarding the placement of the lineage *Amolops* in the nontorrent regime. Model OU3c is based on call similarities observed in our dataset: it assumed a convergence between torrent-dwelling groups, *Amolops* and *Staurois* on one hand, which shared the same regime, and *Odorrana* and *CHM* on the other hand, which shared another regime. The non-torrent-dwelling lineages shared a third regime.

We chose to keep both α and σ^2 constant across the whole phylogeny to avoid overparameterization; preliminary analyses showed that a larger dataset would be needed to test more complex models where one or both of these parameters could vary

among regimes while keeping enough statistical power (data not shown). We tested whether torrent-dwelling groups had optimal trait values different from those of nontorrent species (models BM vs. OU2 and OU1 vs. OU2), and if they all shared the same optimal trait values, or whether they exhibited different “strategies” evolving toward different optimal values (models OU2 vs. OU3 and OU3 vs. OU3c). Finally, we tested whether the torrent lineage *Amolops* shared optimal trait values with non-torrent-dwelling species (models OU2 vs. OU2b and OU3 vs. OU3b).

In order to have a robust selection of models of evolution, we followed the method described in Boettiger et al. (2012) simulating 1000 datasets for each model to take into account phylogenetic uncertainty and variance in parameter estimations. We first estimated the parameters of the chosen models from the data with the function *OUwie*, in the R package *OUwie* (Beaulieu et al. 2012; Beaulieu and O’Meara 2013). We then simulated the data a hundred times with the estimated parameters under each model using the function *rTraitCont* in the R package *ape* (Paradis et al. 2004; Popescu et al. 2012) and fitted both models again on the simulated data. For each simulated dataset, the likelihood ratio between the two models was calculated. To choose the best-fitting model, we finally compared the distribution of likelihood ratios for the simulations under both models to the likelihood ratio derived from the real data. We repeated this protocol on 10 post-burnin trees from the BI analysis. The pairwise models comparisons were chosen in a stepwise manner, comparing increasingly complex models (Fig. 1). This method allows the assessment of the power of our

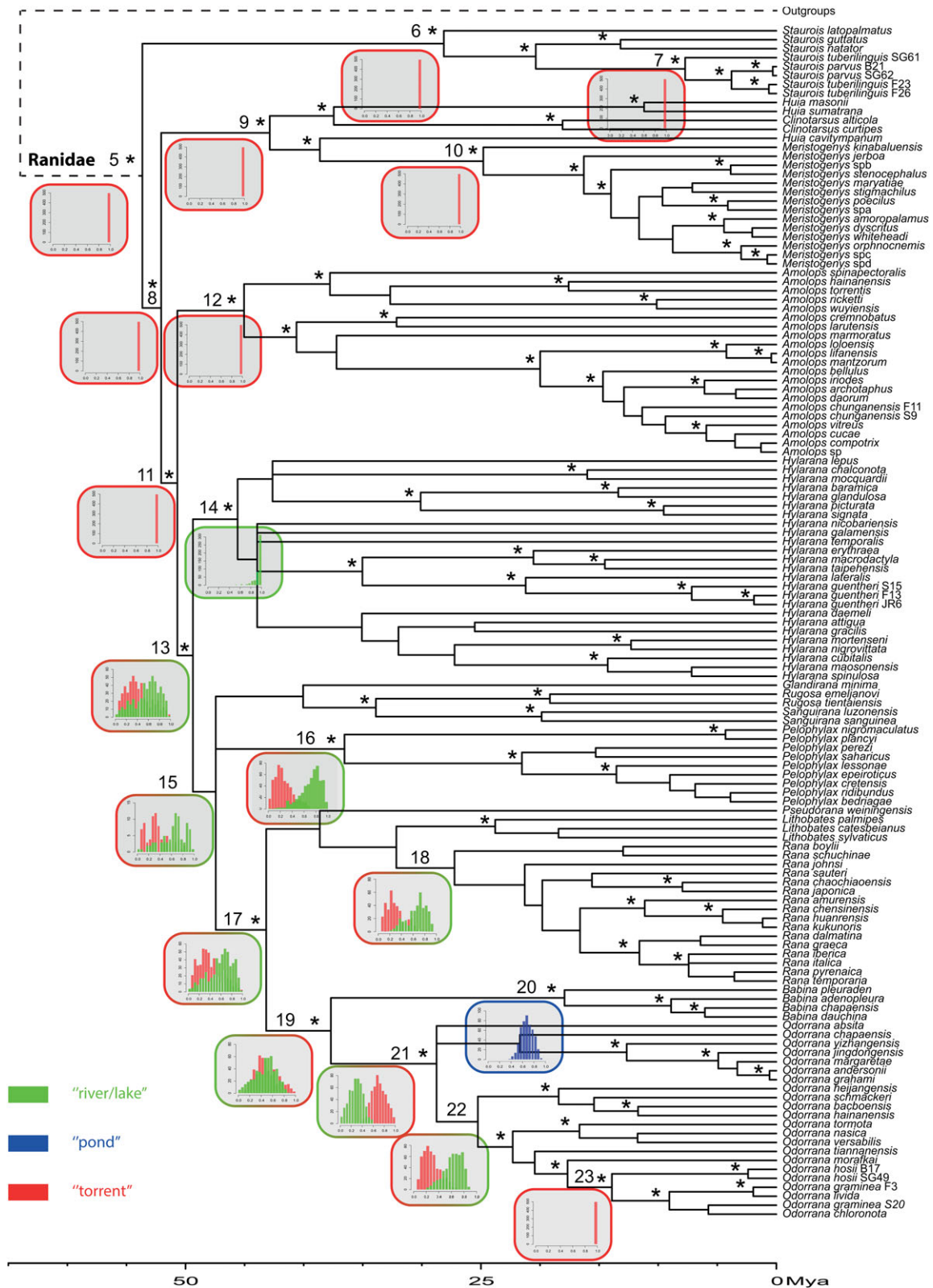


Figure 2. Ancestral state reconstructions of habitat types. Histograms at numbered nodes indicate the probability of each habitat type for 500 trees. Probabilities under 0.2 are not plotted. Asterisks indicate a posterior probability of 1 for the corresponding nodes. Four torrent-dwelling clades are identified (*Staurois*, *Huia*–*Clinotarsus*–*Meristogenys*, *Amolops*, and *Odorrana*), with an ancestral torrent habitat for the family Ranidae. Calmer water bodies are inferred for nodes 13–18 so that torrent-dwelling life for *Odorrana* species corresponds to a reversion toward “torrents.”

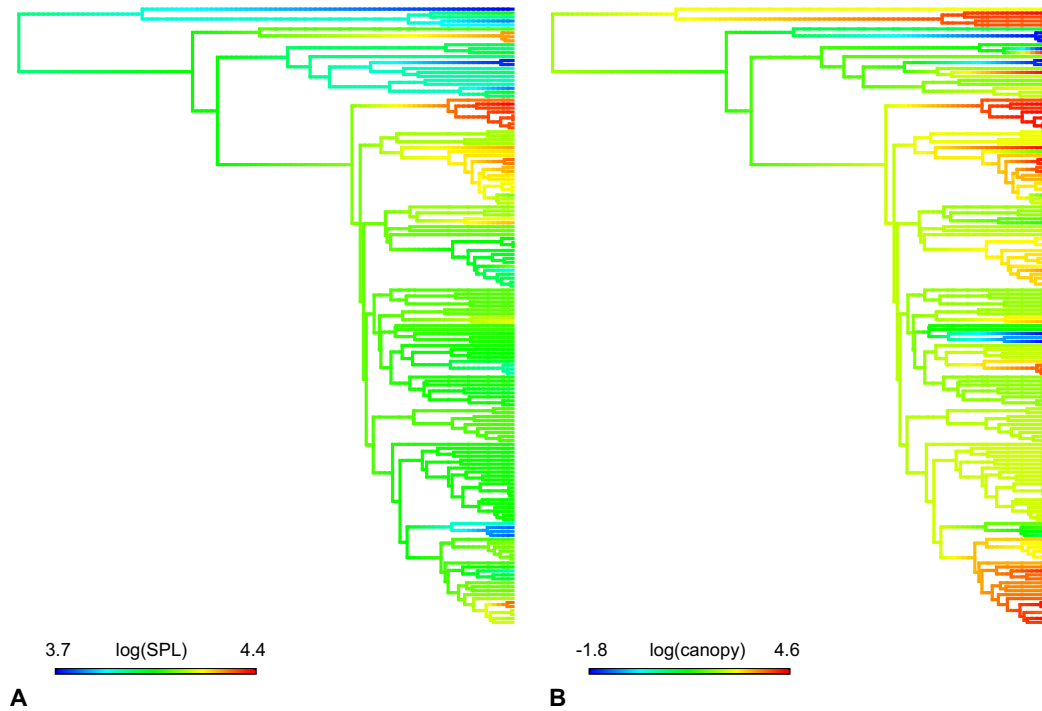


Figure 3. Estimation of ancestral values of habitat characteristics. Panels show heat-coded values for log-transformed (A) ambient noise level (SPL) and (B) canopy coverage across the phylogeny, obtained through ML estimation. Estimated values at the nodes are given in Table S5. Torrent-dwelling clades present higher canopy coverage and louder ambient noise level than non-torrent-dwelling clades with a few exceptions, in particular the genus *Amolops*.

analysis simultaneously to the models comparison. In contrast, AIC scores (Table S9), although allowing the comparison of all models at the same time, can be misleading in favoring more complex but poorly supported models (Boettiger et al. 2012).

Ancestral values' estimation of vocalization characteristics and body size

We estimated ancestral values of vocalization characteristics and body size with the function *fastAnc* in *phytools* (Revell 2012) on a set of 500 trees. Again, each variable was log-transformed, except for body size, from which the square root was taken prior to the analysis. According to the chosen model of evolution (see above), we used either a BM or an OU model of evolution to compute these estimates. In the latter case, we transformed the branch lengths of the tree using the fitted value of α with the function *ouTree* (R package *geiger*) before estimating the ancestral values using BM, thereby obtaining ancestral values under the OU model.

Results

MOLECULAR PHYLOGENY

The resulting topology in BI (Fig. S1) agrees with the most recent published phylogeny of the family Ranidae (Pyron and Wiens 2011). The few differences (*A. bellulus*, *Rana weiningensis*, *R.*

shuchinae, *Glandirana minima*, *Pelophylax saharicus*) were not strongly supported in one or both of the phylogenies (low support values or position method-dependent).

The phylogenetic reconstruction in ML (Fig. S2) resulted in a very similar topology. Differences affected recent nodes, which were not or weakly supported in one or both topologies. These differences have a minimal impact on our conclusions about vocalization evolution as they concern only recent nodes within clades sharing similar environmental constraints and vocalization characteristics.

TAXONOMIC NOTE

In this article, we follow Frost's (2015) taxonomy. As seen in previously published phylogenies (Pyron and Wiens 2011), the genus *Huia* is not monophyletic in our phylogenetic hypothesis, placing the clade containing "*Huia*" *masonii* and "*Huia*" *sumatrana* as sister taxa to the genus *Clinotarsus* (Fig. S1). As *Huia cavitympanum* is the type species of the genus, a taxonomic revision is required for this clade.

The genus *Glandirana*, as defined in Frost (2015), is not monophyletic in our phylogenetic hypotheses, although the problematic position of *Glandirana minima* is not supported in any tree. Here, we follow Fei et al. (2009) and recognize *Glandirana minima*, *Rugosa emeljanovi*, and *R. tientiaensis*.

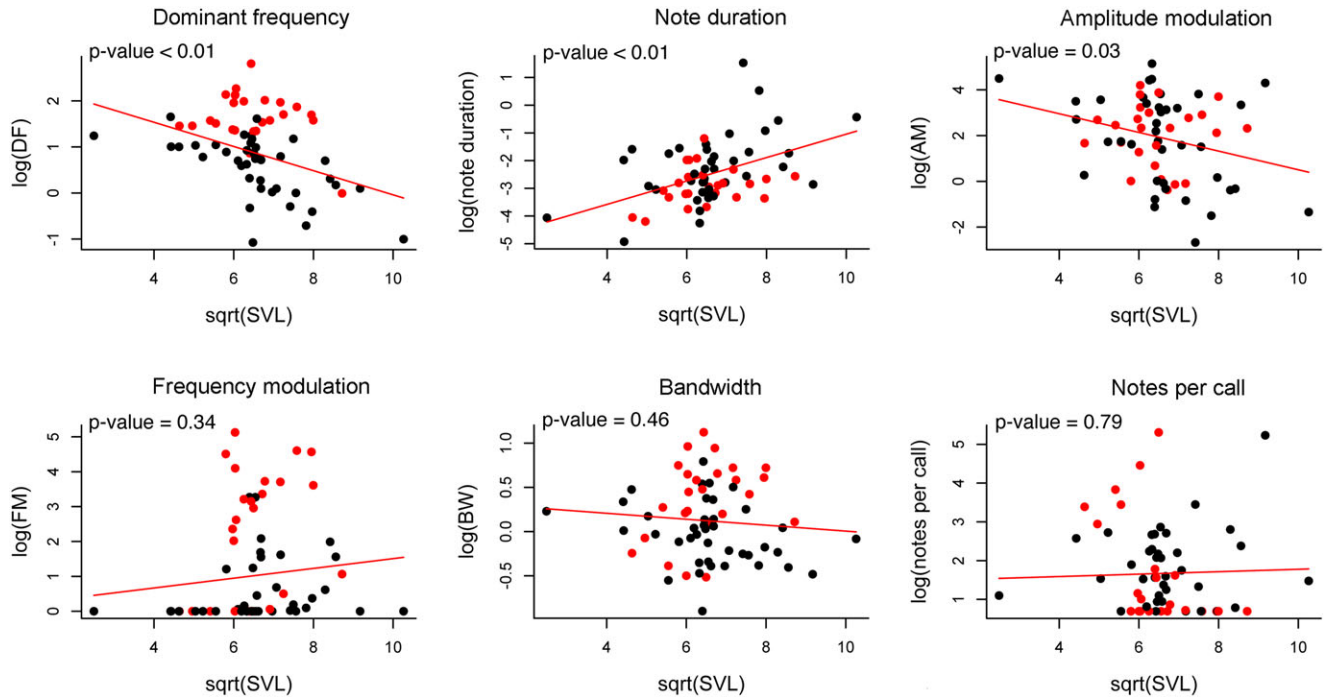


Figure 4. Relationships between body size and call acoustic characteristics. Panels show relationships between body size (SVL, square-rooted) and call acoustic characteristics (log-transformed) through pGLS regression, with regression line in red and associated *P*-values. Red points: torrent-dwelling species; black points: non-torrent-dwelling species. Three call characteristics shows significant relationship to body size (top panels) and most torrent-dwelling species show a higher DF than expected from their body size.

EVOLUTION OF CALLING SITE PREFERENCES AND INFLUENCE OF CODING OPTIONS

The estimation of ancestral calling site values using either the broad-type or the fine-scale coding methods leads to different phylogenetic patterns. Broad-type allowed splitting the phylogeny into clearly defined groups, whereas fine-scale descriptors varied in more complex ways.

The broad-type approach suggests that the ancestor of the Ranidae (sensu Frost et al. 2006) reproduced in fast-flowing streams (Fig. 2, Table S5). Our ancestral reconstruction of calling site for nodes deeper than the Ranidae ancestor may be strongly constrained by the outgroup sampling, which is limited by definition. It is thus difficult to say whether the common ancestor of the Ranidae and Rhacophoridae was already a torrent dweller or whether the Ranidae ancestor pioneered this habitat. The first diverging groups (i.e., *Staurois*, *Clinotarsus*, *Huia*, *Meristogenys*, and *Amolops*) remained in torrents for reproduction. At node 13, we inferred a change in calling site preference toward calmer water bodies. Indeed, species of the genera *Pelophylax*, *Sanguirana*, *Glandirana*, *Rugosa*, *Hylarana*, *Lithobates*, and *Rana* are found calling at ponds, lakes, rice fields, or calmer riverbanks. We inferred a secondary change toward faster-flowing streams at the divergence of the genus *Odorrana*.

Ancestral estimation of the fine-scale characteristics provides a more-detailed scenario on the evolution of calling site prefer-

ences and showed clear variations within the “torrent” category. The patterns of variation of ambient noise level (SPL) and canopy coverage showed some clear similarities (Fig. 3, Table S5). These variables are related to one another, and it is thus not surprising to see a correlation in their variation patterns. Both variables had relatively high values for the ancestor of Ranidae. They decreased gradually through evolutionary time, but increased twice after the divergences of *Staurois* and the *Clinotarsus*–*Huia*–*Meristogenys* (CHM) group.

The canopy coverage increased dramatically again after the divergence of *Odorrana*, whereas the increase in ambient noise level occurred only after the divergence of a subgroup of *Odorrana*. The values of both variables varied much less after the divergence of *Amolops*. We observed a decreasing trend for both variables for the nontorrent groups.

RELATIONSHIP BETWEEN BODY SIZE AND CALL CHARACTERISTICS

Body size was significantly correlated with DF, note duration, and AM (Fig. 4, Table S8). There was no significant correlation with frequency BW, FM, or the number of notes per call. A large majority of torrent-dwelling species had a higher DF than expected from their body size, based on all sampled ranids (Fig. 4), although they did not deviate from other species for all other acoustic variables.

EVOLUTION OF CALLS AND BODY SIZE

Models of evolution

For all acoustic variables except the number of notes per call, the best-fitting model separated the torrent-dwelling ranids from the non-torrent-dwelling groups (Table 1), each group showing different optima (Table 2). The OU and BM models for the number of notes per call were not significantly different. For the FM, the model OU2b was preferred over the model OU2 in nine of the ten trees sampled (Table 1), even though the power of the test remained low (52.8%). For body size, no OU model was significantly better than the BM model (Table 1).

The optimal values (θ) estimated for torrent-dwelling ranids were a higher DF (7.7 vs. 2.0 kHz), a shorter note duration (56 vs. 99 msEC), and a greater FM (threefold increase) than the optima for non-torrent-dwelling lineages (Table 2). The optima for AM were not significantly different between the torrent- and non-torrent-dwelling lineages.

For DF, the estimated value for α was low (Table 2), indicating a weak constraint the estimated optimal value θ ; this constraint was more substantial for the FM and note duration; and the estimated values of α for BW and AM were very high, indicating a strong selective pressure for this trait. As α was estimated for the whole phylogeny and not for each regime, its value does not account for the possibility that some regimes may involve a stronger constraint than others. We will thus refrain from discussing it further.

Ancestral values' estimation

According to our ancestral value estimations (Table 3), the ancestor of the Ranidae (node 5) displayed a multinote call, with a DF high enough to be placed in the torrent-dwelling frogs partition (2.9 kHz). Four increases of DF, linked to the four torrent-dwelling lineages, were estimated: in the *Staurois* lineage (node 6), in the *CHM* group (node 9), after the divergence of *Odorrana* (node 21), and a slighter one in the genus *Amolops* (node 12). On deeper nodes, the DF value decreased gradually from the Ranidae ancestor to the *Odorrana* divergence.

Note duration decreased for the genus *Staurois*. Conversely, note duration increased for the non-torrent-dwelling group nodes, except for the genus *Babina* (node 20). Note duration decreased again after the divergence of *Odorrana* (node 21).

The number of notes per call increased after the divergence of *Staurois* and *Amolops*, and decreased after the divergence of the *CHM* group and *Odorrana*. The frequency BM increased after the divergence of the *CHM* group and *Odorrana*, but decreased after the divergence of the genus *Staurois*. FM increased after the divergence of the *CHM* group and *Odorrana*, but had low values at nodes basal to *Amolops* and non-torrent-dwelling groups, except for the genus *Babina*. Finally, AM showed little variation through the phylogeny. Body size showed an overall

Table 1. Model choice for the call characteristics and body size based on 100 simulations for a set of 10 trees.

Test	AM			DF			Frequency BW			FM			Note duration			Notes per call			Body size		
	Rej.	β		Rej.	β		Rej.	β		Rej.	β		Rej.	β		Rej.	β		Rej.	β	
BM vs. OU1	100	25.6 ± 4.1	0	0	23 ± 4.8	100	100	24.4 ± 3.7	100	25.1 ± 6.5	100	100	24.6 ± 5.2	0	33.6 ± 6.3	0	0.26 ± 0.08				
BM vs. OU2	100	100 ± 0.0	90	90	69.3 ± 9.4	100	100	100 ± 0.0	100	100 ± 0.0	100	100	99 ± 1.4	0	43.6 ± 10.4	0	0.61 ± 0.122				
OU1 vs. OU2	10	2.8 ± 1.1	90	90	9.3 ± 4.1	0	0	3.8 ± 2.4	0	3.9 ± 0.9	0	0	6.5 ± 2.5	0	5.4 ± 2.4	0	0.00 ± 0.00				
OU2 vs. OU2b	0	49.3 ± 14.1	0	0	42.2 ± 4.0	0	0	44.4 ± 15.8	90	52.8 ± 7.4	0	0	53.9 ± 5.8	0	42.8 ± 4.6	0	0.39 ± 0.05				
OU2 vs. OU3	0	6.3 ± 1.4	0	0	5.8 ± 1.6	0	0	5.6 ± 1.7	0	6.8 ± 2.1	0	0	7.9 ± 2.0	0	6.7 ± 1.6	0	0.06 ± 0.03				
OU3 vs. OU3b	0	7.8 ± 2.7	0	0	8.3 ± 2.6	10	10	7.3 ± 2.1	70	5.1 ± 1.7	0	0	4.9 ± 2.0	0	2.6 ± 1.4	0	0.07 ± 0.05				
OU3 vs. OU3c	0	7.9 ± 2.4	0	0	5.4 ± 2.1	100	100	5.7 ± 1.7	0	7.6 ± 2.0	20	20	6.7 ± 2.3	10	5.5 ± 1.8	0	0.06 ± 0.04				

The percentage of rejections of the first model is given (Rej.), with the average and 95% confidence interval of the associated power of the test (β). Abbreviations for call characteristics: AM, amplitude modulation; DF, dominant frequency; BW, bandwidth; FM, frequency modulation.

Table 2. Estimates of the OU2 model.

	AM	DF (Hz)	Frequency BW (Hz)	FM	Note duration (msec)
θ_{pond}	7.12 $\pm$ 1.86	2043.53 $\pm$ 3.94	963.36 $\pm$ 2.21	2.86 $\pm$ 2.18	99.11 $\pm$ 1.85
$\theta_{torrent}$	6.72 $\pm$ 1.46	7712.52 $\pm$ 1.41	1478.17 $\pm$ 1.08	9.27 $\pm$ 1.63	56.15 $\pm$ 1.34
α	232.25	9.22	325.33	36.45	33.08
σ^2	1432.71	6.61	92.19	293.67	71.88

θ estimates have been back-transformed and expressed in the original variable units with standard error. Modulation measures have no unit. Abbreviations for call characteristics: AM, amplitude modulation; DF, dominant frequency; BW, bandwidth; FM, frequency modulation.

Table 3. Ancestral value estimations for vocalization characteristics.

	AM	DF (Hz)	Frequency BW (Hz)	FM	Note duration (msec)	Notes per call
1	7.04 $\pm$ 1.57	2593.10 $\pm$ 1.78	1132.69 $\pm$ 1.12	4.30 $\pm$ 1.90	79.37 $\pm$ 1.40	4.31 $\pm$ 6.66
2	6.89 $\pm$ 1.54	2594.79 $\pm$ 1.67	1132.53 $\pm$ 1.11	4.32 $\pm$ 1.89	79.55 $\pm$ 1.39	4.86 $\pm$ 3.56
3	7.04 $\pm$ 1.57	2630.94 $\pm$ 1.61	1132.69 $\pm$ 1.11	4.31 $\pm$ 1.89	79.38 $\pm$ 1.40	5.32 $\pm$ 2.74
4	7.05 $\pm$ 1.57	2580.21 $\pm$ 1.57	1132.68 $\pm$ 1.11	4.32 $\pm$ 1.91	79.03 $\pm$ 1.41	6.32 $\pm$ 1.90
5	7.03 $\pm$ 1.57	2895.48 $\pm$ 1.41	1132.99 $\pm$ 1.12	4.40 $\pm$ 1.86	80.91 $\pm$ 1.38	5.32 $\pm$ 2.74
6	7.07 $\pm$ 1.82	3883.52 $\pm$ 1.64	1123.83 $\pm$ 1.15	8.88 $\pm$ 3.62	58.78 $\pm$ 1.94	7.55 $\pm$ 1.20
7	7.26 $\pm$ 2.57	4239.53 $\pm$ 1.45	1045.92 $\pm$ 1.23	31.63 $\pm$ 4.88	31.82 $\pm$ 2.21	18.82 $\pm$ 0.70
8	7.02 $\pm$ 1.57	2876.98 $\pm$ 1.38	1133.07 $\pm$ 1.12	4.39 $\pm$ 1.86	81.36 $\pm$ 1.38	5.47 $\pm$ 0.90
9	7.03 $\pm$ 1.59	4610.08 $\pm$ 1.56	1136.83 $\pm$ 1.12	4.85 $\pm$ 2.13	83.20 $\pm$ 1.50	3.11 $\pm$ 1.30
10	7.12 $\pm$ 1.69	5612.64 $\pm$ 1.60	1155.26 $\pm$ 1.13	6.70 $\pm$ 2.92	80.82 $\pm$ 1.75	1.67 $\pm$ 1.19
12	7.02 $\pm$ 1.58	2861.00 $\pm$ 1.48	1133.12 $\pm$ 1.12	4.29 $\pm$ 1.95	80.30 $\pm$ 1.43	9.33 $\pm$ 1.16
14	7.02 $\pm$ 1.57	2449.78 $\pm$ 1.39	1132.80 $\pm$ 1.12	4.39 $\pm$ 1.91	81.97 $\pm$ 1.40	4.79 $\pm$ 0.88
16	6.95 $\pm$ 1.60	1974.88 $\pm$ 1.61	1131.94 $\pm$ 1.12	3.92 $\pm$ 2.25	90.13 $\pm$ 1.54	4.97 $\pm$ 1.43
17	6.96 $\pm$ 1.58	2056.07 $\pm$ 1.43	1132.98 $\pm$ 1.12	4.20 $\pm$ 1.96	85.91 $\pm$ 1.42	4.53 $\pm$ 0.98
18	6.77 $\pm$ 1.62	1323.19 $\pm$ 1.57	1128.94 $\pm$ 1.12	3.52 $\pm$ 2.36	103.03 $\pm$ 1.58	5.14 $\pm$ 1.21
19	6.95 $\pm$ 1.59	2168.58 $\pm$ 1.49	1134.16 $\pm$ 1.12	4.35 $\pm$ 2.09	83.60 $\pm$ 1.48	4.53 $\pm$ 0.98
20	6.72 $\pm$ 2.43	1265.28 $\pm$ 1.47	1115.70 $\pm$ 1.22	6.46 $\pm$ 4.82	77.13 $\pm$ 2.20	6.42 $\pm$ 0.76
21	6.89 $\pm$ 1.65	2875.54 $\pm$ 1.52	1142.92 $\pm$ 1.13	4.85 $\pm$ 2.56	75.47 $\pm$ 1.64	1.93 $\pm$ 1.06
22	6.85 $\pm$ 1.68	3146.11 $\pm$ 1.53	1147.02 $\pm$ 1.13	5.23 $\pm$ 2.75	73.29 $\pm$ 1.69	1.93 $\pm$ 1.06
23	6.41 $\pm$ 1.07	4627.40 $\pm$ 1.49	1210.06 $\pm$ 1.18	9.92 $\pm$ 4.11	60.99 $\pm$ 2.04	1.28 $\pm$ 0.83

Mean and 95% confidence interval are given. Ancestral values for nodes corresponding to torrent-dwelling species are in bold. Estimations were obtained with an OU model, except for the number of notes per call (BM model). Abbreviations for call characteristics: AM, amplitude modulation; DF, dominant frequency; BW, bandwidth; FM, frequency modulation.

increase throughout the phylogeny but no clear pattern emerged (Fig. S3).

Discussion

In this study, we tested whether similarly constraining environments would lead to similar vocalizations as predicted by the AAH (Morton 1975; Ey and Fischer 2009). We showed that (1) the level of details in habitat characterization impacts the interpretations on environmental constraints and (2) these constraints constitute an important factor of call evolution in torrent-dwelling ranids.

EVOLUTION OF CALLING SITE PREFERENCE AND INFLUENCE OF CODING METHODS

Despite straightforward expectations, studies on the AAH have produced contradictory and inconclusive results (Ey and Fischer 2009). This discrepancy might suggest that environmental constraints are not a major selective force on calls or, as suggested lately (Bosch and De la Riva, 2004; Ey and Fischer 2009), that environmental constraints are not suitably characterized to demonstrate their influence. In the AAH framework, the environment is most often broadly defined as “open” or “closed,” which is likely too coarse a definition to account for the microscale habitat variability. Given that any eco-evolutionary pattern is scale-dependent

(Levin 1992), a broad definition process might lead to spurious conclusions, as already noted in ecology and ethology (Robillard et al. 2006; Grandcolas et al. 2011; Goutte et al. 2013; Legendre et al. 2015).

Contrasting two coding options for calling sites, we show here that a fine-scale approach is needed. First, even though both coding methods are based on precise measurements (the broad-type were defined using precise measurements—see Goutte et al. 2013 for details), they suggest different scenarios for the lineage *Amolops*: grouped with torrent-dwelling lineages with “broad” habitat categories, or grouped with non-torrent-dwelling lineages for the fine-scale approach. This implies diverging appraisals on environmental constraints, and consequently on convergence and adaptation (Patterson 1988; Wake 1991). Second, according to the AAH, note duration should be longer in closed habitats than in open habitats, seen as more fluctuant because of air turbulence, temperature gradients, and wind (Morton 1975; Ey and Fischer 2009). Yet, in our dataset, note duration decreases with increasing canopy coverage. We argue, however, that this result does not necessarily contradict the AAH predictions: even in a dense forest, a torrent is acoustically different from a closed habitat as described in the AAH literature. We can expect the atmosphere around a torrent to be highly turbulent, because a torrent constitutes a break through the vegetation allowing the wind in, while cold turbulent water creates temperature gradients. Thus, torrents fit neither with the “open” nor with the “closed” habitat categories, but present some constraints similar to both habitats. The AAH prediction involves air turbulence, wind, and temperature gradients, three variables that should be measured to properly test the aforementioned prediction. In other words, detailed habitat characterization should always be preferred to investigate the AAH. A few studies followed this direction but only at a small taxonomic scale so far (Castellano et al. 2003; Ziegler et al. 2011). Framed in a large phylogenetic scale and using fine-scale environmental data, our study shows that several acoustic features (DF, note duration, BW frequency, and DF modulation) are compatible with the AAH predictions (see below).

CALL EVOLUTION, ENVIRONMENTAL AND MORPHOLOGICAL CONSTRAINTS

The best-fitting model of evolution for all but one vocalization characteristic distinguishes torrent versus non-torrent-dwelling groups as being under different evolutionary constraints despite the existent morphological constraint on some vocalization characteristics (Fig. 4). Although our data did not allow the distinction of different selective regimes among the different torrent dwelling groups, the ancestral state reconstruction of both calling site and call characteristics and the AIC scores of the models of evolution (Table S9) suggest two distinct call types among torrent-dwelling ranids. More complex models, involving several strategies for

torrent-dwelling groups or allowing different alpha parameters for each regime, should be investigated with a larger dataset in order to test them with sufficient power.

In still-water bodies, male vocalizations are relaxed from the torrent-related selective pressures, so that other factors, such as female choice, competition, or predator-based selection may drive the evolution of vocalizations (Ryan and Kime 2003). This could have resulted in the diversity of calls we observed for non-torrent-dwelling species in our data (see Table S4). Torrents are seen as constraining environments regarding acoustic communication (Feng et al. 2006), so that convergences and adaptations are expected (Trontelj et al. 2012; Weir et al. 2012; Stayton 2015).

For torrent-dwelling ranids, we inferred a maximum of two calling “strategies,” divided into two nonmonophyletic groups: *Staurois*–*Amolops* and *CHM*–*Odorrana*. This low diversity in call types supports the hypothesis that torrents environmental properties have channeled the evolution of ranid vocalizations, like environmental factors constrain morphological or other behavioral traits (Trontelj et al. 2012; Roda et al. 2013). The similarities in vocalizations between *Odorrana* and *CHM* species fit with the definition of a convergence (Stayton 2015). Nevertheless, our dataset was insufficient to distinguish between the model of evolution supporting this convergence hypothesis (OU3c) and the one rejecting it (OU3), even though the AIC scores favored the OU3c model for three acoustic characteristics (DF, frequency BW, and number of notes per call). The call characteristics of *Staurois* and *Amolops* are more similar to the estimates of the Ranidae ancestor vocalization. *Staurois* and *Amolops* exhibit retained similarities, inherited from their common ancestor, and not a convergence pattern (Stayton 2015). In torrent ranids, two major options in advertisement calls evolved, one inherited from the Ranidae ancestor and the other appearing convergently in two ranid groups. These two options underline that different solutions might evolve despite similar constraints, which is rarely supposed at a high level of organization (Edelman and Gally 2001; Roda et al. 2013) or that subtle differences among the constraints resulted in different solutions. For instance, the group *CHM* might have evolved a different calling “strategy” than their closely related torrent-dwelling lineages (i.e., *Staurois* and *Amolops*) after a change in calling site characteristics toward larger streams (Table 3). Indeed, the vocalizations of the former are composed of single or very few, modulated notes and present high frequencies up to ultrasonic frequencies, while the latter relatively low-pitch calls are composed of multiple short notes. Although the AIC scores of the models support this hypothesis and the fine-scale coding approach already brings substantial elements, more data are needed to confirm this hypothesis with sufficient power.

The evolution of call DF follows patterns of ambient noise level variation (Fig. 3 and Table 3), indicating a possible

adaptation of the former to the latter: for the lineage *Amolops* both the ambient noise level and the DF increased marginally, whereas both values increased substantially for the other torrent-dwelling groups. The adaptive value of higher-pitched vocalizations in noisy environments has been proposed as an advantageous masking release mechanism for birds (Slabbekoorn and Peet 2003; Narins et al. 2004; Brumm and Slabbekoorn 2005; Nemeth and Brumm 2009; Cardoso and Atwell 2011; Goodwin and Podos 2013) and anurans (Narins et al. 2004; Arch and Narins 2008; Arch et al. 2008; Cunningham and Fahrig 2010; Boonman and Kurniati 2011). Our results support this hypothesis although comparative and experimental approaches are needed to further support these findings. The increase of call DF in *Staurois* is, however, minor compared with the other groups, even though the individuals from this genus vocalize at sites with the highest noise level.

Three other vocalization characteristics show general evolutionary patterns related to SPL: note duration decreases, frequency BW, and FM increase at nodes showing an increase in SPL (Fig. 3 and Table 3). However, for BW and FM, the genus *Staurois* departs from the general trend once again. These differences may be explained by the visual displays these species use during advertisement in addition to vocalizations (Grafe and Wanger 2007; Preininger et al. 2009; Grafe et al. 2012). Visual communication could lower the selective pressure on the acoustic signal, which may then act more as an alerting call than an advertisement call per se (Grafe and Wanger 2007). Such bimodal communication is known only for *Staurois* within the Ranidae, preventing from any statistical test of this hypothesis. However, torrent-dwelling species from other families are known to use bimodal communication (Lindquist and Hetherington 1996; Haddad and Giarretta 1999; Amézquita and Hödl 2004; Gomez et al. 2011; Preininger et al. 2013) and they should be investigated in this respect.

Although body size was significantly correlated with three vocalization characteristics (Fig. 4), calling site categories did not influence the evolution of body size (Table 1). The observed influence of calling site on the evolution of vocalization was therefore not mediated by a change in body size. Moreover, the quasi-totality of torrent-dwelling species had a higher DF than expected for their size based on the relationship observed in all sampled ranids (Fig. 4), indicating that selection of high DFs in torrent habitat occurred in spite of this morphological constraint.

Despite coarsely similar environmental conditions, we determined two calling “strategies” for torrent-dwelling ranids. This result shows that, while investigating the effect of environmental constraints on call evolution, a fine-scale characterization of the habitat, rather than broad categories, is needed. Comparative studies relying on fine-scale measures (environmental but also morphological and behavioral) would certainly improve our un-

derstanding of the evolution of acoustic communication, and reduce the discrepancies between theoretical and empirical studies in the AAH framework.

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DATA ARCHIVING

Original DNA sequences are archived in GenBank database (accession numbers KU840478–KU840796). Calls recorded for this study were deposited at the Fonoteca Zoológica in Madrid (<http://www.fonozoo.com/>; records 9199–9393). The doi for our data is 10.5061/dryad.89j5k.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Phylogenetic hypothesis from the Bayesian Inference analysis with two partitions (arithmetic mean of marginal likelihood = −58545.67).

Figure S2. Topology resulting from the maximum-likelihood analysis with two partitions.

Figure S3. Ancestral value estimations of body size (snout-vent length) by maximum likelihood.

Table S1. Primers.

Table S2. GenBank accession numbers.

Table S3. List of species recordings provided by recorders other than S. Goutte.

Table S4. Call variables' average values per species.

Table S5. Ancestral state reconstructions for the calling site types and the habitat variables.

Table S6. Description of the biological hypotheses for each Ornstein–Uhlenbeck (OU) model used in the analysis.

Table S7. Ancestral value estimation for body size (SVL).

Table S8. Correlations between the body size and acoustic variables by phylogenetic least squares.

Table S9. Fit of the models of evolution for the acoustic variables.

Table S10. Parameter values for the models of evolution fitted to acoustic variables and body size.