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Evolution of the anuran foam nest: trait conservatism and lineage diversification

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Key innovations are organismal traits that trigger adaptive radiation and lineage diversification. The wide range of anuran reproductive strategies from aquatic to terrestrial modes are potential key innovations. One such strategy is the foam nest, a structure with multiple functions that originated independently several times in different continents. Here, we explore several evolutionary aspects associated with the foam nest in multiple lineages to test the hypothesis that this trait is phylogenetically conserved and has influenced diversification. We used the most inclusive anuran phylogeny to date to reconstruct ancestral states, measure phylogenetic signal and evaluate models of trait-dependent diversification. Our results show that the foam nest appeared during the Paleocene–Eocene transition (*c.* 55 Myr) in three major groups (Leptodactylidae, Limnodynastidae and Rhacophoridae) and has been highly conserved ever since. The foam nest probably originated from an ancestor with aquatic reproduction, except in Rhacophoridae (in which it evolved from an ancestor with jelly nests). Despite possessing several ecological functions, we found evidence of the influence of foam nests on diversification rates only in the South American lineage Leptodactylidae. We suggest that the foam nest was an important adaptation to historical climatic changes, enabling reproduction in dry open areas.

ADDITIONAL KEYWORDS: Anura – key innovation – Leptodactylidae – reproductive mode – Rhacophoridae – terrestriality – thermal maximum.

INTRODUCTION

The evolution of a new trait has the potential to generate ecological opportunity by providing species access to physical, ecological and evolutionary spaces previously unreachable (Simpson, 1953). Such traits are referred to as key innovations and have the potential to lead to adaptive radiation (Losos, 2011a). The term key innovation has been applied in different contexts over the years and it is mainly used in a broader sense that maintains the core idea that some organismal attribute has played a major role over a lineage's evolutionary history (Heard & Hauser, 1995). More specifically, the definition of key innovation includes traits that promote diversification, either by increasing speciation or by decreasing extinction rates (Ree, 2005). Some studies have successfully identified such macroevolutionary effects of key innovations (e.g. Zimkus *et al.*, 2012; Pyron & Burbrink, 2014), even though diversification rates can also be influenced

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*Corresponding author. E-mail: elisabpereira@gmail.com by several other factors such as time, environment and species richness (see Morlon, 2014).

Evolution of anuran reproductive traits is particularly interesting in the context of key innovations and macroevolution, owing to outstanding diversity of anuran reproductive attributes and close relationship with environmental conditions (Haddad & Prado, 2005). Indeed, anuran reproductive features can be classified into several modes ranging from aquatic to fully terrestrial (Haddad & Prado, 2005). Reproductive modes with terrestrial eggs and/or tadpoles originated independently several times and with distinct adaptations (Gomez-Mestre, Pyron & Wiens, 2012) as a consequence of multiple selective pressures that include predation risk, larval competition and polyandry in aquatic environments (Heyer, 1969; Zamudio *et al.*, 2016). Although terrestriality is one of the few anuran traits hypothesized as a key innovation, results obtained so far show that it did not influence (Gomez-Mestre *et al.*, 2012) or that it decreased lineage diversification rates (Zimkus *et al.*, 2012). One possible explanation for the latter result is that terrestrial reproduction imposes additional risks, such as desiccation, difficulty in gas exchange and exposure to solar radiation (Touchon & Worley, 2015). Some anuran lineages overcame these limitations of terrestrial reproduction by placing eggs or tadpoles in foam nests on top of water, land or leaves (Haddad & Prado, 2005). The nest-building process is composed of multiple stages, in which the foam is formed by the incorporation of air from moving the fluid released by the female cloaca (Dalgetty & Kennedy, 2010). This movement varies among groups of anurans and can be made by males and/or females using their hind legs or paddling with arms (Furness *et al.*, 2010 and references therein). The foam is known to allow the animals to avoid aquatic predators (Hissa *et al.*, 2008), prevent desiccation (Dobkin & Gettinger, 1985), control oxygen supply (Hissa *et al.*, 2008) and buffer temperature (Méndez-Narváez, Flechas & Amézquita, 2015), to be a food source for larvae (Tanaka & Nishihira, 1987), and to protect eggs from microbial colonization (Fleming *et al.*, 2009) and solar radiation (Hissa *et al.*, 2008).

The foam nest is a potential key innovation and an interesting study case for trait-dependent diversification because it constitutes an exceptional example of convergence, having evolved independently in several anuran lineages on different continents (Wells, 2007). Some species of these lineages lay eggs in jelly masses with different thicknesses, but little is known about the composition and evolution of this attribute. For example, it is still unclear whether the jelly nest is a transitional state towards the foam nest or other terrestrial reproductive modes in all groups (Faivovich *et al.*, 2012; Meegaskumbura *et al.*, 2015). Despite the foam nest having many ecological properties and broad geographical distribution, only a few studies have explored its evolutionary dynamics and none has used a comparative approach or a diversification perspective (Faivovich *et al.*, 2012; Meegaskumbura *et al.*, 2015).

Here, we test the hypothesis that the foam nest is a conserved trait and a key innovation that increased diversification owing to its many ecological properties such as reducing water dependence and buffering temperature, which could have enabled species survival during historical climatic changes. Specifically, we reconstructed ancestral states, measured phylogenetic signal and tested trait-dependent diversification models on four of the richest anuran lineages with foam nests to evaluate whether (1) the foam nest is conserved; (2) jelly is an intermediate state that precedes the foam nest; and (3) the foam nest influenced lineage diversification.

MATERIALS AND METHODS

DATA SETS

We compiled from the scientific literature a list of all anurans that build foam nests, which encompassed 347 species of six families distributed over South America, Africa, Asia and Australia (Table S1 in Supporting Information). To avoid sampling biases (Davis *et al.*, 2013), we selected only lineages containing more than one species with foam nests, namely Cophylinae (Microhylidae), Leptodactylidae, Limnodynastidae and Rhacophoridae. Deposition of eggs in foam nests is the most common reproductive mode in Leptodactylidae (81% of the 202 species) and Limnodynastidae (70% of the 43 species), whereas it is less common in Cophylinae (23% of the 70 species). Rhacophoridae is the lineage with the highest variation in reproductive strategies, ranging from a completely aquatic mode to terrestrial direct development. This last mode makes up nearly half (48%) of the total 393 Rhacophoridae species, followed by reproduction with foam nests (35%). We used the most recent taxonomic list of amphibians (Frost, 2017) to prune the most comprehensively dated anuran phylogeny (Pyron, 2014), which encompasses nearly half of the total known diversity, and derive a phylogenetic hypothesis for each of the four aforementioned groups. Phylogenetic representation for each of the four lineages was moderate, with Limnodynastidae being the best represented group (53.5%), followed by Cophylinae (45.7%), Rhacophoridae (41.7%) and Leptodactylidae (38.6%).

To reconstruct ancestral states (see below), we classified each species as having indirect development without a nest (state 0), indirect development with a foam nest (state 1), indirect development with a jelly nest (state 2) or direct development (state 3). State 0 includes distinct reproductive modes, but for most species it refers to aquatic reproduction. To measure phylogenetic signal and test the influence of the foam nest on lineage diversification, we used only two categories, absence (0) or presence (1) of a foam nest. Every reproductive mode without a foam nest was included in the first category. As a conservative approach, species with jelly nests were considered as not having foam nests given that the functions of jelly are not yet fully understood and it may not have the same adaptive advantages as foam (Wells, 2007).

ANCESTRAL STATE RECONSTRUCTION AND PHYLOGENETIC SIGNAL

We reconstructed ancestral states and estimated the transitions between them by performing 1000 simulations of stochastic character mapping using SIMMAP in the phytools R package (Bollback, 2006; Revell, 2012). We performed this analysis on the entire anuran phylogenetic hypothesis (full tree; Pyron, 2014) using the four reproductive character states mentioned above and then pruned the four focal lineages from the mapped tree to ensure greater rigour in the reconstruction of the basal node of each group.

We measured the phylogenetic signal of foam nests in the full tree and separately for each of the four focal lineages using the D statistic (Fritz & Purvis, 2010) with 1000 permutations in the R package caper (Orme *et al.*, 2013).

DIVERSIFICATION ANALYSIS

State-dependent Speciation and Extinction (SSE) models have received much attention in recent years, including the description and critiques of new methods and concerns about type I and II errors, statistical power and replicas (Magnuson-Ford & Otto, 2012; Davis, Midford & Maddison, 2013; Maddison & Fitzjohn, 2015; Rabosky & Goldberg, 2015; Moore *et al.*, 2016; Beaulieu & O'Meara, 2016a; Alves, Diniz-Filho & Villalobos, 2017). Recently, Beaulieu & O'Meara (2016a) proposed a new method that incorporates more complex patterns of diversification by considering hidden traits to solve the high susceptibility of SSE models to false positives (Maddison & Fitzjohn, 2015; Rabosky & Goldberg, 2015). Here, we applied the Hidden State Speciation Extinction (HiSSE) model (Beaulieu & O'Meara, 2016a), using the R package hisse (Beaulieu & O'Meara, 2016b), to test if the foam nest has influenced diversification rates in each of the four lineages. This method evaluates if a trait, in this case the foam nest, influenced lineage diversification by estimating net turnover (τ), extinction fraction (ε) and transition rates between states (q) associated with the absence or presence of the focal trait (0 or 1, respectively) and/or an unknown hidden trait (states A to D).

We fitted 24 diversification models using a maximum likelihood (ML) approach (Table S2): four Binary State Speciation Extinction (BiSSE; Magnuson-Ford & Otto 2012), four Character-independent (CID) and 16 HiSSE models with varied constraints on parameters, as suggested by Beaulieu & O'Meara (2016a). CID models do not force the diversification rate to be the same across the tree, which makes them adequate null models for trait-dependent diversification (Beaulieu & O'Meara, 2016a). We specified the proportion of foam-producing and non-foam-producing species sampled in each phylogeny to account for missing species and we transformed τ and ε rates into speciation and extinction rates to calculate net diversification (Beaulieu & O'Meara, 2016a). Given that there is no consensus on the best way to compare a set of models (Burnham & Anderson, 2002), we chose to use a pluralist approach by jointly using ΔAIC, Akaike weights (w_i) and evidence ratios. Diversification models with ΔAIC ≥ 2 were considered as having substantial support (Burnham & Anderson, 2002). Additionally, we used Akaike weights (w_i) and evidence ratios to assess the relative likelihood of the best model against each of the other 23 models. Since there is no cut-off point to determine when one model is considerably better than another, we adopted evidence ratios ≥ 10 as indicating at least limited support (Evvett & Weir, 1998). When the difference between models was lower than these ΔAIC and evidence ratio thresholds, we considered the models to be equally probable.

RESULTS

ANCESTRAL STATE RECONSTRUCTION AND PHYLOGENETIC SIGNAL

The foam nest nest is a highly conserved and relatively young trait in Anura. The deposition of eggs in foam nests first appeared around *c*. 55 Myr independently in three disjoint continental lineages (Figs 1, 2): Leptodactylidae (mainly South America), Rhacophoridae (Africa–Asia) and Limnodynastidae (Australia–New Guinea). This period dates back to the Paleocene and Eocene transition, during the thermal maximum (Fig. 1B). In the endemic Madagascar group, Cophylinae, the foam nest appeared more recently, during the second half of the Eocene, *c.* 40 Myr (Fig. 2A). The most ancestral state for all four groups is the absence of a nest, probably with aquatic reproduction. This state probably transitioned to the foam nest once in Cophylinae and Limnodynastidae (Fig. 2A, C) and twice in Leptodactylidae (Fig. 1A). Conversely, aquatic breeding led to the jelly nest in Rhacophoridae (*c.* 65 Myr), which in turn evolved independently to the foam nest and to direct development (Fig. 2B). This last mode has three independent origins in Rhacophoridae, all of which evolved from an ancestor with jelly nests *c*. 45

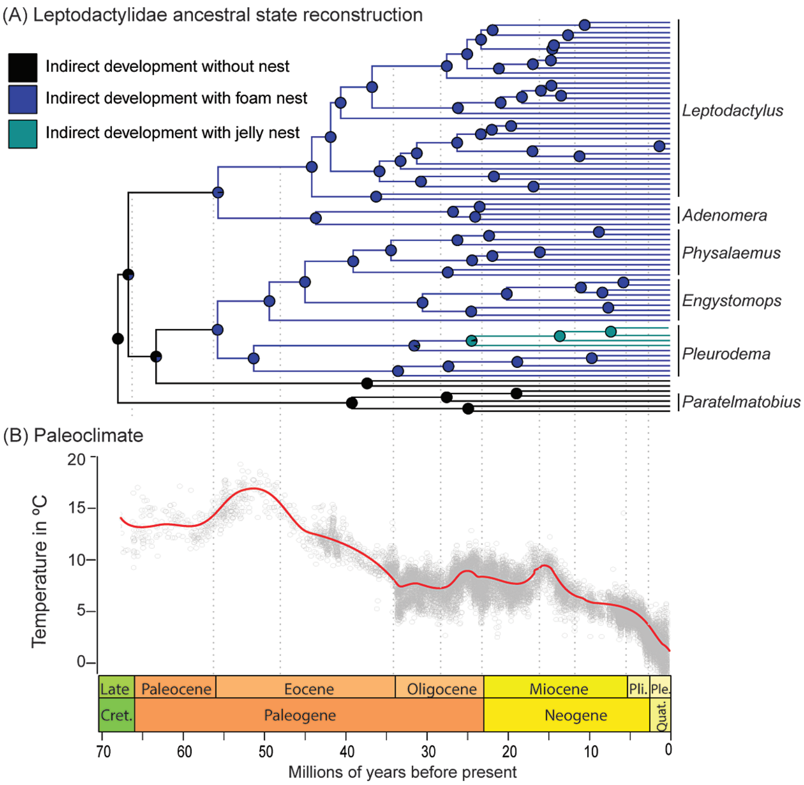

Figure 1. A, ancestral state reconstruction of Leptodactylidae reproductive attributes, with the most representative genera indicated to the right of the phylogeny. Species were classified as having indirect development without a nest (black), with a foam nest (blue) or with a jelly nest (green). B, palaeotemperature graph based on data from Zachos, Dickens & Zeebe (2008), with geological ages indicated by dotted lines.

Myr. While the jelly nest gave rise to the foam nest in Rhacophoridae, in Leptodactylidae the jelly nest is probably a more recent state that evolved from an ancestor with foam nests (*c.* 24 Myr; Fig. 1A). Overall, ancestral state reconstruction demonstrated that the foam nest is considerably conserved in all four groups, transitioning to jelly nests only once in Leptodactylidae (Fig. 1A) and reversing to the aquatic ancestral state twice in Limnodynastidae at *c.* 15 Myr (Fig. 2C). Indeed, the strong phylogenetic signal of the foam nest generated significantly low values of the D statistic for all groups, ranging from −2.78 in Cophylinae to −0.87 in the full phylogeny (Table S3).

DIVERSIFICATION ANALYSIS

The comparison of diversification models using ΔAIC and evidence ratios showed that no single model best explains the diversification in any of the four groups (Tables S2 and S3). The equally possible alternative diversification models included all classes: BiSSE, CID and HiSSE (Tables S2 and S4). The only exception is Rhacophoridae when comparing models using ΔAIC, which led to only two equally possible CID models. Fewer models were considered equally possible when compared by ΔAIC than by evidence ratios: Leptodactylidae 7/11, Limnodynastidae 4/17, Cophylinae 7/16 and Rhacophoridae 2/8, respectively.

Most of the equally possible models for Leptodactylidae indicated a higher diversification rate in lineages with foam nests, followed by models with equal diversification rates or models with unknown hidden characters influencing lineage diversification (HiSSE). Only a single model for Leptodactylidae indicated greater diversification in lineages without foam nests. The model with the highest Akaike weight was a BiSSE model with a higher diversification of lineages that lay eggs in foam nests ($w_i = 21.8\%$; Tables S2, S4, S5 and Fig. S1) due to higher speciation rates of foam-producing lineages (0.062, 95% CI [0.043, 0.081]) than for non-foam-producing ones (0.053, 95% CI [0.032, 0.073]).

Conversely, the set of equally possible diversification models of Limnodynastidae and Cophylinae included more models in which the diversification of the group is related to an unknown hidden trait, followed by models whose lineages without foam nests had increased diversification (Tables S2 and S4). In general,

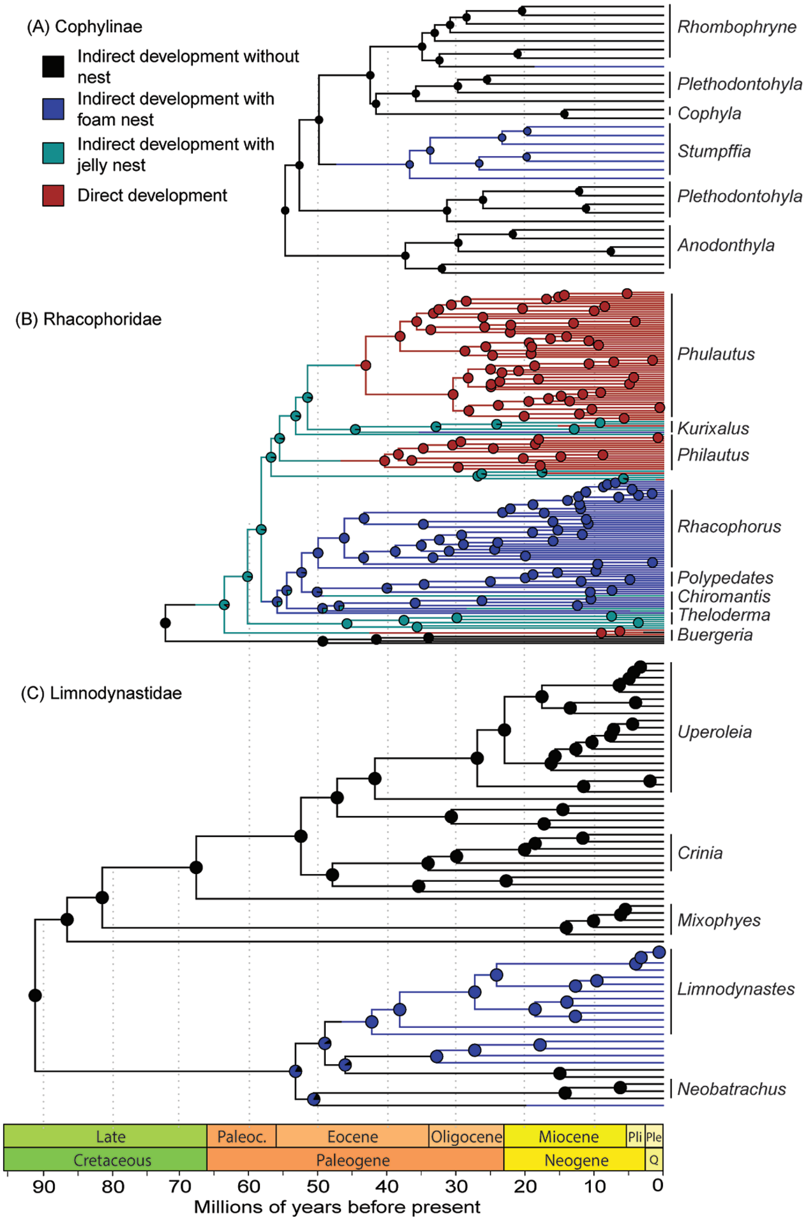

Figure 2. Ancestral state reconstruction of reproductive attributes of (A) Cophylinae, (B) Rhacophoridae and (C) Limnodynastidae, with the most representative genera of each group indicated to the right of the phylogeny. Species were classified as having indirect development without a nest (black), indirect development with a foam nest (blue), indirect development with a jelly nest (green) or direct development (red). Dotted lines mark every 10 million years.

the diversification models for Limnodynastidae and Cophylinae had the lowest Akaike weights among the four lineages studied.

Our results showed an even more mixed scenario for the diversification of Rhacophoridae. Using the criterion of evidence ratios, eight models of diversification are equally possible. In three of these models, diversification is greater in lineages with foam nests and a hidden trait. In three other models, lineages with and without foam nests together with hidden traits had similar rates and in two models the diversification was greater in lineages without foam nests (Tables S2 and S4). Interestingly, when using the ΔAIC criterion, the two most probable models were a CID-4 model with highest AIC weight ($w_i = 30\%$) followed by a CID-2 model ($w_i = 15\%$; Tables S2 and S4). In both cases, the favoured models imply that neither the presence nor the absence of the foam nest alone or together with a hidden trait has driven Rhacophoridae diversification.

DISCUSSION

ORIGIN AND MAINTENANCE OF THE FOAM NEST

Our results show that the foam nest is a relatively young and highly conserved trait in anurans. Its first appearance in Leptodactylidae, Limnodynastidae and Rhacophoridae dates back to the Paleocene–Eocene thermal maximum (*c.* 55 Myr), when an increase in atmospheric greenhouse gases led to high temperatures and high levels of humidity and precipitation (Zachos, 2001, 2003; Jaramillo & Cárdenas, 2013). Such climatic changes influenced biodiversity, leading to major dispersal and migration events of terrestrial vertebrates (Bowen, 2002; Pramuk *et al.*, 2008) and a considerable increase in diversification of the Neotropical flora (Jaramillo & Cárdenas, 2013). Temporal congruence among the three independent origins of the foam nest in anuran lineages, when the Earth's temperature peaked, suggests to us that the foam nest may have played an important role in anuran evolution around this period. Extreme environmental conditions strongly impact physiological processes in anurans, especially during the embryonic and larval phases (McDiarmid & Altig, 1999; Navas, Gomes & Carvalho, 2008). The foam nest is able to prevent desiccation (Dobkin & Gettinger, 1985) and buffer temperature (Méndez-Narváez *et al.*, 2015) while ensuring gas exchange to the early stages of development (Hissa *et al.*, 2008). In fact, a recent study found that the main protein of a *Leptodactylus* foam nest could maintain its conformation even at temperatures as high as 95 °C (Hissa *et al.*, 2016).

Since the Paleocene–Eocene thermal maximum, the Earth has been gradually cooling and becoming drier, with the exception of some periods of warming at the beginning and end of the Oligocene and mid-Miocene (Zachos, 2001). Yet, the foam nest is highly conserved among anuran lineages, indicating that it is an important adaptation to the climatic oscillations that have occurred over their evolutionary history, which have gone from high temperature and humidity to low humidity and low temperature, both with a distinct availability of oxygen (Zachos, 2001). We suggest that the foam's many functions were key to ensuring the survival of foam-producing lineages over the 55 million years since its appearance. Most importantly, maintenance of this trait among lineages could have been possible owing to the versatility of the foam nest. This structure provides thermal stability, being able to keep the embryos' environment colder or warmer than externally and may even accelerate embryonic development (Méndez-Narváez *et al.*, 2015). Additionally, the foam nest reduces water dependence for reproduction and enables oviposition and tadpole development completely out of the water body, such as in some *Leptodactylus* and *Adenomera* species.

ANCESTRAL BREEDING STATE AND SHIFTS TO FOAM NESTS

The most probable ancestral reproductive strategy of the four studied lineages is indirect development without a nest (i.e. aquatic), which later transitioned to foam nests in some Leptodactylidae, Limnodynastidae and Cophylinae. By contrast, in Rhacophoridae the aquatic ancestor gave rise to a jelly nest, which, in turn, is the obligatory ancestral state of two other terrestrial reproductive modes in this lineage: the foam nest and direct development (Meegaskumbura *et al.*, 2015). Interestingly, in Leptodactylidae, the jelly nest probably arose from an ancestor with foam nests. However, considering the effect of extinction in erasing a clade's history and influencing ancestral state reconstruction (Losos, 2011b; Goswami, Mannion & Benton, 2016; Marshall, 2017), we cannot completely rule out the possibility that the foam nest may have originated from ancestors with jelly nests that are now extinct.

Although it is an intriguing trait, so far very little is known about the jelly nest. Among the groups studied here, it is present in several species of Rhacophoridae, in one genus of Leptodactylidae (*Pleurodema*) and in only one species of Limnodynastidae not included in our analyses due to lack of phylogenetic information (*Philoria loveridgei*). It is not yet clear whether jelly is a pre-foam structure or whether it is the same as the foam precursor but without the parental beating behaviour that leads to foam construction (Knowles *et al.*, 2004; Faivovich *et al.*, 2012). This last possibility makes more sense for Leptodactylidae, in which species with jelly nests probably evolved from an ancestor with foam nests. Conversely, both possibilities are potential explanations in Rhacophoridae. In this lineage, the jelly nest may be a pre-foam structure, since it is the ancestral state of the foam nest or may be the same as foam but without the beating behaviour.

To our knowledge, no study has yet evaluated the biochemical composition of jelly, but it is possible that it differs from that of the foam. For example, the foam nest of some species of *Philoria* (Limnodynastidae) disintegrates faster than that of other species (Knowles *et al.*, 2004) and *Pleurodema tucumanum* (Leptodactylidae) has modified foam precursors that also decrease the stability of the nest and cause it to dissolve more quickly (Ferraro *et al.*, 2016). Additionally, anatomical and morphological studies seem to support the behaviour hypothesis. Morphological studies showed that a species of *Pleurodema* (Leptodactylidae) that lays eggs in jelly nests has enlarged posterior convoluta, a typical structure of species that build foam nests (Furness *et al.*, 2010). Also, some populations of *Limnodynastidae tasmaniensis* from south-eastern Australia that have anatomical modifications in the limbs do not construct foam nests and deposit eggs in a jelly mass, while populations without such modifications construct foam nests (Roberts & Seymour, 1989).

The only other ancestral state reconstructions of foam nests were made for *Pleurodema* (Leptodactylidae) (Faivovich *et al.*, 2012) and Rhacophoridae (Meegaskumbura *et al.*, 2015) species, both of which used different phylogenetic hypothesis from ours. Nevertheless, our results agree with these studies that the jelly nest is a transitional state towards more terrestrial reproductive modes in Rhacophoridae (Meegaskumbura *et al.*, 2015), whereas in Leptodactylidae the jelly nest may have derived from an ancestor with foam nests (Faivovich *et al.*, 2012). Additionally, our results also agree that aquatic reproduction is the most probable ancestral mode for Rhacophoridae and that it gave rise to the jelly nest just once, which in turn led to evolution of the foam nest and direct development in this lineage (Meegaskumbura *et al.*, 2015). However, our results may contrast with those previously found in Rhacophoridae because we retrieved a single origin of the foam nest and three origins of direct development, although this last result matches the hypothesis of ‘at least two independent origins’ raised by the authors (Meegaskumbura *et al.*, 2015).

Our ancestral state reconstruction also matches two hypotheses proposed for Limnodynastidae and Leptodactylidae based solely on inspection of the topology (Faivovich *et al.*, 2012; Fouquet *et al.*, 2013). We retrieved two origins of the Leptodactylidae foam nest, one in Leptodactylinae and another in Leiuperinae, and both date back to 55 Myr (Fouquet *et al.*, 2013). Conversely, the foam nest originated only once in Limnodynastidae but reversed twice to an aquatic reproductive mode, one in the most recent common ancestor (MRCA) of *Neobatrachus* and another in the MRCA of *Notaden* (Faivovich *et al.*, 2012).

THE FOAM NEST AS A KEY INNOVATION

The foam nest has been hypothesized as being a key innovation (Heyer, 1975; Fouquet *et al.*, 2013) that generated new ecological opportunities by enabling species access to previously unavailable areas (Simpson, 1953; Stroud & Losos, 2016). Our results indicate a broad set of possible diversification models for the four lineages studied, which makes it difficult to identify a clear evolutionary pathway. Only for the South American lineage Leptodactylidae is there additional evidence pointing to a possible influence of the foam nest in increasing lineage diversification either alone or in conjunction with some hidden trait. By contrast, we have no evidence that the presence of the foam nest increased the diversification of Rhacophoridae, Limnodynastidae or Cophylinae.

The possible higher diversification rate of Leptodactylidae foam-producing lineages was caused by an increase in the speciation rate. This result was expected for this South American lineage given the higher richness of its subfamilies with foam nests (Leptodactylinae and Leiuperinae) in comparison with the non-foam-producing subfamily Paratelmatobiinae (Fouquet *et al.*, 2013). Additionally, species of these two foam-producing subfamilies have considerably larger ranges (Fouquet *et al.*, 2013) and a wider variation in oviposition sites than non-foam-producing species. Since the 1970s, it has been hypothesized that the foam nest in Leptodactylidae increased their diversification due to the ancestral invasion of dry open areas across South America that expanded after the late Oligocene (Simpson, 1983), creating an ecological opportunity for foam-producing species (Heyer, 1969). Again, however, according to our results multiple models are equally possible and support for the models with greater diversification in Leptodactylidae with foam nests is weak.

Rhacophoridae is the richest lineage studied here, corresponding to almost 6% of the total anuran richness, and it is also the one with the greatest variety of reproductive strategies (Meegaskumbura *et al.*, 2015). This lineage of Old World tree frogs includes species with fully aquatic breeding, jelly nests, foam nests and direct development distributed mainly in Asia, but also with the foam-producing genus *Chiromantis* distributed in Africa (Meegaskumbura *et al.*, 2015). This suggests that the foam nest may have fostered lineage diversification in Rhacophoridae by enabling species to enter drier areas. However, our results show that neither the presence nor the absence of the foam nest influenced the diversification of Rhacophoridae. A recent study highlighted that terrestrial direct development in Rhacophoridae, present in half of its species, may be a key innovation allowing tree frogs to lay eggs out of the water and develop directly into froglets (Meegaskumbura *et al.*, 2015). We briefly explored this by performing a HiSSE analysis in which we categorized species solely as having or not having direct development. The best models explaining Rhacophoridae diversification pointed to increased diversification in lineages with direct development due to higher speciation rates. Although more detailed studies are needed, we argue that direct development in Rhacophoridae may be a key innovation, which, in turn, would have overshadowed the influence of the foam nest in lineage diversification.

Multiple diversification models with different biological interpretations are equally plausible to explain Cophylinae and Limnodynastidae diversification. This may be a consequence of a relatively low number of species in the lineage and of the species sampled: 23 of the 43 Limnodynastidae species and 32 of the 70 Cophylinae species. Tests of the BiSSE model have indicated that analysis with few species might not contain enough information to correctly assign a diversification model (Davis *et al.*, 2013), although a recent paper shows otherwise (Gamisch, 2016). In general, the most probable models to explain Cophylinae and Limnodynastidae diversification suggest no influence of the foam nest on this process or even a lower diversification rate in lineages with foam nests. In addition, some models raise the possibility that a hidden trait along with the presence or absence of foam nests could have influenced diversification of these lineages. We propose that other specific attributes of reproductive modes may have influenced diversification of these lineages. For example, the foam structure and nest building characteristics, such as where it is built (e.g. floating on pond or water accumulated in constructed basins, axils of terrestrial bromeliads, subterranean chamber, humid forest floor) and by whom (adult male, female or tadpole), may also explain the opposite results regarding diversification (Wells, 2007). Although this hypothesis can be tested using a multistate approach, the lack of reproductive information for several species of these lineages makes it difficult to evaluate.

The contrasting findings among lineages regarding their diversification associated with foam nests may be related to the fact that this trait confers adaptive advantages only to the early stages of anuran development and also that the independent origins of the foam nest led to evolution under distinct environmental conditions and histories, resulting in distinct patterns of evolution and diversification. Additionally, we stress that most studies demonstrating the various ecological functions of the foam nest have been conducted on species of Leptodactylidae and there are no guarantees that foam nests in other lineages have the same functions. If we consider that only traits that increase the diversification of a lineage could be considered as key innovations, then we cannot state that the foam nest is a key innovation for all anuran lineages. However, if we consider a broader definition of a key innovation, for example 'key innovations are aspects of organismal phenotype important to the origin or subsequent success of a taxonomic group' (Heard & Hauser, 1995), we suggest that the foam nest is a key innovation as it is a highly conserved trait that played an important role over anuran evolutionary history by allowing lineages to survive through periods of climate change.

In summary, we have presented the first comparative study of the anuran foam nest under a macroevolutionary perspective. We demonstrated that it is a highly conserved trait that originated independently in lineages present in different continents with at least three major origins dating from the Paleocene–Eocene thermal maximum *c.* 55 Myr. We hypothesize that the many functions of foam nests were key to ensuring the survival of foam-producing lineages throughout the climatic oscillations encountered during their evolutionary history. Interestingly, the presence of foam nests may have increased the diversification of the South American lineage Leptodactylidae through higher speciation rates, whereas such an increase in diversification was not detected in Rhacophoridae, Limnodynastidae or Cophylinae. We have provided a first comparative study among lineages to unravel the evolutionary pattern of the foam nest, and have presented for the first time the important role that this trait has played over anuran evolutionary history.

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SUPPORTING INFORMATION

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

Table S1. List of reproductive attributes of Cophylinae (Microhylidae), Rhacophoridae, Leptodactylidae and Limnodynastidae.
Table S2. Description of the 24 diversification models fitted to anurans with foam nest.
Table S3. Measures of phylogenetic signal of foam nest.
Table S4. Summary of diversification model results and fit.
Table S5. Estimated diversification rates and confidence intervals of the model with lowest ΔAIC to all four lineages.
Figure S1. Posterior probabilities density of speciation, extinction and net diversification rates estimated by the Leptodactylidae trait-dependent diversification model with lowest ΔAIC. Bars below the histogram represents the 95% confidence interval.