Much more than a clasp: evolutionary patterns of amplexus diversity in anurans

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The evolution and diversification of animal reproductive modes have been pivotal questions in behavioural ecology. Amphibians present the highest diversity of reproductive modes among vertebrates, involving various behavioural, physiological and morphological traits. One such feature is the amplexus, which is the clasp or embrace of males on females during reproduction and is found almost universally in anurans. Hypotheses about the origin of amplexus are limited and have not been tested thoroughly, nor have they taken into account evolutionary relationships in most comparative studies. However, these considerations are crucial to an understanding of the evolution of reproductive modes. Here, using an evolutionary framework, we reconstruct the ancestral state of amplexus in 685 anuran species. We investigate whether the type of amplexus has a strong phylogenetic signal and test whether sexual size dimorphism could have influenced amplexus type or male performance while clasping females. Overall, we found evidence of ≥34 evolutionary transitions in amplexus type across anurans. We found that amplexus type exhibits a high phylogenetic signal and that amplexus type does not evolve in association with sexual size dimorphism. We discuss the implications of our findings for the diversity of amplexus types across anurans.

ADDITIONAL KEYWORDS: Amphibia – ancestral reconstruction – frogs – reproductive modes – sexual dimorphism.

INTRODUCTION

Understanding the evolution and diversification of animal reproductive modes has been a shared interest among evolutionary biologists for decades (e.g. Salthe, 1969; Shine, 1983; Craig, 1987; Alves *et al.*, 1998; Blackburn, 2000; Crespi & Semeniuk, 2004; Haddad & Prado, 2005). In addition to sexual selection, natural selection promotes reproductive diversity by favouring modes of reproduction that maximize the likelihood of successful matings in a given environment (Pianka, 1976; Zamudio *et al.*, 2016). Thus, reproductive modes are one of the most critical life-history traits directly affecting fitness and survival in response to environmental (and other) selective pressures (Angelini & Ghiara, 1984; Lodé, 2012).

Anuran amphibians exhibit one of the highest diversities in reproductive modes among vertebrates (Duellman & Trueb, 1986; Vitt & Caldwell, 2014). These reproductive modes are defined as a combination of ecological, physiological, developmental and behavioural traits that include oviposition site, ovule morphology, clutch size, developmental rate and the presence or absence of (different types of) parental care (Salthe & Duellman, 1973; Duellman & Trueb, 1986). Thus, anuran reproductive modes exhibit a gradient of parental engagement that ranges from no or little parental care, involving mostly aquatic oviposition of clutches with hundreds or thousands of eggs, to elaborate parental care, involving relatively

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few terrestrial eggs with either a free-swimming larval stage or direct development and a reduced or absent tadpole stage (Hödl, 1990; Haddad & Prado, 2005; Crump, 2015).

Previous studies have investigated different aspects of the evolution of anuran reproductive modes using limited phylogenetic comparative methods (Duellman, 2003; Gomez-Mestre *et al.*, 2012; Zamudio *et al.*, 2016; Furness & Capellini, 2019). In these studies, it has been hypothesized that factors of ecological and population structure, such as desiccation in temporary ponds, availability of humid microhabitats in terrestrial environments, and predation, are major selective forces shaping most reproductive modes. However, to our knowledge, none of these studies has addressed the evolution of a key behavioural component in frog reproduction, namely the amplexus or 'mating clasp'. Here, we investigate the evolutionary patterns of this trait across the Anura and reveal major evolutionary transitions in this crucial component of the reproductive behaviour of frogs.

Amplexus is present in most anuran species and consists of a male grasping a female from behind with his forelimbs. Thus, not surprisingly, it has been interpreted as a behaviour by which a male ensures the fidelity of the female during mating, thereby increasing the chance of egg fertilization (Duellman & Trueb, 1986; Wells, 2007). Like other mating traits in anurans, amplexus types are diverse (see Fig. 1). For instance, the inguinal amplexus is considered a basal condition to all anurans, whereas the axillary amplexus and its variations (including the complete lack of amplexus) are considered derived states (Duellman & Trueb, 1986; Wells, 2007; Pough *et al.*, 2016). Several hypotheses have been proposed to address the evolution of such amplexus diversity, suggesting that variants might have evolved as a consequence of sexual size dimorphism (SSD), parental care and the ecological factors affecting the site of oviposition (Duellman & Trueb, 1986; Wells, 2007; Pough *et al.*, 2016). However, these ideas have not been tested under a phylogenetic framework. Such comparative analyses could greatly improve our understanding of the evolutionary patterns of anuran amplexus diversity and offer new baseline data for further comparative studies about the behavioural ecology of reproductive modes among vertebrates.

Here, we use the most complete phylogeny of Anura (Jetz & Pyron, 2018) to map the origins and diversification of the known types of amplexus in 685 species with reported records. Furthermore, we explore the relationship between the evolution of amplexus diversity and SSD [measured as female-to-male snout–vent length (SVL) ratio]. We predict that species with male-biased, little or no SSD would benefit from axillary amplexus, whereas species with a high female-biased SSD would be likely to present inguinal amplexus or another derived type of amplexus (e.g. 'glued' in Fig. 1). These predictions are based on the physical restrictions that a very small male could have to clasp a large female and thereby ensure her fidelity during mating. Overall, our results show that the different types of amplexus, and the lack thereof, are well defined throughout the Anura tree of life. This is a fundamental step forwards in understanding how environmental factors and life history have shaped the amazing diversity of reproductive modes across Anura and other vertebrates.
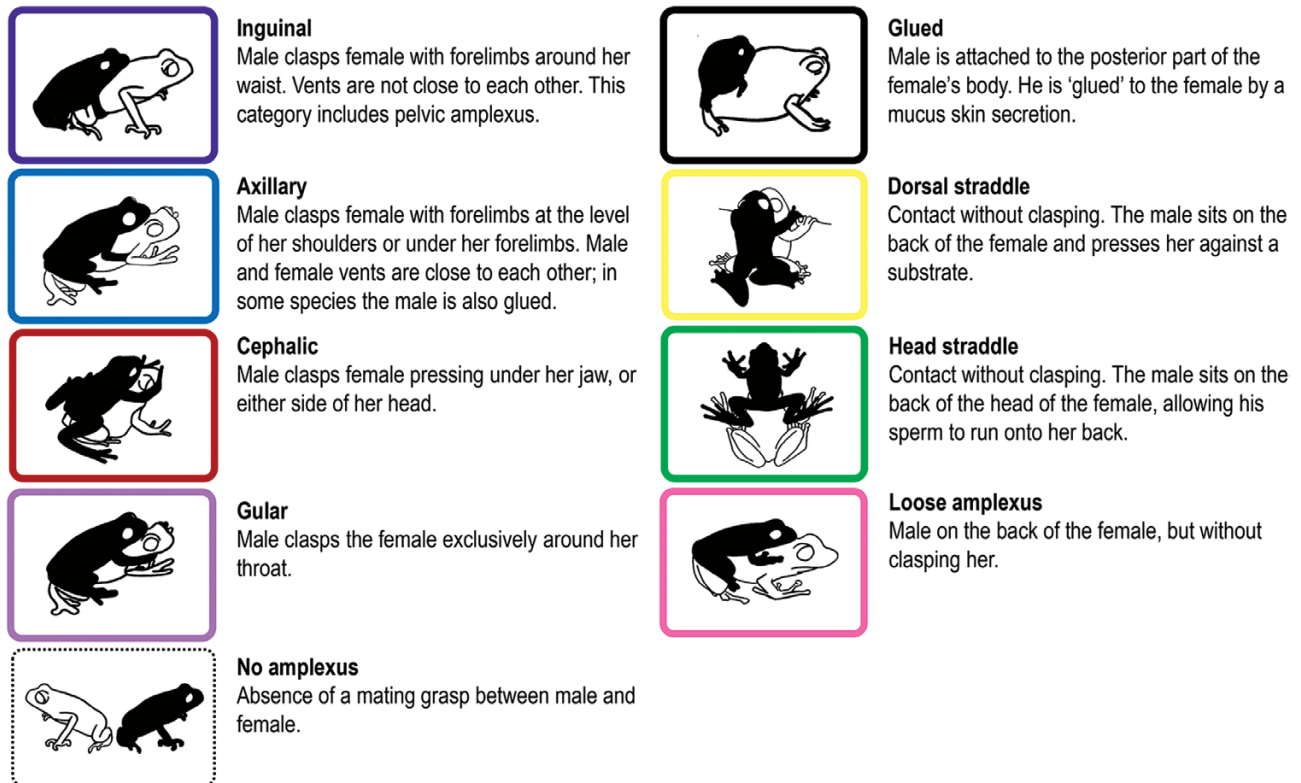
MATERIAL AND METHODS

CHARACTERIZATION OF AMPLEXUS

We searched primary literature (e.g. peer-reviewed articles, books) for information on amplexus types in Anura, using Google Scholar and Web of Science. We used 'amplexus' and 'nuptial clasp' as keywords. Given that we obtained an excess of results unrelated to Anura (the term amplexus has also been used for invertebrates; e.g. Conlan, 1991; Sutherland *et al.*, 2007; Lipkowski *et al.*, 2019), we included the keywords 'anura' and 'frogs'. To narrow down our search further, we also combined previous keywords with the names of anuran families (e.g. "Amplexus" AND "Dendrobatidae"). Within the selected publications, we searched for an account describing male and female behaviour with enough detail (e.g. an observational account of the behaviour or a photograph) to be assigned to an amplexus type.

We defined the documented amplexus types (Fig. 1) following Duellman & Trueb (1986) and Willaert *et al.* (2016), but also considering the following clarifications. First, in several species it has been reported that the type of amplexus might change at the moment of oviposition (e.g. *Anomaloglossus beebei* and *Brachycephalus ephippium*; Bourne *et al.*, 2001; Pombal *et al.*, 1994). In these cases, we considered the preoviposition amplexus type to be the predominant one (i.e. longer duration and most frequently reported in the literature, because in most cases researchers did not wait until oviposition to record the breeding behaviour). Second, some studies include observations of multiple types of amplexus for a given species. In these cases, we used the report(s) with the strongest evidence, which include a textual description of the type of amplexus or visual evidence, such as photographs or videos. Below, we provide a list of some specific examples of conflicting reports and evidence of new types of amplexus.

For *Scaphiophryne gottebei* (Microhylidae), Rosa *et al.* (2011) mention an inguinal amplexus, but

Inguinal
Male clasps female with forelimbs around her waist. Vents are not close to each other. This category includes pelvic amplexus.

Axillary
Male clasps female with forelimbs at the level of her shoulders or under her forelimbs. Male and female vents are close to each other; in some species the male is also glued.

Cephalic
Male clasps female pressing under her jaw, or either side of her head.

Gular
Male clasps the female exclusively around her throat.

No amplexus
Absence of a mating grasp between male and female.

Glued
Male is attached to the posterior part of the female's body. He is 'glued' to the female by a mucus skin secretion.

Dorsal straddle
Contact without clasping. The male sits on the back of the female and presses her against a substrate.

Head straddle
Contact without clasping. The male sits on the back of the head of the female, allowing his sperm to run onto her back.

Loose amplexus
Male on the back of the female, but without clasping her.

Figure 1. Diversity of amplectic positions in anurans (males in black). Definitions are according to Blommers-Schlosser (1975), Duellman & Trueb (1986), Townsend & Stewart (1986), Wells (2007), Zachariah *et al.* (2012), Jungfer *et al.* (2013) and Willaert *et al.* (2016). Given that descriptions of some amplexus types are not clear or are ambiguous, we pooled the category 'independent' (cited by Duellman & Trueb, 1986) as 'no amplexus'. Frame colours relate to the same amplexus types in Figures 2, 3.

their account includes several figures showing a characteristic axillary amplexus. For *Osteocephalus* (Hylidae), we used the 'gular amplexus' definition by Jungfer *et al.* (2013), which refers to a type of amplexus where the male clasps the female exclusively around her throat. Likewise, some species with axillary amplexus have been reported to have a 'glued amplexus' [e.g. *Elachistocleis bicolor* (Cacciali, 2010) and *Chiasmocleis leucosticta* (Haddad & Hödl, 1997)]. Such reports indicate that the males are 'glued' to the female dorsum, but in our analysis we did not consider this clasping behaviour to be different from an axillary amplexus. Our consideration is based on the fact that males of species with axillary amplexus have not been examined thoroughly enough for the presence or absence of glands to suggest that these organs exist or that they produce sticky or glue-like substances. For species such as *Nyctibatrachus aliciae* (Nyctibatrachidae; Biju *et al.*, 2011) or *Mantella aurantiaca* (Mantellidae; Vences, 1999; Glaw & Vences, 2007), the observed amplexus consists of a male sitting on the dorsum of the female for a short period of time, without a clasp; we classified such observations as

'loose amplexus'. Lastly, *Aplastodiscus leucopygius* (Hylidae) and *Ascaphus truei* (Ascaphidae) have a 'dynamic amplexus', which is difficult to categorize because at different moments the same pair (i.e. male and female) exhibits diverse amplexus positions (Stephenson & Verrell, 2003; Berneck *et al.*, 2017). Therefore, these two species were not included in our analysis.

We completed the life-history characterization for all species with amplexus data by including male and female body size (i.e. SVL) and SSD. In instances where only the range of body size was available, we used the median values.

COMPARATIVE METHODS

For the phylogenetic analysis, we obtained 1000 random phylogenies based only on genetic data from Jetz & Pyron (2018). We ran the analysis 1000 times to evaluate the robustness and include uncertainty in topologies in the R software environment (R Core Team, 2018). Later, we made a stochastic ancestral reconstruction (Bollback, 2006) of the character 'amplexus type' using
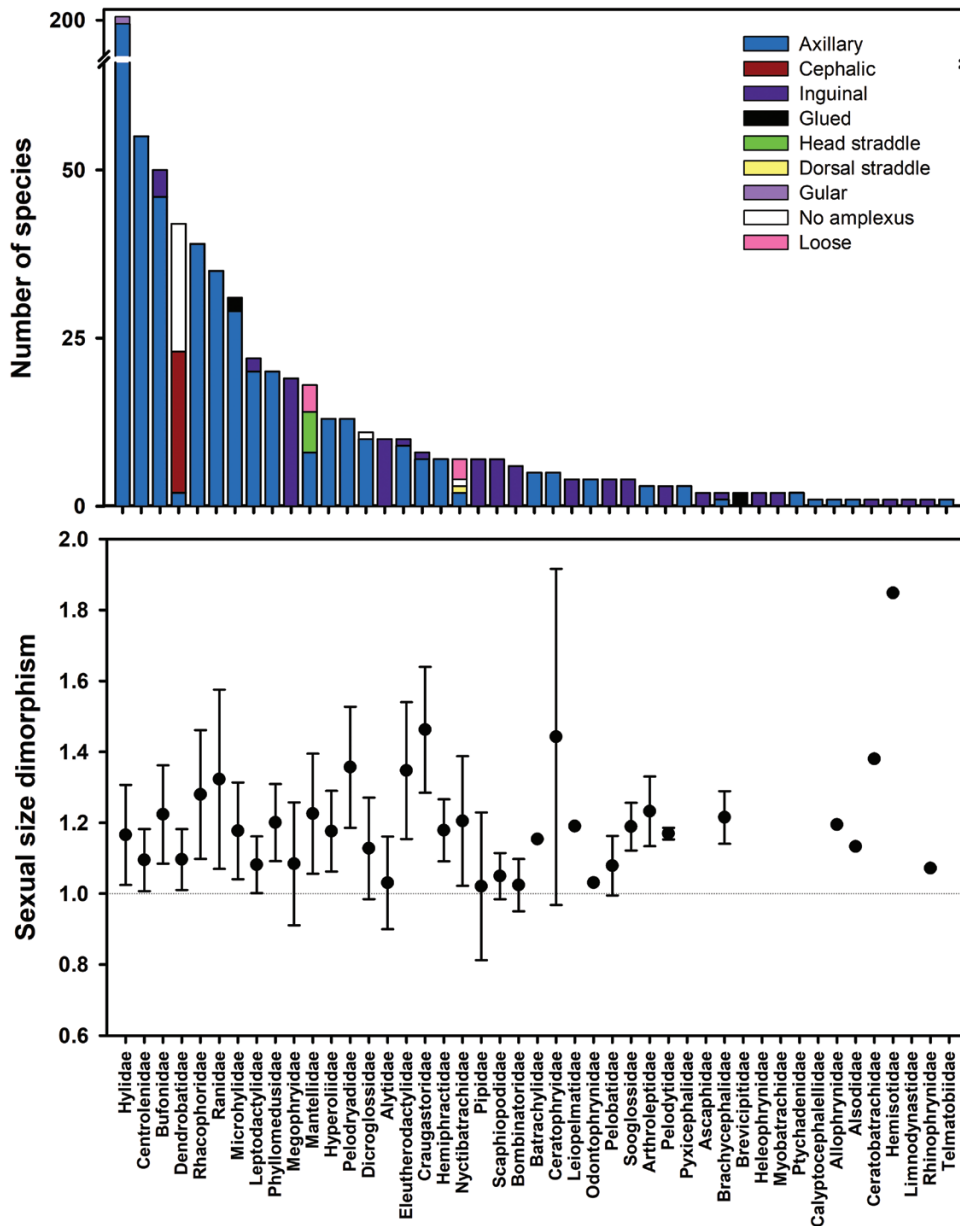
Figure 2. A, summary of the number (685) of anuran species and type of amplexus (or lack thereof). B, sexual size dimorphism (SSD; measure as female-to-male snout–vent length ratio) per family of Anura included in this study. The plot is based on data from the Supporting Information (Table S1). Dots and error bars indicate the mean values and standard deviations, respectively. For some families, it was not possible to calculate SSD because of an absence of data for female or male body size.

1000 trees, with the *make.simmap* function in the R package 'phytools' v.0.6-99 (Revell, 2012), which is a best-fit character evolution model (with only 100 trees). We contemplated an equal rate (ER) model, which assumes that all transitions between traits occur at the same rate (Pagel, 1994; Lewis, 2001), and an all-rate-different (ARD) model, which assumes that all transitions between traits occur at different rates (Paradis *et al.*, 2004). We used the *fitDiscrete* function in the R package 'geiger'

(v.2.0.6.2; Harmon *et al.*, 2008) to compare the ER and ARD models and selected the model with the lowest corrected Akaike information criterion (AICc) value. We did not generate fully sampled phylogenies using the taxonomic imputation method to make our ancestral character reconstruction because this approximation has been demonstrated to be inappropriate for this type of analysis owing to increased bias (Rabosky, 2015; Rocha *et al.*, 2016; Jetz & Pyron, 2018).
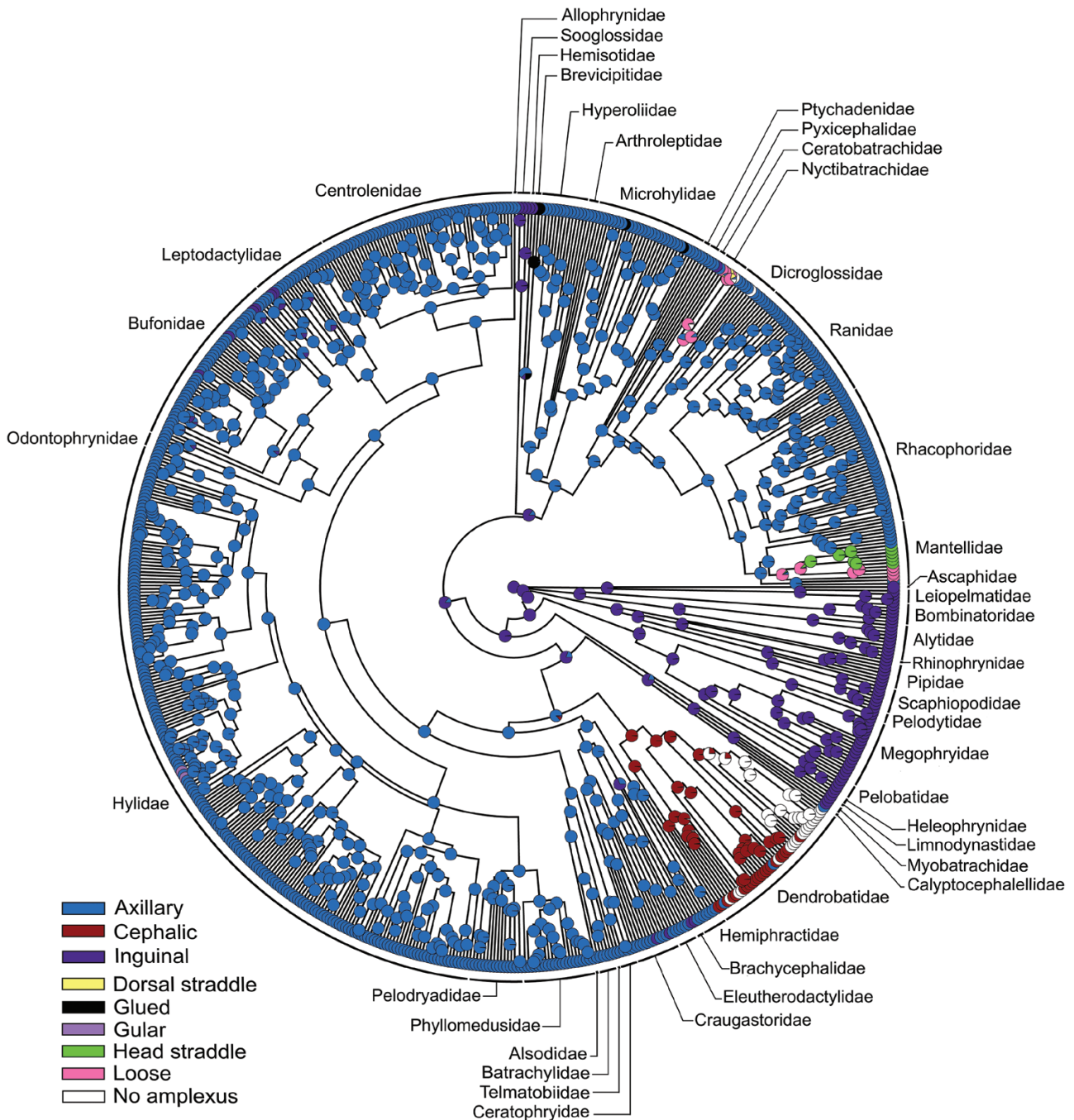
Figure 3. Ancestral reconstruction of amplexus type using 1000 trees for 685 anuran species under an 'equal rates' model of trait evolution. Tips represent the observed type of amplexus for each species, and nodes represent the probability of each type of amplexus (for specific values, see Supporting Information, Table S2). The phylogenetic tree is from Jetz & Pyron (2018).

To test whether amplexus type and SSD are conserved or labile (convergent) traits, we calculated their phylogenetic signal. This property is defined as the tendency of traits in related species to resemble each other more, as a consequence of shared ancestry (Blomberg & Garland, 2003). For this purpose, we used the statistic lambda (λ), proposed by Pagel (1997, 1999) as a measurement of phylogenetic signal. The λ value varies from zero to one; if λ is close to one, it indicates a strong phylogenetic signal (i.e. conserved

trait), whereas if λ is close to zero, it indicates that the evolution pattern of the trait has been random or convergent, i.e. these characters lack phylogenetic signal (Kraft *et al.*, 2007; Revell *et al.*, 2008; Gómez *et al.*, 2010). Calculation of the phylogenetic signal for type of amplexus was carried out using the *fitDiscrete* function of the R package 'geiger'. In addition, we calculated the likelihood of a model with no phylogenetic signal and the maximum likelihood value of λ; later, we used a likelihood ratio test to compare these two models and calculate a *P*-value (significance α = 0.05) under a χ^2 distribution. For SSD, we took a similar approach, comparing the observed phylogenetic signal value against a model with no phylogenetic signal with a likelihood ratio test, using the *fitContinuous* function in the R package 'geiger'.

To test whether the rates of evolution of the type of amplexus have increased or slowed over time, we used a δ model in the *fitDiscrete* function of 'geiger'. In addition, we calculated the likelihood of a model with δ equal to one and a model with observed δ; later we used a likelihood ratio test to compare these two models and calculated a *P*-value (significance α = 0.05) under a χ^2 distribution. If the δ statistic is greater than one, this indicates that recent evolution has been relatively fast; if, in contrast, δ is less than or equal to one, it indicates that recent evolution has been relatively slow. Given that the difference in body size between males and females could promote changes in types of amplexus owing to mechanic incompatibility (e.g. small males might not physically clasp a large female), we used a phylogenetic ANOVA to compare SSD across different types of amplexus. This analysis was performed using the *phylANOVA* function in 'phytools'.

RESULTS

Our analyses included 685 species from 45 anuran families (Supporting Information, Table S1). The distribution of species in our dataset comprised all continents where amphibians are present. Most families (i.e. 34 families) have only one type of amplexus, whereas 11 families have more than one (Fig. 2; Supporting Information, Table S1). The average SSD is 1.17 ± 0.16 (range = 0.70–1.85; *N* = 477 species), and it varies between anuran species and families (Fig. 2; Supporting Information, Table S1); in 42 species (8.80%) male body size is larger than female body size, whereas in most cases (428 species, 89.72%) females are larger than males; in the remaining seven species (1.46%) the males and females exhibit similar body size.

For the ancestral reconstruction of amplexus types, we chose the ER model (log likelihood = −224.722, AIC = 451.450) over the ARD model (log

likelihood = −166.072, AIC = 493.321) based on the lowest AICc value (Fig. 3). Our results support the inguinal amplexus as the basal state for all Anura. For instance, Ascaphidae and other basal frog families (e.g. Leiopelmatidae, Bombinatoridae, Alytidae, Pipidae) present inguinal amplexus. The axillary amplexus was found to be the most frequent state, occurring in 540 of the 685 species (i.e. 78.83%). However, we found 34 evolutionary transitions between all types of amplexus across the whole Anura phylogeny (Fig. 4A). The greatest number of evolutionary transitions (i.e. 12) occurred between axillary and inguinal amplexus states (Fig. 4A). We found a strong phylogenetic signal for amplexus type (Pagel's λ = 0.96, *P* < 0.0001) and a labile phylogenetic signal for SSD (Pagel's λ = 0.811, *P* < 0.0001). We also found that the δ value was equal to 0.77 (SD = 0.384), but when we compared it with a model with δ equal to one, we found no differences (*P* = 0.63). Furthermore, we did not find differences in SSD across types of amplexus (phylogenetic ANOVA: *F* = 2.8971, *P* = 0.803, *N* = 477; Fig. 4B).

DISCUSSION

Different selective pressures are known to shape the behavioural, physiological and morphological traits that characterize the diverse reproductive modes and behaviours in anurans (and fishes) in comparison to other vertebrates. One of those traits is the amplexus, whose evolutionary trends we characterized using a comparative phylogenetic framework. For this purpose, we explored the relationship between the different types of amplexus (or lack thereof) and SSD. Below, we discuss the implications of our findings and generate testable hypotheses for future research on the evolution of reproductive modes in anurans.

We found a significant phylogenetic signal in amplexus type. This result was anticipated, considering that the amplexus is an aspect of the anuran reproductive behaviour expected to have been shaped by various other factors not related to reproduction (e.g. morphology, microhabitat; Duellman & Trueb, 1986; Wells, 2007). Likewise, it is presumed that the type of amplexus affects the reproductive success of both males and females in several ways; for example, it determines the proximity of cloacas between males and females and, possibly, the success of egg fertilization (Davies & Halliday, 1979; Wells, 2007). Altogether, we infer that any change between amplexus types requires several selective factors to act in tandem and weighed by their combined selective force. Although we found a slight indication of a slowing down in the rate of amplexus evolution (δ = 0.694), this was not supported when we tested whether this parameter estimate was different from δ = 1. In other words,
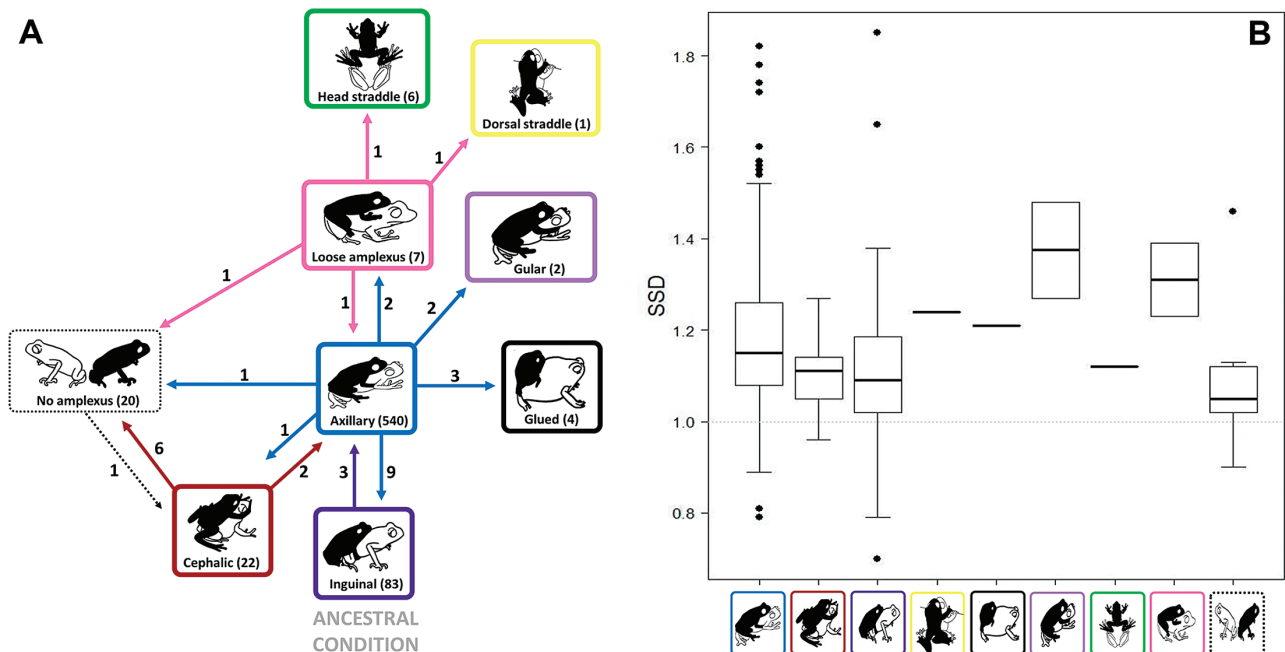
Figure 4. A, estimated number of independent evolutionary transitions in type of amplexus for the 685 anuran species included in the present study. In parentheses are indicated the number of species with each type of amplexus. Estimates are based on 1000 phylogenetic trees, and an ancestral state reconstruction performed with the 'phytools' package (for details, see main text and Fig. 2). B, variation in sexual size dimorphism (SSD; measured as female-to-male snout–vent length ratio) in 477 species with different types of amplexus. Phylogenetic ANOVA ($F = 2.8971$, $P = 0.803$, $N = 477$).

the rate of change in amplexus follows a Brownian motion model that does not support a slowing down, but instead a random change (either slowing down or acceleration) in its rate of evolution throughout the evolutionary history of Anura.

In contrast to amplexus type, the labile phylogenetic signal in SSD suggests that alternative selective forces might be promoting disparities in body size across Anura. For instance, in most frogs the females are larger than the males because there is a strong positive relationship between fecundity and body size (i.e. selection favours larger size in females; Shine, 1979; Wells, 2007); in contrast, a larger body size in males is not necessarily tied to higher attractiveness to females or higher dominance across species (Halliday & Tejedo, 1995). Besides, intersexual differences in age at first reproduction and survival greatly influence the extent of SSD (Monnet & Cherry, 2002; Vargas-Salinas, 2006).

Previous reports support our results showing that all basal lineages (e.g. Ascaphidae, Pipidae and Myobatrachidae) have an inguinal amplexus (Lynch, 1973; Rabb, 1973; Weygoldt, 1976), which we found to be the ancestral state across anurans. Likewise, axillary amplexus appears to have been derived from the inguinal type, as hypothesized by others (Duellman & Trueb, 1986; Wells, 2007; Vitt & Caldwell, 2014; Pough *et al.*, 2016). Most importantly, our analysis

revealed that ≥34 transitions have occurred between amplexus types across the anuran phylogeny. Notably, we found that transitions from axillary to other type(s) of amplexus have occurred at high frequency (i.e. ≥18 times). These results suggest that axillary amplexus might be a key intermediate type that eventually diversified into almost all other amplexus types.

Axillary amplexus is the most widespread type across Anura and, according to our analysis, it evolved from the ancestral inguinal amplexus at least three times independently. The prevalence of axillary amplexus suggests its high versatility in different ecological contexts that can relate effectively to the reproductive success of males. For instance, in many species, especially those denominated by explosive breeders, where males and females congregate to reproduce (Wells, 1977; Lengagne *et al.*, 2007), it is common to find uncoupled males trying to remove amplectant males from the dorsum of their partner [Halliday & Tejedo, 1995; Wells, 2007; for an example in *Rhinella castaneotica* (Caldwell, 1991), see Supporting Information, Video S1]. In this context, amplectant males would benefit from grasping the female as strongly as possible, and axillary amplexus is the most effective for this [for an example with *Rhinella marina* (Linnaeus, 1758), see Lee & Corrales, 2002; Vargas-Salinas, 2005). Male–male competition

might also favour axillary amplexus where the intense competition during short breeding seasons results in the evolution or persistence of traits that reinforce mate-guarding behaviour by males (e.g. axillary amplexus plus being 'glued'). In contrast, most other types of amplexus might be related to taxa with prolonged breeding seasons, where amplectant males are exposed to lower risks of being displaced by competing males (Duellman & Trueb, 1986; Wells, 2007; Willaert *et al.*, 2016).

If the evolution of the axillary amplexus has been so successful, and this type of amplexus is so versatile, what could promote the evolution of other types of amplexus? Furthermore, why do reversions to the inguinal amplexus occur? Surely, the type of amplexus in a given species or clade has been shaped by multiple environmental, physiological and morphological factors and particular social contexts (see possible scenarios below). This pattern is also true for other aspects of reproductive modes, and this is likely to apply to vertebrate groups other than amphibians. Testing all the possible scenarios is beyond the scope of the present study, but we propose some inferences, as follows. For example, effective antipredator chemical defenses (i.e. toxic alkaloids), such as those in Dendrobatidae (poison frogs), can promote amplexus diversification. Several lineages of poison frogs have evolved aposematic coloration (Santos *et al.*, 2003; Rojas, 2017), which is associated with a high diversification in acoustic communication signals as an alleged indirect effect of a reduction in predation pressure (Santos *et al.*, 2014); thus, aposematism could also allow an increase in the complexity of courtship behaviours, promoting matings where axillary amplexus is not necessary. Our results support such intuition, because ≥22 dendrobatid species exhibit cephalic amplexus, whereas 18 species exhibit no amplexus (Weygoldt, 1987; Castillo-Trenn & Coloma, 2008). Moreover, most species of Dendrobatidae are prolonged breeders (Wells, 1977), mostly terrestrial, highly territorial and whose oviposition occurs in hidden places under leaf litter and tree roots (Wells, 1978; Pröhl, 2005; Summers & Tumulty, 2014; Rojas & Pašukonis, 2019). In these conditions, it might be assumed that aposematic males have fewer risks of predation or losing a female because of the action of an intruder male (Zamudio *et al.*, 2016). Thus, if the cost of predation is minimized, aposematic species could evolve complex mating behaviours and diversity of amplexus types. In contrast, species that rely on avoiding detection by predators might have evolved mating strategies that offer a balance between attracting females and avoiding enemies.

Microhabitat, or the environment where males court, is an important factor often overlooked in discussions about the evolution of amplexus diversity.

Axillary amplexus may function well in diverse microhabitats (arboreal, terrestrial and aquatic; see Supporting Information, Fig. S1). In arboreal species, for example, when a female jumps from leaf to leaf or across branches, an axillary amplexus would not prevent her from achieving the highest jumping performance; thus, this type of amplexus would be selectively advantageous. A similar performance in arboreal microhabitats would be difficult for inguinal or cephalic amplexus, because the movement of the male's body during jumping would be erratic and unbalanced with respect to that of the female's. This hypothesis warrants further investigation from the perspective of the functional association, e.g. between locomotor performance and a specific amplexus type in a particular microhabitat. Likewise, performance experiments would be required to account for the diameter, inclination and type of substrate, which significantly affect the kinematics of locomotion and thus select for specific morphologies and behaviours across a variety of taxa (Andersson, 1994; Losos, 2009; Herrel *et al.*, 2013).

We found a few instances of reversal from the axillary to the inguinal amplexus. A possible explanation for such transitions is that inguinal amplexus promoted morphological adaptations related to thermoregulation (Ashton, 2002; Meiri & Dayan, 2003; Zamora-Camacho *et al.*, 2014). Alternatively, such rare transitions might be an adaptation to fossorial habits (e.g. Microhylidae and Hyperoliidae). In animals with inguinal amplexus, this behaviour allows the female to avoid digging a wider burrow on the ground (Duellman & Trueb, 1986). We hypothesize that this mechanical limitation might explain the evolution of this type of amplexus in *Osornophryne*, a genus of toads with relatively short limbs, occurring at medium to high elevations in the South American Andes (Frost, 2019). Given that these species are adapted to cold climates, their globular body shape and short legs require a type of amplexus that provides better grasping potential for males.

Contrary to our predictions, we found no relationship between SSD and amplexus type. Differences in body size between the sexes can impose physical restrictions on males for clasping females in such a way that cloacas are aligned and egg fertilization is optimized (Davies & Halliday, 1977; Ryan, 1985; Robertson, 1990; Bourne, 1992). Moreover, differences in body size between sexes could reduce the strength with which a male can clasp a female, hence reducing the likelihood of a male being displaced by competing males (Bruning *et al.*, 2010). It is possible that the type of amplexus is related to sexual dimorphism in body shape or to the interaction between body shape and size, rather than to SSD alone. For instance, in species with globular bodies and short

limbs (e.g. genus *Breviceps*), the axillary amplexus is less feasible mechanically. In such species, the male is often very small with respect to the female and, thus, the evolution of alternative strategies to enhance amplexus might have been advantageous. We propose that the evolution of mucous skin secretions could have increased the effectiveness of gamete transfer in the absence of a clasp.

Compared with other amphibian orders, our results reveal some interesting differences. For instance, a recent study involving 114 salamandrid species reported that the ancestral states for this clade were mating on land, oviparity and lack of amplexus (Kieren *et al.*, 2018). The authors also suggested that the presence or absence of amplexus might be unrelated to the mating habitat. Our results suggest that anurans exhibit many more reproductive modes, as a consequence of a higher species diversity, diverse morphology and more diverse reproductive strategies (Vitt & Caldwell, 2014; Hutter *et al.*, 2017; Frost, 2019). Furthermore, this diversity increases towards tropical regions (e.g. Duellman, 1988; Hödl, 1990; Haddad & Prado, 2005). Therefore, complex habitats, such as the tropics, might offer more opportunities for adaptation in the context of reproductive characteristics, where environmental conditions affect the evolutionary patterns of amplexus diversity. This habitat complexity might also have influenced the diversity in reproductive modes found in other ectothermic vertebrates.

The type of amplexus, or lack thereof, in anurans is related to behavioural features that can clearly affect the reproductive success of an individual (Duellman & Trueb, 1986; Wells, 2007; Buzatto *et al.*, 2017). Our findings highlight not only the value of implementing phylogenetic comparative approaches for recording the evolutionary history of reproductive traits in vertebrates, but also the importance of making detailed field observations of reproductive behaviour and natural history. Precisely the lack or infrequency of such types of reports is one of the main difficulties faced by researchers aiming to carry out analyses like ours on amphibians and other taxa. Surely, the diversity of amplexus and associated behaviours is much higher than what has been reported to date in the scientific literature (Pombal *et al.*, 1994; Stephenson & Verrell, 2003; Berneck *et al.*, 2017). For example, a pectoral amplexus has been mentioned for *Nasikabatrachus sahyadrensis* (Nasikabatrachidae) (Zachariah *et al.*, 2012), but based on the detailed description by the authors, we consider that this behaviour might be a new type of amplexus (not included in our analysis because more evidence is necessary to support this hypothesis).

Overall, our study represents a unique, large dataset on amplexus types in anurans and allows us to highlight two types of amplexus (loose amplexus and gular amplexus) that have been overlooked in key literature references (i.e. Duellman & Trueb, 1986; Wells, 2007; Vitt & Caldwell, 2014; Pough *et al.*, 2016; Willaert *et al.*, 2016). We hope that further studies about breeding behaviour in anurans include detailed observations and descriptions that could reveal new aspects associated with the diversity of breeding strategies in vertebrates, even in those lineages considered as well studied.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Axillary amplexus in species in different microhabitat conditions (arboreal, terrestrial or aquatic). A, *Atelopus favescens* (Bufonidae). B, *Centrolene savagei* (Centrolenidae). C, *Ceratophrys calcarata* (Ceratophrydae). D, *Dendropsophus triangulum* (Hylidae). E, *Engystomops pustulosus* (Leptodactylidae). F, *Agalychnys callidryas* (Phyllomedusidae). G, *Pristimantis orpacobates* (Pristimantidae). H, *Lithobates vaillanti* (Ranidae). Pictures by: B. Rojas (A), F. Vargas-Salinas (B, D, E, G, H), L. A. Rueda-Solano (C) and A. M. Ospina-L (F).

Table S1. Amplexus type, Body size (snout-to-vent lenght) and Sexual size dimorphism (female to male snout-vent length ratio) for 685 anuran species.

Table S2. Mean posterior probabilities of amplexus types in each node in the phylogenetic tree for the 685 anuran species shown in Figure 3.

Video S1. Scramble behaviour and attempt of amplexus displacement in the toad *Rhinella* castaneotica. Video by B. Rojas.

Table S1 and references, Video S1 and Table S2 can also be found at DOI: 10.6084/m9.figshare.11595216