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18

ABSTRACT

19 The evolution and diversification of animal reproductive modes have been pivotal questions
20 in behavioral ecology. Amphibians present the highest diversity of reproductive modes
21 among vertebrates, involving various behavioral, physiological and morphological traits. One
22 of such features is the amplexus, the clasp or embrace of males on females during
23 reproduction, which is almost universal to anurans. Hypotheses about the origin amplexus are
24 limited and have not been thoroughly tested, nor had they taken into account evolutionary
25 relationships in most comparative studies. However, these considerations are crucial to
26 understand the evolution of reproductive modes. Here, using an evolutionary framework, we
27 reconstruct the ancestral state of amplexus in 686 anuran species; investigate whether the
28 amplexus type is a conserved trait; and test whether sexual size dimorphism (SSD) could have
29 influenced the amplexus type or male performance while clasping females. Overall, we found
30 evidence of at least 35 evolutionary transitions in amplexus type across anurans. We also
31 found that amplexus exhibits a high phylogenetic signal (it is conserved across Anura
32 evolutionary history) and the amplexus type does not evolve in association with SSD. We
33 discuss the implications of our findings on the diversity of amplexus types across anurans.

34 **Keywords:** Amphibia - ancestral reconstruction – frogs - reproductive modes - sexual
35 dimorphism.

36

INTRODUCTION

37 Understanding the evolution and diversification of reproductive modes in animals has been a
38 shared interest among evolutionary biologists over decades (e.g., Salthe, 1969; Shine, 1983;
39 Craig, 1987; Alves *et al.*, 1998; Blackburn, 2000; Crespi & Semeniuk, 2004; Haddad &
40 Prado, 2005). In addition to sexual selection, natural selection promotes reproductive
41 diversity by favoring modes of reproduction that maximize the likelihood of successful
42 matings in a given environment (Pianka, 1976; Zamudio *et al.*, 2016). Thus, reproductive
43 modes are one of the most critical life-history traits directly affecting fitness and survival via
44 physiological, morphological and behavioral adaptations that increase each individual's
45 ability to find mates and producing viable offspring, in response to environmental (and other)
46 selective pressures (Angelini & Ghiara, 1984; Lodé, 2012).

47 Anuran amphibians exhibit one of the highest diversities in reproductive modes among
48 vertebrates (Duellman & Trueb, 1986; Vitt & Caldwell, 2014). These reproductive modes are
49 defined as a combination of ecological, developmental and behavioral traits that include
50 oviposition site, ovule morphology, clutch size, developmental rate, and presence or absence
51 of (different types of) parental care (Salthe & Duellman, 1973; Duellman & Trueb, 1986).
52 Thus, anuran reproductive modes exhibit a gradient of parental involvement which ranges
53 from no or little parental care, involving mostly aquatic oviposition of clutches with hundreds
54 or thousands of eggs; to elaborate parental care with relatively few terrestrial eggs, decisive
55 parental involvement or direct development with reduced or absent tadpole stage (Hödl, 1990;
56 Haddad & Prado, 2005; Crump, 2015).

57 Previous studies have investigated different aspects of the evolution of reproductive modes in
58 anurans using limited phylogenetic comparative methods (Duellman, 2003; Gomez-Mestre *et*
59 *al.*, 2012; Zamudio *et al.*, 2016; Furness & Capellini, 2019). These studies have hypothesized

60 that ecological and population structure factors such as desiccation in temporary ponds,
61 availability of humid microhabitats in terrestrial environments and predation are major
62 selective forces shaping most reproductive modes. However, to our knowledge, none of these
63 studies has addressed the evolution of a key behavioral component in frog reproduction: the
64 amplexus or ‘mating clasp’. Here, we investigate the evolutionary patterns of this trait across
65 the Anura and reveal major evolutionary transitions in this crucial component of frogs’
66 reproductive behavior.

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

82 Here, we use the most complete phylogeny of Anura (Jetz & Pyron, 2018) to map the origins
83 and diversification of the known types of amplexus in 686 species with reported records.

84 Furthermore, we explore the relationship between the evolution of amplexus diversity and
85 sexual dimorphism in body size (measured as female-to-male snout-vent length ratio,
86 hereafter SSD). We predict that species with a male-biased, little or no SSD, would benefit
87 from axillary amplexus, whereas species with a high female-biased SSD would likely present
88 inguinal amplexus or another derived type of amplexus or strategy (e.g. ‘glued’ in Fig. 1).
89 These predictions are based on the physical restrictions that a very small male could have to
90 clasp a large female and, hence, to ensure her fidelity during mating. Overall, our results
91 show that the different types of amplexus, and the lack thereof, are well-defined throughout
92 the Anura tree of life. This is a fundamental step forward to understanding how
93 environmental factors and life history have shaped the amazing diversity of reproductive
94 modes across Anura and other vertebrates.

95

96 MATERIAL AND METHODS

97 AMPLEXUS CHARACTERIZATION

98 We looked for information on types of amplexus in Anura from primary literature (e.g., peer-
99 reviewed articles, books) located by using Google Scholar and Web of Science (WoS). We
100 used “amplexus” and “nupcial clasp” as keywords. Because we obtained an excess of results
101 unrelated to Anura (the term amplexus has also been used for invertebrates; Conlan, 1991),
102 we included the keywords “anura” and “frogs”. To further narrow down our search, we also
103 combined previous keywords with anuran families’ names (e.g., “Amplexus” AND
104 “Dendrobatidae”). Within the selected publications, we searched for an account describing
105 male and female behavior with enough detail (e.g. an observational account of the behavior or
106 photograph) to be assigned to an amplexus type.

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

119 For *Nyctibatrachus humayumi* (Nyctibatrachidae), it was reported that this species lacks
120 amplexus (Kunte, 2004), but it was later observed that *N. humayumi* has a dorsal straddle
121 amplexus (Willaert *et al.*, 2016). In another case, the authors cite a pectoral amplexus for
122 *Nasikabatrachus sahyadrensis* (Sooglossidae) (Zachariah *et al.*, 2012), but based on the
123 detailed description by the authors, we consider this behavior to be a new type of amplexus.
124 We define this clasp as a ‘scapular amplexus’, where the male presses his fists into each side
125 of the female’s vertebral column. For *Scaphiophryne gottebei* (Microhylidae), the authors
126 mention an inguinal amplexus, but this account includes several figures showing a
127 characteristic axillary amplexus (Rosa *et al.*, 2011). For *Osteocephalus* (Hylidae), we used the
128 ‘gular amplexus’ definition by Jungfer *et al.*, (2013), which refers to a type of amplexus
129 where the male clasps the female exclusively around her throat. Likewise, some species with
130 axillary amplexus have been reported to have a ‘glued amplexus’ (e.g. *Elachistocleis bicolor*
131 (Cacciali, 2010) and *Chiasmocleis leucosticta* (Haddad & Hödl, 1997)). Such reports indicate

132 that the males are ‘glued’ to the female dorsum but, in our analysis, we did not take this
133 clasping behavior as different from an axillary amplexus. Our consideration is based on the
134 fact that males of species with axillary amplexus have not been examined thoroughly enough
135 for the presence or absence of glands to suggest that these organs exist, or produce sticky or
136 glue-like substances. For species like *Nyctibatrachus aliciae* (Nyctibatrachidae, Biju *et al.*,
137 2011) or *Mantella aurantiaca* (Mantellidae, Vences, 1999, Glaw & Vences, 2007), the
138 observed amplexus consists of a male sitting on the dorsum of the female by a short period of
139 time, without an actual clasp; we classified such observations as ‘loose amplexus’. Lastly,
140 *Aplastodiscus leucopygius* (Hylidae) and *Ascaphus truei* (Ascaphidae) have a ‘dynamic
141 amplexus’, which is difficult to categorize because at different moments the same pair (i.e.
142 male and female) exhibits diverse amplexus positions (Stephenson & Verrell 2003; Berneck
143 *et al.* 2017). Therefore, these two species were not included in our analysis.

144 We completed the life history characterization for all species with amplexus data by including
145 male and female body size (i.e. snout-vent-length, SVL) and sexual size dimorphism (SSD).
146 In cases where only the range of body size was available, we used the median values.

147

148

COMPARATIVE METHODS

149 For the phylogenetic analysis, we obtained 1000 random phylogenies based only on genetic
150 data from Jetz & Pyron (2018). Then, we ran the analysis 1000 times to evaluate the
151 robustness and include uncertainty in topologies in the R software environment (R Core
152 Team, 2018). Later, we made a stochastic ancestral reconstruction (Bollback, 2006) of the
153 character “type of amplexus” using 1000 trees, with the *make.simmap* function in ‘phytools’
154 R-package version 0.6-99 (Revell, 2012) which is a best-fit character evolution model (Only
155 with 100 trees). We contemplate an “Equal Rate model” (ER), which assumes that all

156 transitions between traits occur at the same rate (Pagel 1994; Lewis 2001); and an “All-Rate-
157 Different model” (ARD), which assumes that all transitions between traits occur at different
158 rates (Paradis *et al.*, 2004). We used the *fitDiscrete* function in the ‘geiger’ R-package (v.
159 2.0.6.2; Harmon *et al.*, 2007) to compare the ER and ARD models, and selected the model
160 with the lowest AICc value. We did not generate fully-sampled phylogenies using the
161 taxonomic imputation method to make our ancestral character reconstruction because this
162 approximation has been demonstrated to be inappropriate for this kind of analysis due to
163 increased bias (Rabosky, 2015; Rocha *et al.*, 2016; Jetz & Pyron 2018).

164 To test whether the type of amplexus and SSD are conserved or labile (convergent) traits, we
165 calculated their phylogenetic signal. This property is defined as the tendency of traits in
166 related species to resemble each other more as a consequence of shared ancestry (Blomberg &
167 Garland 2003). For this purpose, we used the statistic lambda (λ) proposed by Pagel (1997,
168 1999) as a measurement of phylogenetic signal. The λ value varies from 0 to 1; if $\lambda \sim 1$, it
169 indicates a strong phylogenetic signal (i.e. conserved trait), whereas if $\lambda \sim 0$, it indicates that
170 the evolution pattern of the trait has been random or convergent; that is, these characters lack
171 phylogenetic signal (Gómez *et al.*, 2010, Kraft *et al.*, 2007; Revell *et al.*, 2008). Calculation
172 of the phylogenetic signal for type of amplexus was made using the *fitDiscrete* function of
173 ‘geiger’ R-package. In addition, we calculated the likelihood of a model with no phylogenetic
174 signal and the maximum likelihood value of λ ; later we used a likelihood ratio test to compare
175 these two models and calculate a p-value (significance alpha 0.05) under a chi-square
176 distribution. For SDD, phylogenetic signal was tested with the *phylosig* function in ‘phytools’
177 (Revell, 2012).

178 To test whether the evolution rates of type of amplexus have increased or slowed over time,
179 we used a delta model in *fitDiscrete* function of ‘geiger’. In addition, we calculate the

180 likelihood of a model with delta equal to one and a model with observed delta; later we used a
181 likelihood ratio test to compare these two models and calculated a p-value (significance alpha
182 0.05) under a chi-square distribution. If delta statistic > 1 , this indicates that recent evolution
183 has been relatively fast; in contrast, if delta ≤ 1 , it indicates that recent evolution has been
184 relatively slow. Because the difference in body size between males and females could
185 promote changes in types of amplexus due to mechanic incompatibility (e.g., small males
186 might not physically clasp a large female), we used a phylogenetic ANOVA to compare
187 sexual size dimorphism across different types of amplexus. This analysis was performed
188 using the *phylANOVA* function in 'phytools'.

189

190

RESULTS

191 Our analyses included 686 species from 46 anuran families (Table S1). The distribution of
192 species in our dataset comprised all continents where amphibians are present. Most families
193 (i.e. 35 families) have only one type of amplexus, while 11 families have more than one (Fig.
194 2, Table S1). The average SSD is 1.17 ± 0.16 (range = 0.70-1.85; n= 478 species), and it
195 varies between anuran species and families (Fig. 2, Table S1); in 42 species (8.78%) male
196 body size is larger than female body size, while in most cases (429 species, 89.74%) the
197 female is larger than the male; in the remaining seven species (1.46%) males and females
198 exhibit similar body size.

199 For the ancestral reconstruction of types of amplexus, we chose the ER model ($LnL = -$
200 227.438 , $AIC = 456.882$) over ARD model ($LnL = -165.484$, $AIC = 538.497$) based on the lowest
201 AICc value (Fig. 3). Our results support the inguinal amplexus as the basal state to all Anura.
202 For instance, Ascaphidae and other basal frog families (e.g., Leiopelmatidae, Bombinatoridae,
203 Alytidae, Pipidae) present inguinal amplexus. The axillary amplexus was found to be the

204 most frequent state, occurring in 540 out of the 686 species (i.e. 78.72%). However, we found
205 35 evolutionary transitions between all type of amplexus across the whole Anura phylogeny
206 (Fig. 4A). The greatest number of evolutionary transitions (i.e., 12) occurred between axillary
207 and inguinal amplexus states (Fig. 4A). Likewise, we found a strong phylogenetic signal
208 amplexus (Pagel's $\lambda = 0.963$, $P < 0.0001$). In contrast, we found that SSD has a weak
209 phylogenetic signal (Pagel's $\lambda = 0.266$, $P = 0.043$). We also found that the delta value is equal
210 to 0.694 (SD = 0.208), but when we compare it with a model with delta equal to 1, we found
211 no differences ($P = 0.63$). Furthermore, we did not find differences in SSD across types of
212 amplexus ($F = 1.2086$, $P = 0.4215$, $n = 478$; Fig. 4B).

213

214

DISCUSSION

215 Different selective pressures are known to shape the behavioral, physiological and physical
216 traits that characterize the diverse reproductive modes and behaviors in anurans (and fishes)
217 in comparison to other vertebrates. One of s traits is the amplexus, whose evolutionary trends
218 we characterized using a comparative phylogenetic framework. For this purpose, we explored
219 the relationship between the different types of amplexus (or lack thereof) and sexual size
220 dimorphism. Below, we discuss the implications of our findings and generate testable
221 hypotheses for future research on the evolution of reproductive modes in anurans.

222 We found a significant phylogenetic signal in amplexus. This result was anticipated,
223 considering that the amplexus is an aspect of the anuran reproductive behavior that is
224 expected to have been shaped by other various factors not related to reproduction (e.g.
225 morphology, microhabitat; Duellman and Trueb 1986, Wells 2007). Likewise, it is presumed
226 that the type of amplexus affects the reproductive success of males and females in several
227 ways. For example, the type of amplexus determines the proximity of cloacas between males

228 and females and, possibly, the success of egg fertilization (Davis and Halliday 1979; Wells
229 2007). Altogether, we infer that any change between types of amplexus requires several
230 selective factors to act in tandem and weighed by their combined selective force. While we
231 found a slight indication of a slow down in the rate evolution of amplexus (delta value equal
232 to 0.694), this was not supported when we tested if this parameter estimate was different from
233 $\delta = 1$. In other words, the amplexus rate of change follows a Brownian motion model
234 which does not support a slow-down, but rather a random change (either slow-down or
235 acceleration) in its rate of evolution through the evolutionary history of Anura.

236 In contrast to amplexus type, the weak phylogenetic signal in SSD suggests that
237 selective forces might be promoting body size disparities to be the result of convergent
238 evolution across Anura. For instance, in most frogs, females are larger than males because
239 there is a strong positive relationship between fecundity and body size (i.e., selection favors
240 larger size in females); in contrast, a larger body size in males is not necessarily tied to higher
241 attractiveness to females or higher dominance across species (Halliday and Tejedo 1995;
242 Wells 2007).

243 Previous reports support our results that all basal lineages (e.g., Ascaphidae, Pipidae,
244 and Myobatrachidae) have an inguinal amplexus (Lynch, 1973; Rabb, 1973; Weygoldt,
245 1976), which we found to be the ancestral state across anurans. Likewise, axillary amplexus
246 appears to have been derived from the inguinal type, as hypothesized by others (Duellman &
247 Trueb, 1986; Wells, 2007; Vitt & Caldwell, 2014; Pough *et al.*, 2016). Most importantly, our
248 analysis revealed that at least 35 transitions have occurred between amplexus types across the
249 anuran phylogeny. Notably, we found that transitions from axillary to other type(s) of
250 amplexus have occurred in high frequency (i.e., at least 18 times). These results suggest that

251 axillary amplexus might be a key intermediate type that eventually diversified in almost all
252 other amplexus types.

253 Axillary amplexus is the most widespread type across Anura and, according to our
254 analysis, it evolved at least three independent times from the ancestral inguinal amplexus. The
255 prevalence of axillary amplexus suggests its high versatility in different ecological contexts
256 that can effectively relate to the reproductive success of males. For instance, in many species,
257 especially those denominated by explosive breeders where males and females congregate to
258 reproduce (Wells, 1977), it is common to find uncoupled males trying to get off amplexant
259 males from the dorsum of their partners (Halliday & Tejedo, 1995; Wells, 2007; see video S1
260 for an example in *Rhinella castaneotica* (Caldwell, 1991)). In this context, amplexant males
261 would benefit from grasping the female as strongly as possible and only the axillary amplexus
262 is the most effective (see Lee & Corrales, 2002; Vargas-Salinas, 2005 for an example with
263 *Rhinella marina* (Linnaeus, 1758)). Intrasexual competition between males may also favor
264 axillary amplexus where the intense competition during short breeding seasons results in the
265 evolution or persistence of traits that reinforce mate guarding behavior by males (e.g. axillary
266 amplexus plus been glued). In contrast, most other types of amplexus might be related to taxa
267 with prolonged breeding seasons where amplexant males are exposed to lower risks of being
268 displaced by competing males (Duellman & Trueb, 1986; Wells, 2007; Willeart *et al.*, 2016).

269 If the evolution of the axillary amplexus has been so successful, and this type of
270 amplexus is so versatile, what could promote the evolution of other types of amplexus?
271 Furthermore, why do reversions to the inguinal amplexus occur? Surely, the type of amplexus
272 in a given species or clade has been shaped by multiple environmental, physiological and
273 morphological factors and particular social contexts (see possible scenarios below). This
274 pattern is also true for other aspects of reproductive modes, and likely applicable to vertebrate

275 groups other than amphibians. Testing all the possible scenarios is beyond the scope of this
276 study, but we propose some inferences, as follows. For example, effective antipredator
277 chemical defenses (i.e., toxic alkaloids) such as those in Dendrobatidae (poison frogs) can
278 promote amplexus diversification. Several lineages of poison frogs have evolved aposematic
279 coloration (Santos *et al.*, 2003; Rojas, 2017), which is associated with a high diversification in
280 acoustic communication signals as an alleged indirect effect of a reduction in predation
281 pressure (Santos *et al.*, 2014); thus, aposematism could also allow an increase in the
282 complexity of courtship behaviors, promoting matings where axillary amplexus is not
283 necessary. Our results support such intuition, as at least 22 dendrobatid species exhibit
284 cephalic amplexus, whereas 18 species exhibit no amplexus (Weygoldt, 1987; Castillo-Trenn
285 & Coloma, 2008). Moreover, most species of Dendrobatidae are prolonged breeders (Wells,
286 1977), mostly terrestrial, highly territorial and whose oviposition occurs in hidden places
287 under leaf litter and tree roots (Wells, 1978; Pröhl, 2005; Summers & Tumulty, 2014; Rojas &
288 Pašukonis, 2019). Under these conditions, it might be assumed that aposematic males have
289 fewer risks of predation and losing a female, attracted via acoustic and visual signals, because
290 of the action of an intruder male (Zamudio *et al.*, 2016). Thus, aposematic species could
291 evolve complex mating behaviors and diversity of amplexus types if the cost of predation is
292 minimized. In contrast, species that rely on avoiding detection by predators may have evolved
293 mating strategies that offer a balance between attracting females and avoiding enemies.

294 Microhabitat, or the environment context where males court, is an important factor
295 often overlooked in discussions about the evolution of amplexus diversity. Axillary amplexus
296 may function well in diverse microhabitats (arboreal, terrestrial, aquatic; see Fig. S1). In
297 arboreal species, for example, when a female jumps from leaf to leaf or across branches, a
298 male clasping her in an axillary amplexus would not prevent her from achieving the highest
299 jumping performance; thus this kind of amplexus would be selectively advantageous. A

300 similar performance in arboreal microhabitats would be difficult for inguinal or cephalic
301 amplexus, as the movement of the male's body during jumping would be erratic and
302 unbalanced with respect to that of the female's. This hypothesis warrants further investigation
303 from the perspective of the functional association, e.g., between locomotor performance and a
304 specific amplexus type in a particular microhabitat. Likewise, performance experiments
305 would be required to account for the diameter, inclination and type of substrate, which
306 significantly affect the kinematics of locomotion and thus select for specific morphologies
307 and behaviors across a variety of taxa (Andersson, 1994; Losos, 2009; Herrel *et al.*, 2013).

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

320 Contrary to our predictions, we found no relationship between sexual size dimorphism
321 (SSD) and type of amplexus. Differences in body size between the sexes can impose physical
322 restrictions to males for clasping females in a way that cloacas are aligned and egg
323 fertilization is optimized (Davis & Halliday, 1977; Ryan, 1985; Robertson, 1990; Bourne,

324 1992). Moreover, differences in body size between sexes could reduce the strength with
325 which a male can clasp a female, hence reducing a male's likelihood of being displaced by
326 competing males (Brunning *et al.*, 2010). It is possible that amplexus type is related to sexual
327 dimorphism in body shape, or the interaction between body shape and size, rather than size
328 dimorphism alone. For instance, in species with globular bodies and short limbs (e.g. genus
329 *Breviceps*; *Nasikabatrachus sahyadrensis*), the axillary amplexus is mechanically less
330 feasible. In such species, the male is often very small with respect to the female and, thus, the
331 evolution of "alternative strategies" to enhance amplexus may have been advantageous. We
332 propose that the evolution of mucus skin secretions could have increased the effectiveness of
333 gamete transfer in the absence of an actual clasp.

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

346 The type of amplexus in anurans, or the lack thereof, is related to behavioral features that
347 clearly can affect the reproductive success of individuals (Duellman & Trueb, 1986, Wells,

348 2007; Buzatto *et al.*, 2017). Our findings highlight not only the value of implementing
349 phylogenetic comparative approaches for recording the evolutionary history of reproductive
350 traits in vertebrates, but also the importance of doing detailed field observations of
351 reproductive behavior and natural history. Precisely the lack or infrequency of such kind of
352 reports is one of the main difficulties faced by researchers aiming to do analysis like ours on
353 amphibians and other taxa. Surely, the diversity of amplexus and associated behaviors is
354 much higher than what has been reported to date in the scientific literature, not only among
355 species but even at intraspecific level (Pombal *et al.*, 1994; Stephenson & Verrell, 2003;
356 Berneck *et al.*, 2017). Our study represents a unique large dataset on amplexus types for
357 anurans, and allows to highlight three amplexus types (loose amplexus, gular amplexus,
358 scapular amplexus) that have been overlooked in key literature references (i.e. Duellman &
359 Trueb, 1986; Wells, 2007; Vitt & Caldwell, 2014; Pough *et al.*, 2016; Willaert *et al.*, 2016).
360 We hope that further studies about breeding behavior in anurans include detailed observations
361 and descriptions that could reveal novelty aspects associated to diversity of breeding
362 strategies in vertebrates, even in those lineages considered as well studied.

363

364

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371

Supplementary data

372 Table S1 and references, Video S1, and table S2 can be found in DOI:
373 10.6084/m9.figshare.11050685

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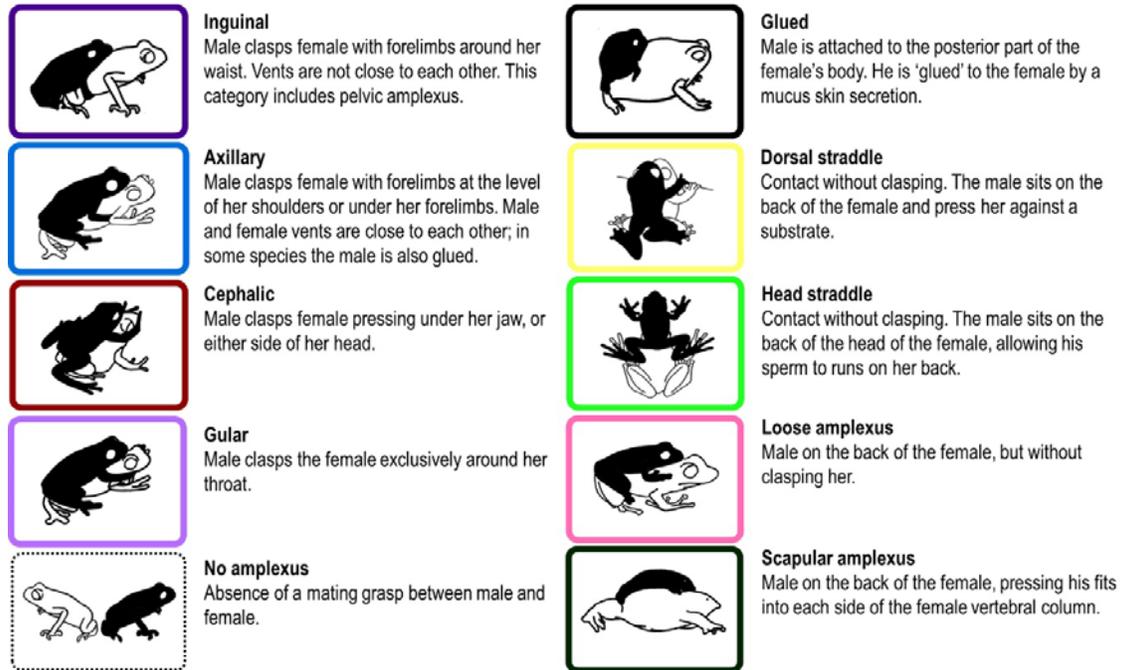
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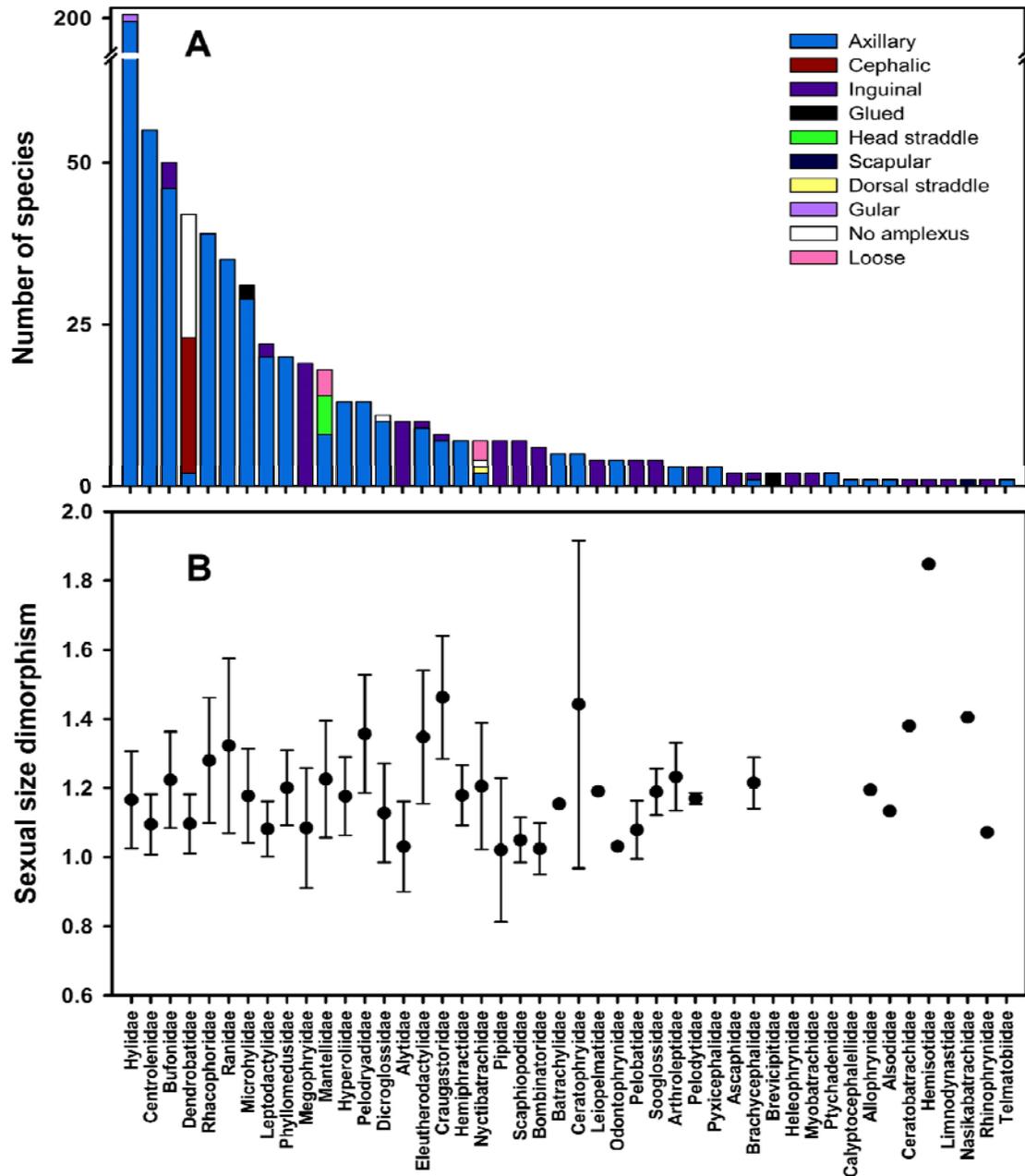
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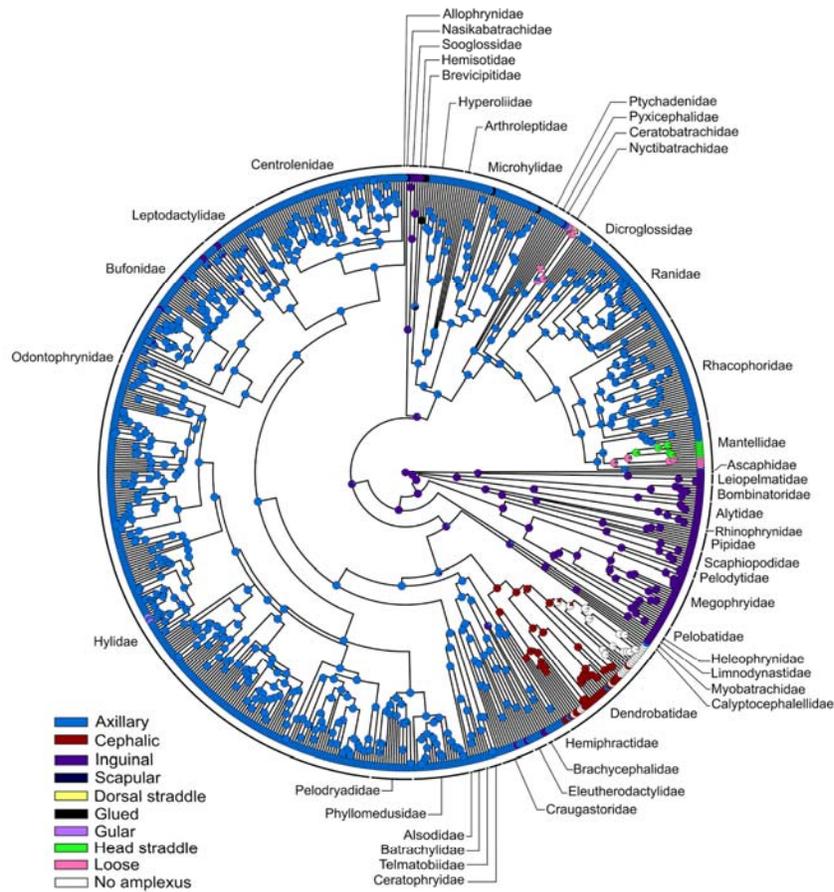
573

574 **Figure 1.** Diversity of amplexic positions in anurans (males in black color). Definitions
575 according to Blommers-Schlösser (1975), Townsend & Stewart (1986), Duellman & Trueb
576 (1986), Wells (2007), Zachariah *et al.*, (2012), Jungfer *et al.*, (2013) and Willeart *et al.*,
577 (2016). Because descriptions of some amplexus types are not clear or are ambiguous, we
578 pooled the category “Independent” (cited by Duellman & Trueb, 1986), as “No amplexus”.
579 Frame colors make references to the same amplexus types shown in Fig. 2 and 3.



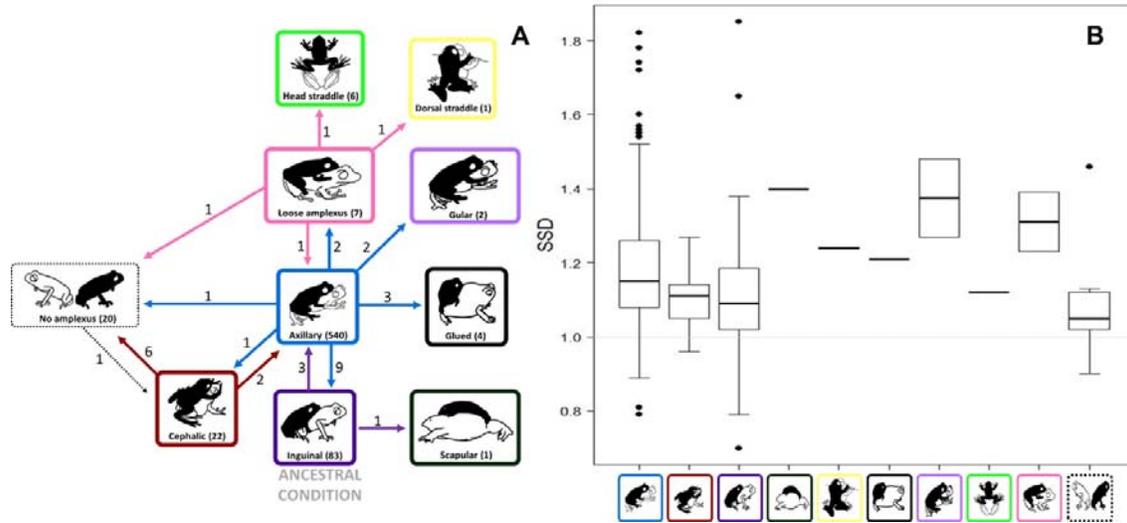
580

581 **Figure 2.** A. Summary of the number (686) of anuran species, type of amplexus (or lack
 582 thereof), and B. sexual size dimorphism (female-to-male snout-vent length ratio; SSD) per
 583 Family of Anura included in this study. Plot based on data from Table S1. Dots and bars in
 584 the plot B indicate mean values and standard deviation, respectively; for some families was
 585 not possible to calculate SSD because absence of data for female or male body size.



586

587 **Figure 3.** Ancestral reconstruction of amplexus type using 1000 trees for 686 anuran species
588 under an “equal rates” model of trait evolution. Tips represent actual type of amplexus for
589 each species and nodes represent the probability of each type of amplexus (Table S2 for
590 specific values). Phylogenetic tree from Jetz & Pyron (2018).



591

592 **Figure 4. A.** Estimated number of independent evolutionary transitions in type of amplexus
 593 for the 686 anuran species included in this study. In parentheses are pointed the number of
 594 species with each type of amplexus. Estimates are based on 1000 phylogenetic trees, and an
 595 ancestral state reconstruction performed with phytools packages (see Fig. 2 and text for
 596 details). **B.** Variation in sexual size dimorphism (female-to-male snout-vent length ratio,
 597 SSD) in 478 species with different types of amplexus. Phylogenetic ANOVA: ($F = 1.2086$, P
 598 $= 0.4215$, $n = 478$).

599

600

601 **Figure S1.** Axillary amplexus in species under different microhabitat conditions (Arboreal,
602 Terrestrial, Aquatic). **A.** *Atelopus faveszens* (Bufonidae), **B.** *Centrolene savagei*
603 (*Centrolenidae*), **C.** *Ceratophrys calcarata* (*Ceratophryidae*), **D.** *Dendropsophus triangulum*
604 (*Hylidae*), **E.** *Engystomops pustulosus* (*Leptodactylidae*), **F.** *Agalychnys callidryas*
605 (*Phyllomedusidae*), **G.** *Pristimantis orpacobates* (*Pristimantidae*), **H.** *Lithobates vaillanti*
606 (*Ranidae*). Pictures by B Rojas (A), F Vargas-Salinas (B,D,E,G,H), LA Rueda-Solano (C),
607 and AM Ospina-L (F).

608

609