

1 RH: Developmental plasticity and morphology

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5 **Morphological consequences of developmental plasticity in *Rana temporaria***
6 **are not accommodated into among-population or among-species variation**

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19 Abstract: Environmental induced developmental plasticity occurs in many organisms and it
20 has been suggested to facilitate biological diversification. Here we use ranid frogs to examine
21 whether morphological changes derived from adaptive developmental acceleration in
22 response to pool drying within a species are mirrored by differences among populations and
23 across species. Accelerated development in larval anurans under pool drying conditions is
24 adaptive and often results in allometric changes in limb length and head shape. We examine
25 the association between developmental rate and morphology within population, among
26 populations in divergent environments, and among species inside the Ranidae frog family,
27 combining experimental approaches with phylogenetic comparative analyses. We found that
28 frogs reared under decreasing water conditions that simulated fast pool drying had a faster
29 development rate compared to tadpoles reared on constant water conditions. This faster
30 developmental rate resulted in different juvenile morphologies between the two pool drying
31 conditions. The association between developmental rate and morphology found as a result of
32 plasticity was not mirrored by differences among populations that differed in development,
33 neither was it mirrored among species that differed in development rate. We conclude that
34 morphological differences among populations and species were not driven by variation in
35 developmental time per se. Instead, selective factors, presumably operating on locomotion
36 and prey choice, seem to have had a stronger evolutionary effect on frog morphology than
37 evolutionary divergences in developmental rate in the ranid populations and species studied.

38

39 INTRODUCTION

40 Over the last decade there has been a renewed interest in the role that phenotypic and
41 developmental plasticity might play in generating evolutionary innovation and triggering
42 biological diversification (West-Eberhard 2003, 2005; Pfennig et al., 2010; Moczek et al.,
43 2011). Phenotypic plasticity can favour diversification by permitting populations to colonize
44 new environments while reducing the associated bottlenecks and preserving greater genetic
45 variation (Draghi & Whitlock, 2012, Gomez-Mestre & Jovani, 2013). Phenotypic plasticity
46 may thus allow populations to rapidly move from one adaptive peak to another (Gomez-
47 Mestre & Jovani, 2013). Once established, the traits that were once plastic might become
48 genetically assimilated or accommodated (West-Eberhard, 2005), resulting in changes in
49 phenotypic trait values and hence in biological diversification under selection (Pfennig e al.,
50 2010). Genetic assimilation refers to a trait that was originally plastic and that has become
51 under strict genetic regulation, i.e. has become constitutive through canalisation (Waddington,
52 1942; Braendle & Flatt, 2006). Genetic accommodation is a broader concept that
53 encompasses genetic assimilation. It refers to the expression of phenotypes resulting from
54 previously cryptic epigenetic or genetic variation, which increases in frequency under
55 selection resulting in divergent reaction norms, whether increased or decreased plasticity
56 (West-Eberhard, 2003; Crispo 2007; Fusco & Minelli 2010).

57 It is unclear how common genetic accommodation may have been in evolution, but we
58 can identify potential cases of this evolutionary process looking for patterns where trait
59 diversity among species or populations exposed to divergent environments mirrors phenotypic
60 plasticity within taxa in response to those same environmental factors (Gomez-Mestre &
61 Buchholz, 2006; Wund et al., 2008). This, however, is not a definitive test of genetic
62 accommodation, which would require mechanistic understanding of how phenotypic plasticity
63 affects biological diversification. For example, tests of genetic accommodation would require

64 assessing if different genes are expressed in organisms of the same phenotype, whether it is
65 expressed constitutively or plastically (Snell-Rood et al., 2010; Johansson et al., 2013).
66 Nevertheless, identifying patterns congruent with genetic accommodation is a necessary first
67 step in the study of this process.

68 Here we use a combination of experimental and phylogenetic comparative approaches
69 to test for associations between developmental rate and morphology in frogs across different
70 levels of organization (within population, among populations, and among species). We
71 quantify plasticity in developmental rate and morphology within species and compare it
72 among populations and species. We hypothesize that the correlation between morphology and
73 development rate seen within a species as a result of phenotypic plasticity, also can be seen
74 across different population and species that differ in development rate. This would be
75 consistent with evolved differences through genetic accommodation, if not a direct test for it.

76 Amphibians are excellent study systems for studying the role of phenotypic plasticity
77 in promoting biological diversity because larval amphibians can greatly vary their growth and
78 differentiation rates depending on the conditions experienced. This plasticity allows
79 decoupling of growth and differentiation so that they can have extended growth with little
80 progress in developmental stages under benign conditions, but also accelerated development
81 to evade adverse conditions (Rose, 2005). One environmental factor that has strong impact on
82 growth, development, and morphology within frog species is pool drying (Newman, 1992).
83 Many amphibian species breed in ephemeral and temporary pools, where pool drying poses a
84 high risk of desiccation to larvae (Newman, 1992; Denver et al., 1998). Consequently, having
85 the ability to decouple growth from differentiation and being able to accelerate development
86 when at risk of pool drying is critical for larval amphibians (Wilbur & Collins, 1973; Altweg
87 & Reyer, 2003).

88 It has been suggested that metamorphosis has evolved to unlink distinct functional and
89 morphological stages allowing their independent adaptation to distinct niches: the adaptive
90 decoupling hypothesis (Eberman 1992; Moran 1994). However, increasing evidence
91 suggests that life stages are not independent (Pechenik 1999). Laboratory and field studies
92 have shown that tadpoles from many taxa have a faster development and usually a lower
93 growth rate under decreasing water levels (Newman, 1992; Richter-Boix et al., 2011;
94 Kulkarni et al., 2011). The relative changes in larval growth and developmental rates, result in
95 predictable allometric changes in the morphology of postmetamorphic individuals,
96 particularly on head shape and hind limb length (Richter-Boix et al., 2006; Gomez-Mestre et
97 al., 2010; Tejedo et al., 2010). Such allometric responses are common across amphibian
98 species and result from developmental shifts in response to several environmental factors
99 (Gomez-Mestre et al., 2010; Tejedo et al. 2010). Under high risk of desiccation,
100 developmental acceleration induces shorter hind limbs and wider heads of the emerging
101 juveniles (Richter-Boix et al. 2006, Gomez-Mestre et al., 2010; Tejedo et al., 2010, Johansson
102 & Richter-Boix, 2013). If these plastic responses became fixed during population
103 differentiation, it would result in morphological diversification (Fig. 1). Hence, the
104 morphological footprint of evolutionary variation in developmental rate is sometimes
105 detectable among populations or among species with broadly divergent developmental rates
106 (Gomez-Mestre & Buchholz, 2006; Johansson & Richter-Boix, 2013). Generally, lineages
107 (populations or species) with slower developmental rates typically show relative longer hind
108 limbs and narrower and/or longer heads than those having faster developmental rates
109 (Emerson, 1986; Gomez-Mestre & Buchholz, 2006; Johansson & Richter-Boix, 2013). We
110 can thus test for associations between larval development and postmetamorphic morphology
111 at various taxonomic levels, and determine if variation in developmental rate and morphology

112 across populations and species is congruent with the expectations of accommodation of
113 ancestral plasticity.

114 We use ranid frogs to ask whether developmental acceleration and the associated
115 morphological changes occurring within population are mirrored among populations and
116 species with divergent developmental rate. To answer this question we examined the
117 association between developmental rate and hind limb length and head shape within
118 populations, among populations in divergent environments, and among ranid species,
119 combining experimental approaches with phylogenetic comparative analyses. We predicted
120 that if evolved through genetic accommodation, plastic shifts in developmental rate and
121 morphology within population in response to pond drying would be mirrored among
122 populations occupying ponds of different duration so that populations breeding in more
123 ephemeral ponds would have evolved overall faster developmental rates and shorter hind
124 limbs. We also expected that if genetic accommodation had occurred among species, they
125 would have evolved different larval periods, spurred by variation in the duration of breeding
126 habitats. If evolutionary divergence in larval period among species was large and the causal
127 link between larval developmental rate and post-metamorphic morphology remained
128 unchanged, we would observe a pattern of constitutive differences among species so that
129 faster developing species would also show shorter hind limbs (Gomez-Mestre & Buchholz
130 2006). Alternatively, if differences in developmental rate among populations did not reflect
131 differences in pond duration, or species differences in developmental rate did not explain
132 morphological variation, the pattern would not be congruent with genetic accommodation. In
133 that case, direct selection on morphology related to its effects on performance aspects such as
134 locomotion or prey capture may be alternative explanations for phenotypic variation.

135

136 MATERIAL AND METHODS

137 *Study species and system*

138 First, we compared the developmental responsiveness against simulated pool drying among *R.*
139 *temporaria* populations adapted to divergent hydroperiod regimes, and tested for differences
140 among sibships within those populations. Second, we compared the relationship between
141 developmental time and morphology among those *R. temporaria* populations varying in their
142 developmental rate. Third, we compared the relationship between developmental time and
143 morphology in 22 species of ranids.

144 ***Laboratory Experiments: influence of developmental rate plasticity on morphology***

145 We studied 5 populations of the common frog, *R. temporaria*, from islands in the Gulf of
146 Bothnia [see Lind & Johansson (2007) and Lind et al. (2011) for more details on these island
147 populations]. In short, frog populations on these islands have adapted their developmental rate
148 to time metamorphosis to the duration of pools they breed on, such that populations from
149 islands with short-lasting pools have a shorter developmental time than those with permanent
150 water pools (Lind & Johansson 2007). In addition, these island populations are genetically
151 differentiated because they have limited gene flow and the degree of quantitative genetic
152 differentiation (Q_{st}) for life history traits is greater than the degree of neutral genetic
153 differentiation (F_{st}) (Lind et al., 2011).

154 The purpose of the laboratory experiment was to estimate the relationship between
155 developmental time and morphology in *R. temporaria*, within and between island populations.
156 We collected egg clutches from five different island populations in northern Sweden.
157 Estimates on developmental time and choice of island populations were made from data in
158 Lind and Johansson (2007). Because we wanted to compare populations differing in
159 developmental time, we sampled eggs from three islands with permanent pools where frogs

160 typically show long developmental times (Lillhaddingen, Storhaddingen and Petlandsskär),
161 and two islands with temporary pools where frogs show faster (10 % faster on average)
162 development (Åhällan and Ålgrundet). On May 10th 2013, we sampled 10 egg clutches from
163 each island except Ålgrundet, were only 6 clutches were found. The clutches were sampled
164 from 1-3 pools depending on the island, but when more than one pool was sampled, pools
165 were always within 10 m of each other. The clutches were in identical developmental stage
166 and were transported to the laboratory at Uppsala University where the experiment was
167 performed in a walk-in climatic room. Each clutch was assumed to represent one female, as
168 female *R. temporaria* lay only one egg clump per season (Savage, 1961) and will hereafter be
169 referred to as a sibship.

170 Egg clutches were kept at 12°C (light: dark cycle of 18 h : 6 h) in 3 litre plastic
171 containers filled with water until tadpoles had developed to Gosner stage 25 (Gosner, 1960).
172 At this stage we started the experiment by individually introducing 6 individuals from each
173 sibship into plastic containers (9.5 cm x 9.5 cm, height 10 cm) filled with 750 mL of
174 reconstituted soft water (RSW; APHA 1985). For three individuals per sibship we simulated
175 pool drying by lowering water level in the containers by 33% every fourth day, starting at day
176 5 and continued until day 25, after which the water volume was kept constant at 66 ml. This
177 simulated pool drying conditions allowed us to compare morphology between an induced
178 accelerated developmental time and the constitutive developmental time achieved at the
179 constant water level, i.e. developmental plasticity. Each individual was placed in a randomly
180 chosen place in the constant-temperature room to reduce effects of small temperature
181 differences observed inside the room. The constant water level treatment allowed us to
182 compare development and morphology among islands under a controlled “common garden”
183 condition, reducing environmental variance among populations, and revealing the genetic
184 variance among them. The water was changed every fourth day, and tadpoles were fed

185 chopped spinach: with 0.045g (± 0.005 SD) dry spinach weight during first week, increased to
186 0.071 \pm 0.009 the second week and to 0.097 \pm 0.011 from the third week until the end of the
187 experiment. When the forelimbs emerged at Gosner stage 42 water level in the containers was
188 reduced to 100 ml and a wet terrestrial habitat was provided for the metamorphosed froglets
189 until the end of the experiment.

190 The experiment ended when individuals had reached Gosner stage 46, i.e. the tailed
191 resorbed, since at this stage metamorphosis is completed and larval period is over. At this
192 stage froglets were anaesthetised with immersion in an aqueous solution of MS222 and
193 ventrally photographed from a standardized distance together with a scale. We estimated
194 morphological traits from these photographs using the software ImageJ (Abramoff, 2004).
195 The morphological traits measured were: snout-vent length (SVL), head width (HW), head
196 length (HL) femur length (FeL), fibio-tibula length (TL) and foot length (FoL) (see Richter-
197 Boix et al. 2006a for details). SVL was used as an overall measure of body size; other
198 metamorphic traits were size-corrected. For size-corrected traits we first regressed each trait
199 on SVL and retrieved the trait residuals, which reflected whether individual traits were
200 relatively short or long for their size. Our experimental design does not allow us to take
201 maternal effects of traits into account. However, maternal genetic variance in life history traits
202 are low in *R. temporaria* (Laugen et al., 2005), and Lind & Johansson (2007) showed that it
203 was only 5 % in the Storhaddingen population, one of the populations used in the current
204 study.

205

206 ***Morphological comparisons among species***

207 For this comparative study we included 22 species of ranid frogs belonging to five different
208 genera. Ranidae is one of the most widespread frog families in the world, ranging across all

209 continents except Antarctica, and in a wide range of habitats encompassing wetlands in
210 deserts, tundra, boreal- and deciduous forests, and breeding in marshes, streams, pools and
211 lakes (Frost et al., 2006).

212 The species included are found across Europe, North America, Asia, and Africa,
213 where they occupy a wide array of aquatic habitats, from temporary pools and ditches, to
214 lakes and streams (Table 1). Some of the species included are rather habitat specialists (e.g.
215 stream dwelling *Rana pyrenaica* or vernal pool specialist *Rana sylvatica*) whereas other
216 species exploit diverse habitats (e.g. *Rana temporaria*, *Rana sphenocephala*). Therefore,
217 morphology in these species is likely to have evolved under selection driven by various
218 environmental factors including predation, diet, temperature, competition, or water current, in
219 addition to the influence that developmental rate may or may not have exerted over it. To
220 conduct explicit tests regarding the possible association of habitat preference and
221 morphology, we coded each species as belonging to either one of three categories: typical of
222 lentic systems (pool, lakes, swamps, flooded plains), restricted to lotic systems (streams and
223 rivers), or found in both.

224 Morphological measurements were obtained from specimens deposited at the Museo
225 Nacional de Ciencias Naturales (Madrid, Spain) and at Estación Biológica de Doñana
226 (Seville, Spain). Using callipers, we took the same morphological measurements from
227 ethanol-preserved specimens as those taken for experimental individuals. The number of
228 individuals studied for each species was determined by specimen availability in the
229 collections visited, and varied between 1-10 (Table 2), with an average of two populations
230 included per species. Only adults were measured for the study, the majority of which were
231 males, as they tend to be overrepresented in museum collections given the biased sex ratios
232 observed in the field during reproduction for these species. The original measurements,
233 together with museum identifiers are given in supplementary Table S1. Duration of the larval

234 period for each species was obtained from the literature and the public database
235 AmphibiaWeb (<http://amphibiaweb.org>, accessed March 2014). This search resulted in a
236 range of values for duration of larval period for each species, encompassing natural
237 observations from field guides to controlled laboratory experiments. For each species we used
238 the range median for phylogenetic analyses.

239

240 *Statistical analyses*

241 *Laboratory Experiments: influence of developmental rate plasticity on morphology*

242 To test if average pool duration in each island affected developmental time, we ran a general
243 linear model (GLM) using island category as a factor with two levels (permanent or
244 temporary) and population as random factor nested within island category. Estimates of
245 average pool duration were taken from Lind and Johansson (2007). We used a one tailed
246 hypothesis for this test since we predicted that developmental time should be shorter on
247 islands with temporary pools. Because island distribution across the hydroperiod did not show
248 a perfect segregation between well-defined transitions (the island Åhällan did not behave as a
249 temporary pool island with regard to developmental time as in our past studies: larval
250 developmental time was almost as long for this island as it was for the permanent islands) we
251 also analysed if developmental plasticity and morphology differed among islands considering
252 pool duration as a continuum. For this we fitted a model using treatment and island as fixed
253 effects (since island choice was made after their average pond duration), and developmental
254 time and morphology as dependent variables. Finally, to examine the relation between
255 development time and morphology among islands, we also run a linear regression of
256 morphological traits against developmental time in individuals subjected to constant water
257 using each island as a replicate. Given the multiple test performed on morphological traits, we

258 applied the Benjamini and Hochberg (BD) false discovery rate (Benjamini and Hochberg
259 1995) with the library *sgof* (Carvajal-Rodriguez et al. 2009) in R. For all other analyses we
260 used the *nlme* library (Pinheiro et al., 2008) in R (version 2010.1, Core Development Team)
261 using the *corCompSymm* argument in the *gls* function.

262

263

264 ***Morphological comparisons among species***

265 To determine if developmental time of species affects their morphology we performed a
266 comparative phylogenetic analysis. We obtained a time-calibrated phylogeny of 22 ranid
267 species (Fig. 2) for which we could collect matching data on six morphological traits and on
268 duration of the larval period, by trimming down the large-scale phylogeny of Pyron and
269 Wiens (2013). All morphological traits were log-transformed prior to analyses. Given that all
270 morphological variables were susceptible of measurement error, we obtained size-corrected
271 length estimates for femur length, fibio-tibula length, foot length, total hindlimb, head length
272 and head width, extracting the residuals of phylogenetic reduced major axis regressions
273 (RMA) between each of these variables and snout-to-vent length. These models were fit using
274 *phyl.RMA* from the *phytools* package (Revell, 2010). We then used the resulting residuals as
275 size-corrected variables in subsequent analyses.

276 We estimated the phylogenetic signal for each of the morphological traits and for
277 larval period calculating Blomberg's K and Pagel's λ using the *phylosig* function from the
278 *phytools* package (Revell, 2011). We then tested alternative models of evolution of
279 developmental rate and morphological traits. We used *fitContinuous* in the *ape* package to
280 compare three models of trait evolution: a Brownian Motion (BM) model where trait variation

281 is fully explained by phylogenetic distances; an Ornstein-Uhlenbeck model (OU), where traits
282 were modelled to have varied under stabilizing selection around a single optimum); and an
283 estimated λ model where the level of phylogenetic signal is estimated. We compared the
284 goodness of fit of these models by means of their corrected Akaike Information Criterion
285 parameter (AICc). Differences in AICc between competing models were considered
286 negligible if < 3 , moderately strong between 4 and 7, and very strong if > 10 (Anderson &
287 Burnham, 2002). We then performed analyses using the best-fitting model. We tested for
288 phylogenetic associations between duration of the larval period and body size, and between
289 larval period and size-corrected morphological traits through phylogenetic generalized linear
290 models (PGLS) using the *ppls* function from the *caper* package (Orme et al., 2012) using the
291 complete data set. PGLS analyses were conducted allowing estimation of the λ parameter
292 through maximum likelihood. Finally, in contrast to similar studies on other amphibian
293 groups, ranid species differ broadly in habitat use. As an alternative hypothesis of
294 morphological evolution associated to accommodated differences in larval period among
295 species, we also tested if habitat type (lentic, lotic, or both) explained morphological
296 differences among species using phylogenetic analyses of variance using the *phylANOVA*
297 function from the *phytools* package (Revell, 2011). This analysis thus compares
298 morphological differences among species across habitats, using species as data points and
299 habitat as factor.

300 We are currently lacking experimental determination of developmental plasticity for
301 most of the ranid species included in this analysis. However, the duration of the larval period
302 is highly plastic across even distantly related species (Gomez-Mestre & Buchholz 2006;
303 Gomez-Mestre et al. 2010; Richter-Boix et al 2011), and therefore it is safe to assume that
304 plasticity is ancestral to most, if not all, anurans and could have the potential to affect
305 morphological diversification.

306

307 RESULTS

308 *Laboratory Experiments*

309 Populations from temporary islands (Åhällan and Ålgrundet), tended to have shorter
310 developmental times (Fig 3), and a generalized linear model with island category (permanent
311 and temporary) as fixed factor suggested a trend for a faster development in islands with
312 temporary pools ($F_{1,3,119} = 3.69$, $P = 0.07$). A mixed model ANOVA using island (population)
313 as fixed factor rather than island category, showed that developmental time differed among
314 populations and that our water level treatment affected developmental time significantly so
315 that tadpoles under drying water conditions significantly shortened their time to
316 metamorphosis (Table 3, Fig 3).

317 There was a trend for a significant differences in body length among populations, and
318 the island Ålgrundet, which had temporary pools, showed the shortest body length, being 5%
319 shorter than those of the permanent pool island Lill Haddingen which had the longest body
320 length (Table 3, Fig 4). Tadpoles raised under simulated drying pool conditions showed a
321 significantly shorter body length (Table 3), being on average 9 % shorter. There was no
322 significant interaction between populations and pool drying.

323 Relative head length differed significantly between islands with the Lill Haddingen
324 population having the shortest relative head width and being 9% shorter than that of the
325 temporary pool island Ålgrundet, which had the longest head width (Table 3, Fig. 4).
326 Treatment had significant effect on head length with simulated pool drying condition causing
327 longer head lengths in 2 populations and shorter in 3 populations. However, the interaction
328 effect between the two factors was not significant.

329 Relative head width differed significantly among islands and the island Petlandsskär
330 having permanent pools, had the narrowest head, with a 6% shorter head width than that of
331 the temporary pool island Ålgrundet which had the longest (Table 3, Fig. 4). There was no
332 significant effect related to treatment and no significant interaction between populations and
333 treatment.

334 Relative femur length and relative fibio-tibula length showed no significant difference
335 among islands (Table 3, Fig 4). In contrast, simulated pool drying conditions caused a
336 significant treatment effect in those traits, with shorter relative femur (8%) and relative fibio-
337 tibula (8%) under decreasing water conditions. There was no significant island x treatment
338 interaction effect.

339 Relative foot length differed significantly among islands and the permanent pool
340 island Lill Haddingen had the longest foot length, being 23% longer than that of the
341 temporary pool island Ålgrundet, (Table 3, Fig. 4). Treatment had a significant effect on foot
342 length; being 10% shorter under decreasing water conditions. No significant interaction
343 between populations and treatment was found. After adjusting p-values for multiple
344 comparisons the effect of treatment was non-significant.

345 The linear regression between morphological traits and development using island as
346 replicate showed no significant relationship for any of the five morphological traits (relative
347 head length: $P = 0.165$, $r^2 = 0.53$; relative head width: $P = 0.06$, $r^2 = 0.74$; relative femur
348 length: $P = 0.48$, $r^2 = 0.17$; relative fibio-tibula length: $P = 0.76$, $r^2 = 0.04$; relative foot length:
349 $P = 0.87$, $r^2 = 0.02$).

350 Overall, all populations accelerated development in response to reduced water levels
351 but did so to a similar extent across all populations. Populations differed in their mean
352 duration of the larval period. Developmental acceleration resulted in changes in morphology

353 within population, but environmentally induced morphological variation was not mirrored by
354 differences among populations.

355

356 *Comparison among species*

357 We detected substantial phylogenetic signal for most of the morphological traits measured,
358 but not for larval period, body length or head width (Table 4). Estimated lambda and OU
359 models showed a better fit than Brownian models for the evolution of morphological traits in
360 all cases, with differences in AIC in favour of the lambda and OU models being low for foot
361 and head length, very strong for femur and overall hindlimb length and head width, and
362 moderate for the rest. Since goodness of fit was nearly identical for OU and estimated lambda
363 models with very slight improvement towards lambda-estimated models, we opted for these
364 latter for further phylogenetic least squares tests of trait associations. However, we found no
365 significant relationships between any of the size-corrected morphological traits and
366 developmental time (all $P > 0.20$). Phylogenetic ANOVAs, however, indicated that head
367 length ($F=9.545$, $P=0.002$) and foot length ($F=4.697$, $P=0.046$) varied significantly across
368 habitat types with fibio-tibula showing the same trend, albeit with a marginally non-
369 significant result ($F = 3.976$, $P=0.064$), (Fig 5). Species specialised in lotic environments
370 tended to have a more elongated shape, with longer fibiotibulas, longer feet, and greater head
371 length. All other morphological traits failed to show any variation among habitat types.

372

373 DISCUSSION

374 We found that our island populations responded to simulated pool drying by speeding up their
375 development (Fig. 3). Such acceleration in development is adaptive because it allows tadpoles

376 to metamorphose before the pools dry up. This adaptive response has been shown previously
377 in this species (Laurila & Kujasalo, 1999; Lind & Johansson, 2007), and other amphibians
378 (reviewed in Richter-Boix et al., 2011). The accelerated development resulted in shorter limb
379 length for the three limb traits. In contrast, head length and width showed no consistent
380 pattern of variation among populations. The morphological changes we found for limb length
381 is typical for frogs subjected to simulated pool drying conditions (Richter Boix et al. 2006,
382 Johansson & Richter-Boix 2013). In contrast head width does not show a consistent pattern
383 across study species because wider as well as narrower responses to pool drying have been
384 found (Tejedo et al., 2010).

385

386 *Mirrored pattern within and among populations*

387 We predicted that, if evolved through genetic accommodation, the morphological changes
388 caused by developmental acceleration in response to simulated pool drying would be mirrored
389 across populations locally adapted to divergent hydroperiods. Specifically, we predicted that
390 populations adapted to longer lasting ponds, and hence with longer developmental times,
391 typically would have relatively longer limbs and narrower and/or longer heads than those
392 having faster developmental rates. We found that islands differed significantly in
393 developmental time and there was a trend for a significant shorter developmental time in
394 islands with temporary pools as has been shown in previous studies of these islands (Lind &
395 Johansson, 2007). Island populations also showed differences in morphology, but we found
396 no association between morphology and developmental rate among populations. Therefore,
397 although the observed among-islands differentiation in developmental rate is congruent with
398 genetic accommodation of ancestral developmental plasticity, such accommodation of larval
399 period does not seem to have caused a concomitant accommodation of morphological plastic

400 changes. For example, the island Ålgrundet, which has temporary pools and showed the
401 fastest development under constant water conditions, did not show a consistent shorter limb
402 length for any of the three limb traits measured compared to the other populations. Neither did
403 this population show a shorter head length, as often induced by pool drying. Similarly, the
404 permanent pool island Petlandsskär, which had a long developmental time, showed a short
405 relative foot length compared to the other permanent islands. Thus, we found morphological
406 differentiation among islands, but it was seemingly unrelated to differences in developmental
407 rate.

408 The lack of mirrored pattern between plastic responses within population and variation
409 among populations is unlikely to be due to a low number of islands being considered. In 2010
410 we performed a similar experiment using three other islands from the same archipelago that
411 also differ in pool permanence. That study also showed that the plastic responses to simulated
412 pool drying within islands were not mirrored across island with regard to morphology,
413 although the developmental response was mirrored (see figure S1 in supplementary material).
414 Altogether, these results suggest that larval life-history traits and frog morphological traits are
415 evolutionarily dissociated and with capacity to respond independently.

416

417 *Among species*

418 We also predicted that the morphological changes caused by phenotypic plasticity responses
419 to simulated pool drying should be mirrored across species such that species with longer
420 development typically should have longer limbs and narrower and/or longer heads than those
421 having faster developmental rates. Gomez-Mestre and Buchholz (2006) observed that plastic
422 responses in developmental rate within spadefoot toad species were mirrored in among
423 species differences. In a study comparing within and among populations of Eurasian

424 spadefoot toads Johansson and Richter-Boix (2013) found that morphological changes
425 induced by decreasing water level was mirrored among populations of *Pelodytes punctatus*
426 that differed in breeding pool hydroperiod and developmental rate. In the first study
427 developmental acceleration was induced by temperature and in the latter by pool drying, but
428 both environmental stimuli would enhance differentiation over growth, triggering an early
429 metamorphosis and were thus expected to result in the same morphological consequences
430 (Gomez-Mestre et al., 2010). Interestingly, both studies (Gomez-Mestre & Buchholz, 2006;
431 Johansson & Richter-Boix, 2013) were conducted on pelobatoids frogs, which included
432 scaphiopodids (North American spadefoot toads), pelobatids (Eurasian spadefoot toads) and
433 pelodytids (Eurasian parsley frogs) (Frost et al., 2006; Pyron & Wiens 2011), whereas our
434 study was performed on ranids. One potential explanation for the absence of support in our
435 study could be that ranid frogs do not experience as extreme variation in pool duration among
436 species as pelobatid frogs do. Consequently, larval period does not vary among the ranid
437 species included here to the extent that it varies among spadefoot toads and parsley frogs
438 (Buchholz & Hayes 2002; Zeng et al. 2014). In consequence, a very large sample size of
439 ranids would be needed to find the mirrored pattern found in pelobatoid frogs. An alternative
440 explanation for absence of a mirrored pattern is that selection on juvenile and adult
441 morphology could be masking the relationship between larval period and morphology,
442 perhaps through compensatory growth in the post-metamorphic stage. Relative shorter limbs
443 are a consequence of growing and developing under stressful conditions like pool drying, but
444 under certain environmental conditions it might be adaptive in the adult stage, or simply
445 evolve neutrally.

446 Limb length in frogs is related to mode of locomotion (Emerson 1978) and climate
447 conditions (Vidal-García et al. 2014). Shorter legs are common in species that walk or in
448 burrowing species (Vidal-García et al. 2014), whereas long limbs are common in species

449 inhabiting very wet environments, to enhance swimming ability (James and Wilson 2008), or
450 in riparian and stream breeders to resist high flow rates (Lewis and Rohweder 2005; Hoskin
451 2010). Though we did not find a relationship between development time and morphology in
452 our comparative analyses, we found an association between morphology and habitat type,
453 despite the low number of species in our analysis. Species specialised in lotic systems tended
454 to have longer feet and fibio-tibula, and greater head lengths than species typical of lentic
455 systems or than generalist species that occupy both lentic and slow-flowing lotic systems.
456 Therefore, in at least the ranid species sampled, selective factors, presumably operating on
457 locomotion and prey choice, seem to have had a stronger evolutionary effect on frog
458 morphology than evolutionary divergences in developmental rate. Under this scenario even if
459 species breed and develop in temporary pools and faster development is selected for, the
460 juvenile morphology will not be genetically accommodated if there is strong selection for an
461 alternative phenotype (Pigliucci et al. 2006; Braendle & Flatt 2006). That is, a mirrored
462 pattern in plasticity among populations or species would only be expected when the plastic
463 morphological trait is adaptive, or neutral and linked to an adaptive trait (West-Eberhard,
464 2003; Gomez-Mestre & Buchholz, 2006).

465

466 *Summary*

467 We found no strong link between morphology and development within, among and
468 population or among species of ranid frogs, and thus no support for genetic accommodation in
469 the organism group studied. The absence of a strong pattern partly supports the adaptive
470 decoupling hypothesis (Hanken, 1992; Moran, 1994) that predicts that larval and adult traits
471 should be independent of each other, and that metamorphosis has evolved to unlink distinct
472 stages and allows their independent adaptation to distinct niches (Moran 1994). However,

473 such decoupling is far from complete and organisms cannot escape carry-over effects of the
474 growing conditions experienced, as shown here in the form of morphological changes due to
475 developmental acceleration induced by pond drying. Future studies about phenotypic
476 integration across stages and independence between developmental modules will help us
477 understand internal constraints in morphology, and evolution and diversification of organisms
478 with complex life cycles, and reveal how and why patterns differ among organism groups,
479 and whether these differences are due to adaptation or phylogenetic constraints.

480

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486

487

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647 Table 1. Species used in the comparative analyses and their distribution and habitat use.
 648 Species names follow AmphibiaWeb (accessed Nov 2014; <http://amphibiaweb.org/>).

Species	Region	Breeding	Description
<i>Fejervarya cancrivora</i>	SE Asia	lentic	Mangrove swamps, edges of tidalprawn pools, and in fresh water areas.
<i>Hylarana galamensis</i>	Central Africa	lentic	Permanent deep waters in the Savannah
<i>Pelophylax bedriagae</i>	E Mediterran	lentic	Shallow, sheltered water bodies
<i>Pelophylax nigromaculatus</i>	Eastern Asia	lentic	Including river pools, channels, lakes, pools, swamps, ditches and rice fields
<i>Pelophylax perezii</i>	SW Europe	both	Both lotic and lentic waters
<i>Pelophylax ridibundus</i>	C and E Europe	both	A wide variety of flowing and stagnant water habitats, from shallow puddles and pools to large lakes and rivers, as well as mountain streams
<i>Pelophylax saharicus</i>	NW Africa	both	Lakes, pools and puddles to flowing streams and rivers
<i>Ptychadena mascareniensis</i>	C Africa	lentic	Temporary pools, car tracks, road ditches and swamps
<i>Rana areolata</i>	SE N. America	lentic	Shallow ditches, temporary pools, flooded overflows from small streams, pasture pools, and prairie wetlands
<i>Rana arvalis</i>	N Europe and W Asia	lentic	Shallow, well-warmed pools
<i>Rana catesbeiana</i>	N. America (introduced elsewhere)	lentic	Vegetation choked shallows of permanent bodies of water
<i>Rana clamitans</i>	E N. America	lentic	Shorelines of lakes and permanent wetlands such as pools, bogs, fens, marshes, swamps, and streams.
<i>Rana dalmatina</i>	C and SE Europe	both	Well-illuminated and warm swamps in the forest or on forest edges. In the south of its range, the species reproduces not only in stagnant but also

			in slowly running waters
<i>Rana graeca</i>	SE Europe	lotic	Streams and springs
<i>Rana iberica</i>	NW Iberia	lotic	Cold streams and small rivers with preference for places with abundant riparian vegetation
<i>Rana macrocnemis</i>	Caucasus and SW Turkmenia	both	Pools, lakes, swamps, ditches and stream pools with stagnant and semi-flowing water
<i>Rana palustris</i>	E N. America	lentic	Woodland pools and pools, stream overflow pools, farm pools, sinkhole pools, floodplain wetlands, marshes, and flooded quarries
<i>Rana pipiens</i>	C N. America	both	Quiet or slow-moving water along streams and rivers, wetlands associated with lakes or tidal areas, permanent or temporary pools, beaver pools, and human-constructed habitats such as borrow pits, agriculture, and cattle pools
<i>Rana pyrenaica</i>	Pyrenees	lotic	Stream dwellers that do not inhabit pools or lakes
<i>Rana sphenocephala</i>	E N. America	both	Temporary pools, pools, lakes, ditches, irrigation channels, and stream and river edges
<i>Rana sylvatica</i>	N N. America	lentic	Ephemeral woodland pools
<i>Rana temporaria</i>	Europe and NW Asia		Quite diverse habitats: under forest cover, in glades bushlands, dry and swampy meadows, swamps and different kinds of anthropogenic landscape

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657 Table 2. Development time and morphology for the species used in the comparative analyses.
 658 The figure after the species name denotes number of individuals from which morphological
 659 measurements were taken.

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Species	DTime	SVL	HeadL	HeadW	FemL	TibiaL	FootL
<i>Fejervarya cancrivora</i> (3)	77	62.10	22.92	21.15	26.48	30.32	17.43
<i>Hylarana galamensis</i> (2)	120	37.30	15.12	13.87	66.20	16.75	8.82
<i>Pelophylax bedriagae</i> (6)	62	54.38	20.34	19.52	25.60	26.14	14.00
<i>Pelophylax nigromaculatus</i> (1)	90	70.70	27.40	26.25	34.10	35.75	18.35
<i>Pelophylax perezii</i> (10)	70	64.255	24.15	22.65	30.825	34.74	18.37
<i>Pelophylax ridibundus</i> (10)	90	155.83	57.57	54.15	73.88	80.64	43.99
<i>Pelophylax saharicus</i> (10)	120	232.46	86.77	80.36	110.62	118.84	64.49
<i>Ptychadena mascareniensis</i> (10)	63	59.48	24.09	18.32	28.4	37.73	16.6
<i>Rana areolata</i> (1)	69	64.25	25.10	25.75	26.7	30.9	17.5
<i>Rana arvalis</i> (7)	57.8	45.80	16.95	15.28	22.42	24.64	13.58
<i>Rana catesbeiana</i> (7)	135	96.07	31.73	37.17	43.10	44.78	26.51
<i>Rana clamitans</i> (9)	94.5	66.52	23.49	24.14	30.91	32.46	18.5
<i>Rana dalmatina</i> (10)	110	52.99	17.86	17.98	30.37	33.44	17.6
<i>Rana graeca</i> (3)	75	62.30	19.65	23.21	35.70	38.71	19.83
<i>Rana iberica</i> (10)	90	50.22	16.40	16.97	26.34	29.97	15.27
<i>Rana macrocnemis</i> (7)	90	97.72	36.97	32.66	48.60	53.21	28.8
<i>Rana palustris</i> (3)	75	37.41	15.30	12.63	18.75	20.80	10.65
<i>Rana pipiens</i> (2)	120	44.27	15.20	15.57	22.35	24.62	13.02
<i>Rana pyrenaica</i> (2)	62.5	47.17	20.25	15.47	25.20	28.65	15.27
<i>Rana sphenoccephala</i> (4)	97.5	50.13	18.70	17.71	25.57	27.2	15.08
<i>Rana sylvatica</i> (10)	66.5	56.21	20.10	20.07	28.76	31.16	16.49
<i>Rana temporaria</i> (5)	70.2	60.69	21.4	20.79	31.48	33.88	18.64

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662 Table 3. Mixed model ANOVA for larval development time, body length, relative head
663 length, relative head width, relative femur length, relative tibia length, and relative foot
664 length. When we adjusted our p-values for multiple comparisons on the morphological traits
665 using the Benjamini and Hochberg (BD) false discovery rate (Benjamini and Hochberg 1995,
666 the critical value for a * difference became 0.007, and hence the two ** effects disappeared.

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669 Trait	Model term	df	F-value	P-value
670 Larval period	Island population	4	3.287	0.0121**
	Treatment	1	37.685	<0.0001***
	Island × Treatment	4	1.142	0.3374
673 Body length	Island population	4	2.21	0.0683
	Treatment	1	125.61	<0.0001***
	Island × Treatment	4	0.91	0.4564
677 Head length	Island population	4	10.168	<0.0001***
	Treatment	1	12.996	0.0004***
	Island × Treatment	4	1.4971	0.2040
681 Head width	Island population	4	6.107	0.0001***
	Treatment	1	1.439	0.2315
	Island × Treatment	4	1.476	0.2103
685 Femur length	Island population	4	0.9097	0.4590
	Treatment	1	25.511	<0.0001***
	Island × Treatment	4	0.2814	0.8898
689 Fibio-tibula length	Island population	4	2.2504	0.0646
	Treatment	1	34.240	<0.0001***
	Island × Treatment	4	1.2075	0.3084
693 Foot length	Island population	4	22.370	<0.0001***
	Treatment	1	6.109	0.0142**
	Island × Treatment	4	1.268	0.2831

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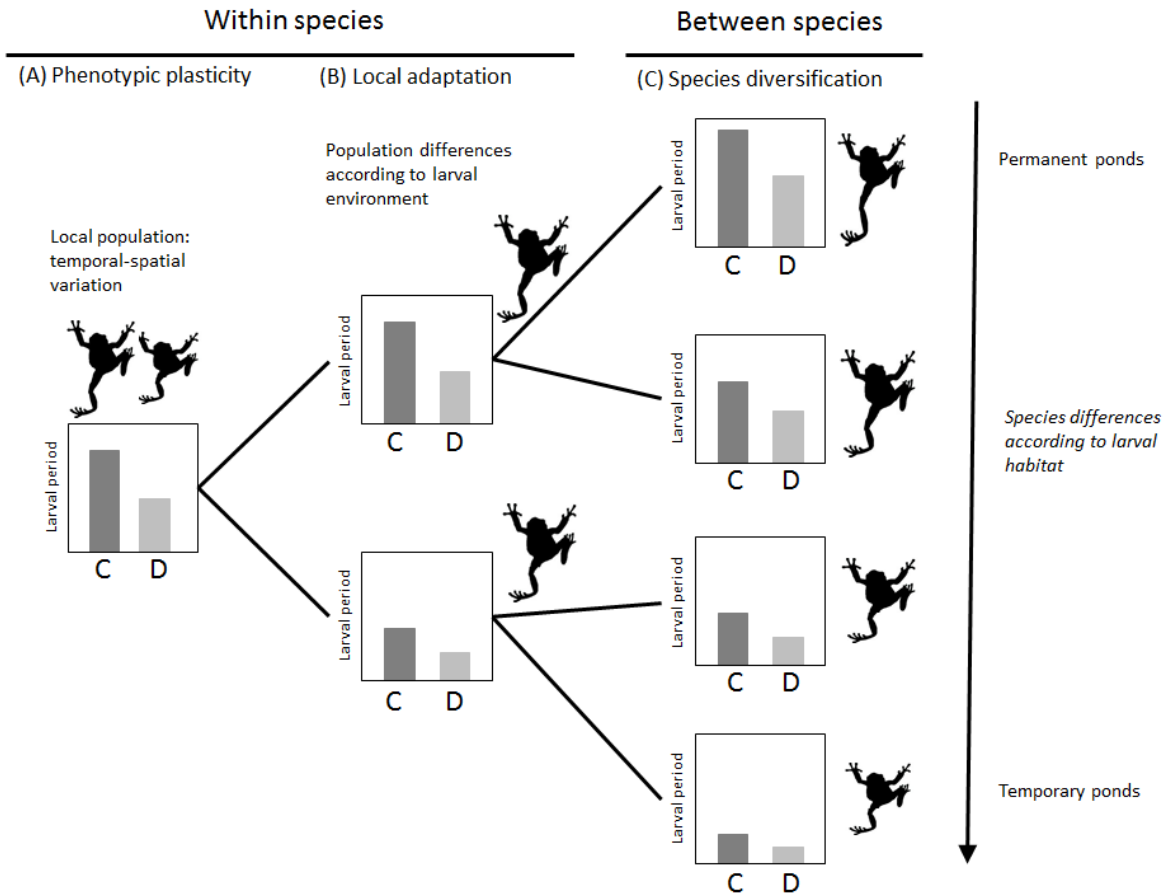
698 Table 4. Summary statistics of phylogenetic signal (estimated by Blomberg's K and Pagel's λ ,
699 see text) and goodness of fit of alternative models of evolution for each dependent variable
700 considered. We provide the Aikaike Information Criterion (AIC) for the competing models,
701 including a Brownian Motion model, an Orstein-Uhlenbeck model, and an estimated λ model.
702 OU and estimated λ models were rather similar, with a slight improvement of OU in a few
703 cases. Consequently, we chose to conduct all comparative analyses assuming an OU model.

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705 Trait	K	Pagel's λ	BM (AIC)	OU (AIC)	λ (AIC)
706					
707 Larval period	0.538	0	11.212	6.703	6.707
708 Snout-vent-length	0.495	0	9.570	3.543	3.545
709 Femur length	0.916	1.070	23.862	14.625	14.627
710 Fibio-tibula	0.826	0.868	-30.265 -	33.631	-33.813
711 Foot length	0.970	0.849	-56.706	-54.796	-54.705
712 Hind limb	0.867	1.136	-9.794	-20.099	-20.084
713 Head length	1.047	0.785	-41.841	-43.089	-44.348
714 Head width	0.648	0	-52.526	-64.458	-64.458

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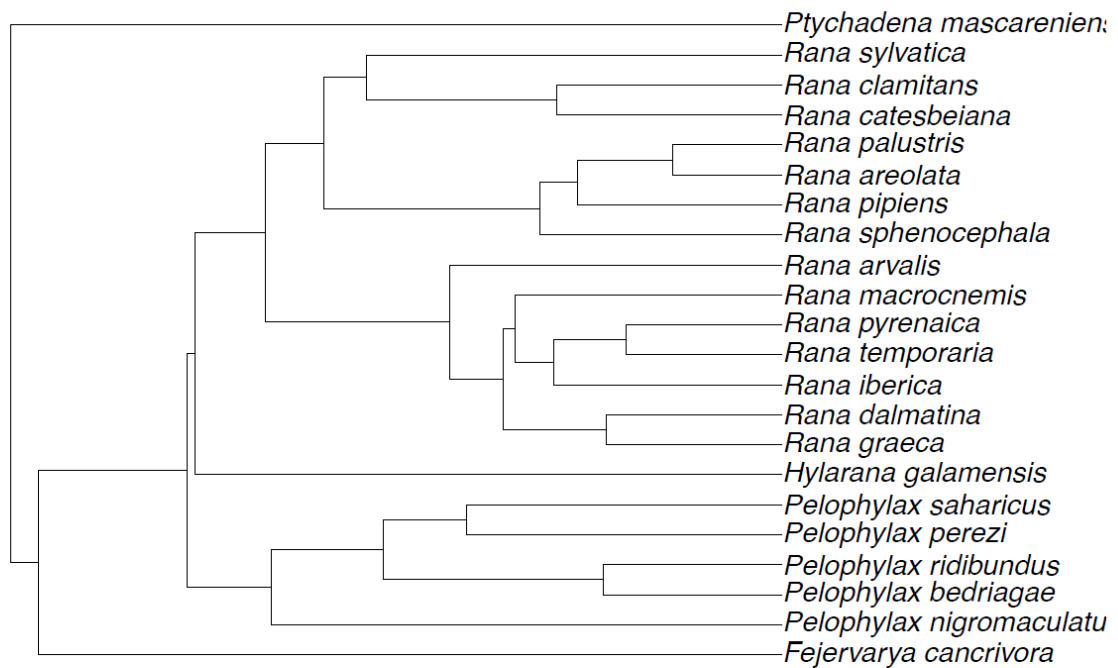
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720 Fig. 1. Schematics of how developmental plasticity might allow populations and species to
721 adapt along a gradient of pool drying, and the expected associated changes in morphology
722 (compare morphology of frog cartoons). (A) Plasticity in developmental rate seems to be
723 widespread in amphibians: larvae accelerate metamorphosis when faced with risk of
724 desiccation. Such developmental acceleration, however, results in shorter hind limbs and
725 either shorter or wider heads. (B) Selection acting on populations adapting to divergent
726 hydroperiods (i.e. to either ephemeral or permanent pools) may drive differences in
727 developmental rate but responsiveness to environmental stimuli may be kept, such that during
728 adaptation, plasticity can be increased or decreased. (C) As environments stabilise in
729 divergent conditions plasticity can become canalised among speciating lineages, and
730 ultimately result in trait divergence among species. C and D in figures denotes constant and
731 decreasing water levels respectively during development time (larval period).

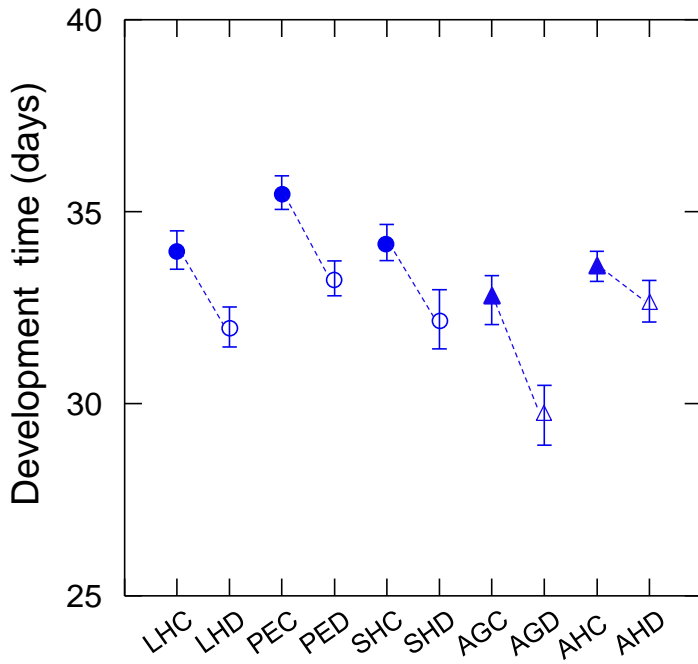
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735 Fig. 2. Time-calibrated phylogeny indicating the relationships among the frog species
 736 included in our comparative analyses. The tree was extracted from Pyron and Wiens (2013),
 737 trimming down from a large-scale phylogeny including 2871 amphibian species.

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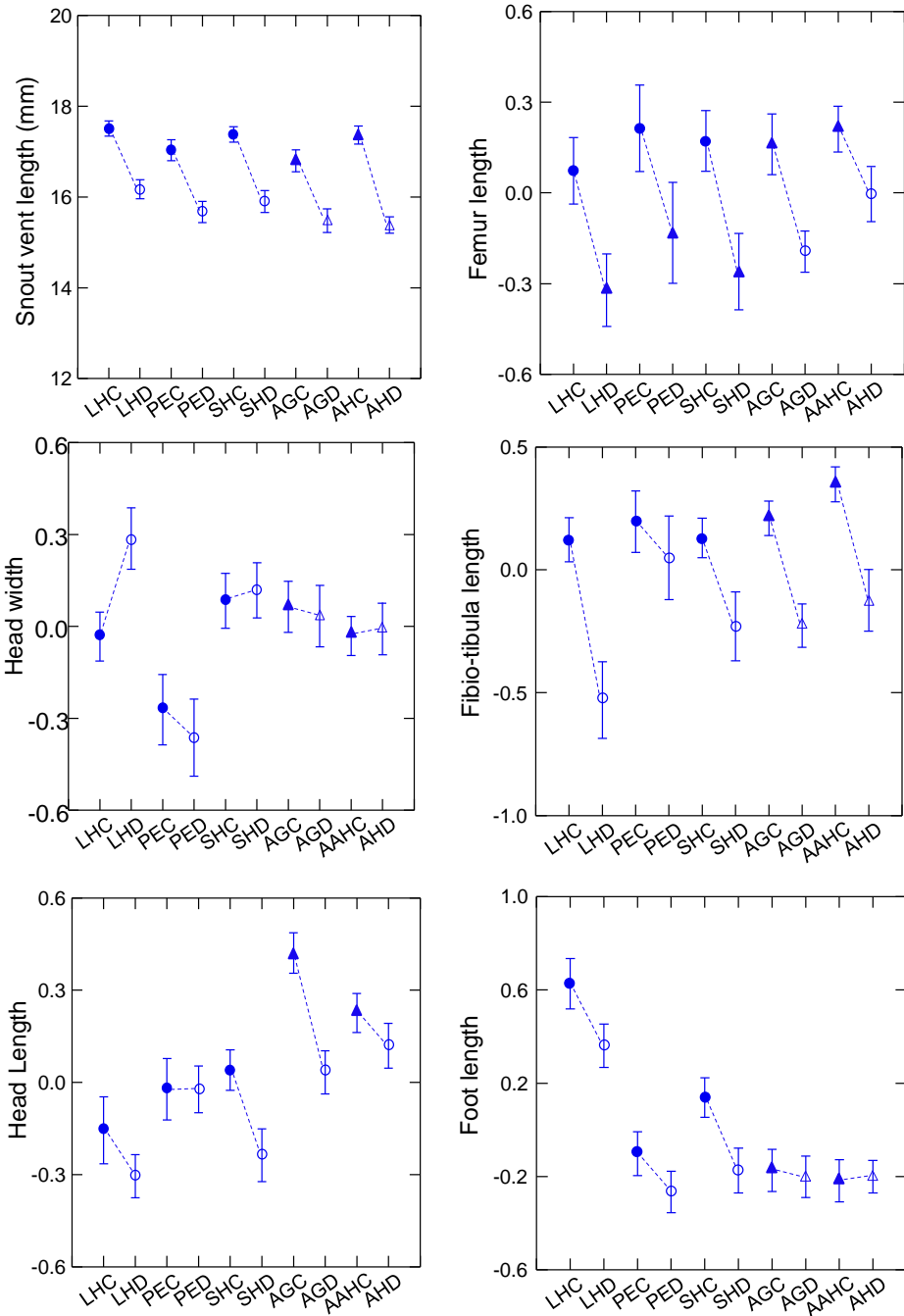


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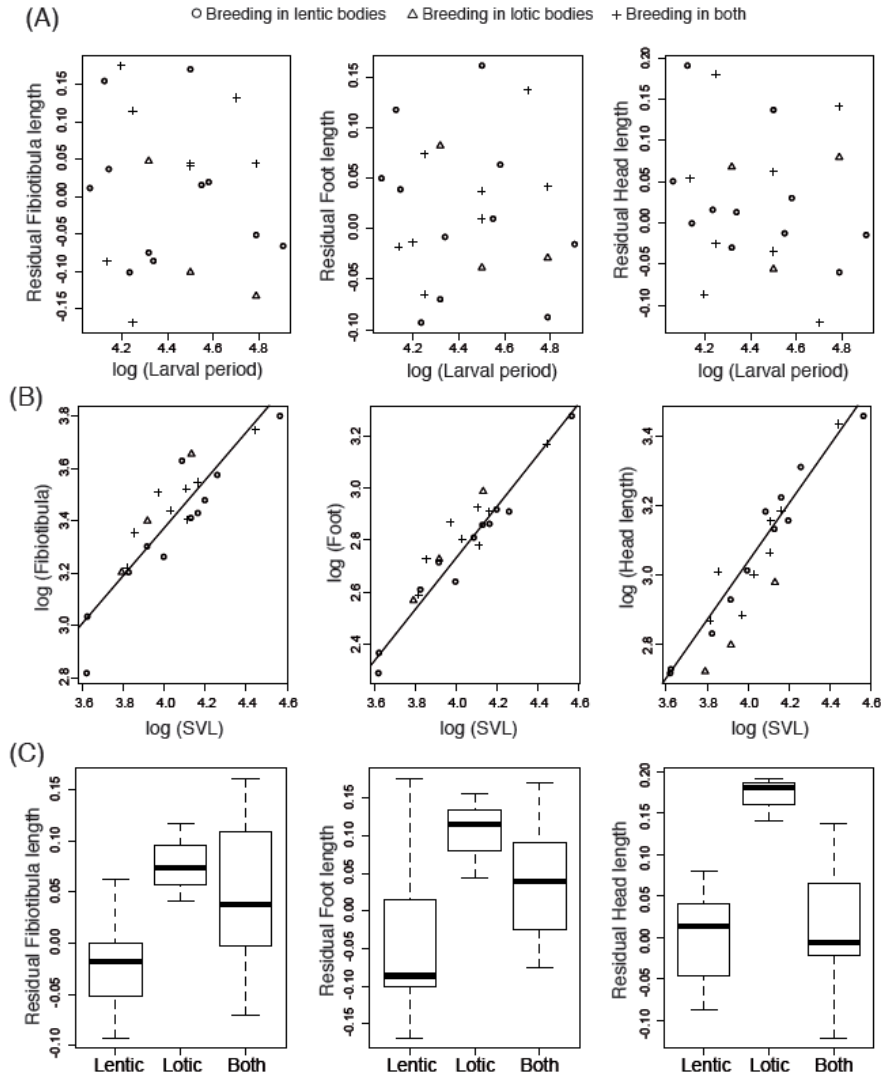
741 Figure 3. Larval development time for individuals from island population raised under constant and
 742 decreasing water level. Dots are islands with permanent pools and triangles islands with temporary
 743 pools: Lillhadingen (LH), Petlandsskär (PE), Stor Haddingen (SH), Ålgrundet (AG) and Åhällan (AH).
 744 Filled and open symbols denote constant and decreasing water level conditions and dotted lines are
 745 drawn to connect these two conditions for each island and the slope of the lines represents the amount
 746 of phenotypic plasticity. Error bars denote S.E.

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750 Figure 4. Snouth vent length, relative head width, relative head length, relative femur length, relative,
 751 fibio-tiula length, and relative foot length for individuals from island population raised under constant
 752 and decreasing water level. The relative lengths and widths are residuals, see text. Dots are islands
 753 with permanent pools and triangles islands with temporary pools: Lillhadingen (LH), Petlandsskär
 754 (PE), Stor Haddingen (SH), Ålgrundet (AG) and Åhällan AH). Filled and open symbols denote
 755 constant and decreasing water level conditions and dotted lines are drawn to connect these two
 756 conditions for each island and the slope of the lines represents the amount of phenotypic plasticity.
 757 Error bars denote S.E.



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760 Figure 5. Relationships among morphology, larval period, and habitat type in 22 ranid species studied.

761 (A) Phylogenetic general least square regressions between larval period and size-adjusted

762 morphological traits. The plotted values from morphological traits are residuals from phylogenetic

763 reduced major axis (RMA) regressions between each original trait and snout-to-vent length. Dots, plus

764 and triangles denote lentic, both and lotic species respectively. We found no evidence for an effect of

765 developmental rate on among-species morphology. (B) Phylogenetic least square regressions between

766 morphological traits and snout-to-vent length. (C) Boxplots showing differences among species

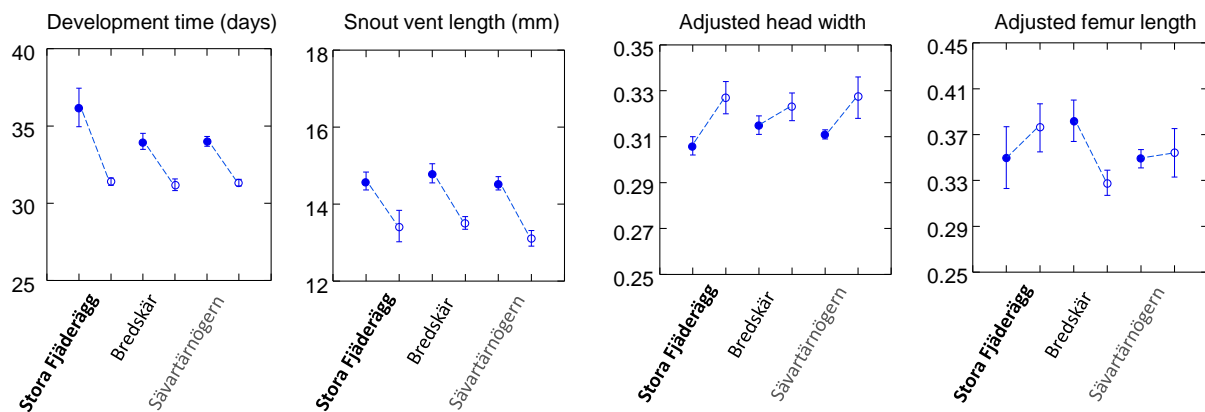
767 grouped as being specialists of lentic environments, lotic environments, or occupying both types of

768 habitats. Species from lotic environments tended to have a more elongated shape, with longer

769 fibiotibulas, longer feet, and greater head length.

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775 Figure S1. Development time to metamorphoses, snout vent length, relative head width and relative
 776 femur length of tadpoles raised under constant (filled dots) and simulated (open dots) pool drying
 777 condition in the laboratory. Islands differ in their pool permanence with the island Stora Fjäderägg
 778 having permanent pools, Sävertärnögern temporary pools, and Bredskär pools with intermediate pool
 779 drying. Results show that tadpoles from the island Stora Fjäderägg with permanent pools has the
 780 longest development time and that simulated pool drying results in a shorter development time. Note
 781 that the simulated pool drying which cause shorter development time and changes in morphology is
 782 not mirrored among populations which differ in development time. For example Stora Fjäderägg has a
 783 longer development time than the other islands but still the islands do not differ in morphology at
 784 constant water conditions from the other two islands. Similarly, simulated pool drying conditions in
 785 the laboratory cause different response in relative femur length among populations. Error bars denote
 786 standard error.

788 Table S1. Identifier and morphology of all individuals used for comparison of species.

IDENTIFIER	SPECIES	COLLECTION	LOCALITY	COUNTRY	SVL	HEADL	HEADW	FEMURR	TIBR	FOOTR
B-8795	Hylarana_albolabris	EBD	San Joaquin de Ndyacom	Guinea	64.50	24.70	21.00	27.70	32.00	18.50
B-8794	Hylarana_albolabris	EBD	San Joaquin de Ndyacom	Guinea	67.10	23.30	20.90	28.10	33.55	19.10
B8995	Hylarana_albolabris	EBD	San Joaquin de Ndyacom	Guinea	73.10	29.10	23.90	34.70	36.60	19.80
B8641	Hylarana_albolabris	EBD	San Joaquin de Ndyacom	Guinea	55.00	17.20	16.55	28.35	29.95	16.85
18632	Hylarana_albolabris	EBD	Miboman km27	Guinea	51.70	17.30	16.60	23.85	29.50	16.45
18624	Hylarana_albolabris	EBD	Miboman km28	Guinea	61.45	20.05	18.85	25.00	35.00	19.00
18631	Hylarana_albolabris	EBD	Miboman km29	Guinea	51.75	15.80	16.20	22.10	28.15	16.10
18625	Hylarana_albolabris	EBD	Miboman km30	Guinea	48.50	17.55	15.50	21.70	25.15	14.65
18630	Hylarana_albolabris	EBD	Miboman km31	Guinea	53.80	17.75	16.70	22.95	31.40	17.05
18628	Hylarana_albolabris	EBD	Miboman km32	Guinea	50.30	16.70	15.45	24.40	27.30	15.15
3860	Hylarana_erythraea	MNCN	Isla de Samar	Philippines	41.50	15.90	13.50	21.15	23.65	12.90
3859	Hylarana_erythraea	MNCN	Isla de Samar	Philippines	39.65	16.35	12.10	22.10	26.10	12.95
3862	Hylarana_erythraea	MNCN	Isla de Samar	Philippines	33.55	12.90	10.10	18.40	20.00	10.00
3861	Hylarana_erythraea	MNCN	Isla de Samar	Philippines	38.80	15.10	11.45	19.15	22.70	12.40
3858	Hylarana_erythraea	MNCN	Isla de Samar	Philippines	39.35	15.30	11.75	20.25	21.65	12.20
3863	Hylarana_erythraea	MNCN	Isla de Samar	Philippines	32.80	12.20	9.90	17.15	18.95	10.35
18395	Hylarana_malabarica	EBD	Akurenan	Guinea	64.25	24.85	18.85	36.35	40.15	20.25
18463	Hylarana_malabarica	EBD	Akurenan	Guinea	49.15	19.60	14.00	25.40	29.05	14.85
18399	Hylarana_malabarica	EBD	Akurenan	Guinea	63.85	22.75	19.35	36.00	41.35	20.40
18397	Hylarana_malabarica	EBD	Akurenan	Guinea	48.40	19.00	15.25	25.50	30.75	15.30
18398	Hylarana_malabarica	EBD	Akurenan	Guinea	61.95	24.70	18.90	34.90	38.20	18.90
18436	Hylarana_malabarica	EBD	Akurenan	Guinea	45.00	19.45	14.90	25.15	27.50	14.35
18439	Hylarana_malabarica	EBD	Akurenan	Guinea	47.75	18.95	14.10	24.55	29.15	15.25
18400	Hylarana_malabarica	EBD	Akurenan	Guinea	63.60	22.35	18.85	34.50	39.75	19.60
18434	Hylarana_malabarica	EBD	Akurenan	Guinea	58.40	21.70	17.65	31.45	35.40	18.00
18396	Hylarana_malabarica	EBD	Akurenan	Guinea	63.25	24.75	18.75	34.45	36.30	19.75
9209	Lithobates_catesbeianus	EBD	Vt. Windsor Co	USA	110.15	34.30	43.00	46.30	52.70	30.50
9211	Lithobates_catesbeianus	EBD	Vt. Windsor Co	USA	108.65	27.45	43.85	44.25	49.10	29.25
9195	Lithobates_catesbeianus	EBD	Vt. Windsor Co	USA	68.60	24.40	26.20	31.75	30.70	18.85
40305	Lithobates_catesbeianus	MNCN	Welsford, Queens	Canada	99.90	33.50	37.85	48.85	46.85	27.75
40304	Lithobates_catesbeianus	MNCN	Welsford, Queens	Canada	89.90	33.75	36.10	40.70	41.90	25.10
3914	Lithobates_catesbeianus	MNCN		Cuba	64.15	23.15	22.45	32.30	30.80	16.90
3920	Lithobates_catesbeianus	MNCN		USA	131.20	45.60	50.80	57.55	61.45	37.25
9218	Lithobates_clamitans	EBD	Vt. Windsor Co	USA	63.60	22.20	25.45	27.10	30.25	17.50
9085	Lithobates_clamitans	EBD	Vt. Windsor Co	USA	61.60	21.85	24.90	28.95	30.50	17.95

9214	Lithobates_clamitans	EBD	Vt. Windsor Co	USA	71.90	25.50	26.70	29.60	34.55	19.45
9083	Lithobates_clamitans	EBD	Vt. Windsor Co	USA	82.35	27.30	27.45	38.80	38.80	22.35
9201	Lithobates_clamitans	EBD	Vt. Windsor Co	USA	70.00	25.85	27.15	33.30	32.70	19.00
11457	Lithobates_clamitans	MNCN	Fayette, Pensilvania	USA	54.85	20.40	18.55	27.20	28.90	14.75
11458	Lithobates_clamitans	MNCN	Fayette, Pensilvania	USA	51.35	20.75	19.25	25.05	27.10	14.80
3947	Lithobates_clamitans	MNCN		USA	65.65	20.75	21.50	27.60	31.05	17.75
26956	Lithobates_clamitans	MNCN	Welsford, Queens	Canada	77.40	26.85	26.35	40.60	38.30	22.95
45048	Lithobates_palmipes	MNCN			91.35	38.45	34.55	43.25	46.05	25.40
3579	Pelophylax_bedriagae	MNCN	Anatolia Central	Turkey	52.10	19.50	18.35	24.50	25.30	13.30
3576	Pelophylax_bedriagae	MNCN	Puerto Topakli	Turkey	46.20	18.30	16.00	22.05	22.05	11.15
3575	Pelophylax_bedriagae	MNCN	Puerto Topakli	Turkey	41.10	15.90	15.00	20.35	21.80	11.20
3600	Pelophylax_bedriagae	MNCN	Lago Aksehir	Turkey	111.15	39.75	40.35	51.10	51.70	29.50
3732	Pelophylax_bedriagae	MNCN	Iago Balik	Turkey	38.05	14.05	13.70	17.40	17.25	9.25
3734	Pelophylax_bedriagae	MNCN	Iago Balik	Turkey	37.70	14.55	13.75	18.25	18.75	9.65
17179	Pelophylax_nigromaculatus	MNCN	Lago Taesonh-ho,Pyongyang Sur	North Corea	70.70	27.40	26.25	34.10	35.75	18.35
27084	Pelophylax_perezi	EBD	Granada	Spain	60.20	22.00	21.60	27.55	32.55	17.80
27091	Pelophylax_perezi	EBD	Granada	Spain	84.55	30.05	30.15	39.30	46.15	24.40
27085	Pelophylax_perezi	EBD	Granada	Spain	63.55	23.15	22.15	28.20	33.45	17.45
27083	Pelophylax_perezi	EBD	Granada	Spain	61.10	24.30	21.40	31.05	33.95	18.60
27086	Pelophylax_perezi	EBD	Granada	Spain	57.55	21.55	19.90	27.95	30.40	15.60
27092	Pelophylax_perezi	EBD	Granada	Spain	76.75	28.40	27.00	38.25	39.60	21.70
27089	Pelophylax_perezi	EBD	Granada	Spain	80.55	29.75	28.90	40.95	42.55	22.60
25193	Pelophylax_perezi	EBD	Grimaldo, Caceres	Spain	52.90	20.60	17.70	21.05	29.40	15.05
25195	Pelophylax_perezi	EBD	Grimaldo, Caceres	Spain	51.40	20.75	18.40	28.00	29.05	15.15
25194	Pelophylax_perezi	EBD	Grimaldo, Caceres	Spain	54.00	21.00	19.30	25.95	30.30	15.35
19070	Pelophylax_ridibundus	MNCN	Rio Usuchezaj, Dagestan	Russia	94.35	32.85	34.20	43.00	48.15	26.60
19071	Pelophylax_ridibundus	MNCN	Rio Usuchezaj, Dagestan	Russia	99.00	34.05	34.90	44.40	46.00	25.55
19067	Pelophylax_ridibundus	MNCN	Rio Usuchezaj, Dagestan	Russia	76.80	30.80	27.10	40.60	40.55	21.80
19072	Pelophylax_ridibundus	MNCN	Rio Usuchezaj, Dagestan	Russia	111.90	34.60	36.80	48.10	50.80	29.25
19068	Pelophylax_ridibundus	MNCN	Rio Usuchezaj, Dagestan	Russia	90.70	34.45	29.40	42.95	42.80	23.90
19069	Pelophylax_ridibundus	MNCN	Rio Usuchezaj, Dagestan	Russia	77.45	32.05	26.90	36.50	42.20	23.30
17188	Pelophylax_ridibundus	MNCN	Montes Witoscha	Bulgaria	77.50	28.65	25.10	35.95	36.90	22.75
17190	Pelophylax_ridibundus	MNCN	Bakonypeterd, Veszprem	Hungary	81.10	29.80	30.05	39.50	42.75	23.30
17185	Pelophylax_ridibundus	MNCN	Rawnopole, Sofia	Bulgaria	67.40	24.95	22.80	32.95	36.05	19.85
17186	Pelophylax_ridibundus	MNCN	Rawnopole, Sofia	Bulgaria	75.30	27.85	25.15	35.85	38.15	21.55
20102	Pelophylax_saharicus	EBD	Laachaaich	Morocco	66.50	26.15	20.50	29.90	31.15	17.25
20100	Pelophylax_saharicus	EBD	Laachaaich	Morocco	54.25	22.45	19.00	24.65	27.95	14.80
20101	Pelophylax_saharicus	EBD	Laachaaich	Morocco	57.35	23.30	20.95	29.25	30.85	16.00
20099	Pelophylax_saharicus	EBD	Laachaaich	Morocco	48.30	20.60	17.25	25.05	23.80	12.80
19507	Pelophylax_saharicus	EBD	Ouel-Laoud	Morocco	64.70	22.30	22.45	30.80	31.50	16.45
19508	Pelophylax_saharicus	EBD	Ouel-Laoud	Morocco	45.20	17.70	15.70	21.75	22.60	11.95

19105	Pelophylax_saharicus	EBD	Ajmata-Abjelil	Morocco	75.60	26.80	25.50	37.60	36.70	20.10
19106	Pelophylax_saharicus	EBD	Ajmata-Abjelil	Morocco	67.75	25.70	23.45	32.70	32.15	17.65
19108	Pelophylax_saharicus	EBD	Ajmata-Abjelil	Morocco	57.50	22.50	18.70	27.65	29.40	15.10
19110	Pelophylax_saharicus	EBD	Ajmata-Abjelil	Morocco	73.35	26.95	24.40	34.10	35.25	18.95
27892	Ptychadena_mascareniensis	EBD		Guinea	62.65	22.00	19.40	25.60	40.90	19.80
27890	Ptychadena_mascareniensis	EBD		Guinea	61.05	26.30	19.45	28.35	43.05	13.25
27391	Ptychadena_mascareniensis	EBD		Guinea	63.20	24.10	16.20	29.55	41.55	12.45
27883	Ptychadena_mascareniensis	EBD		Guinea	65.95	26.30	19.50	33.30	38.85	18.10
27894	Ptychadena_mascareniensis	EBD		Guinea	59.85	25.20	21.20	31.10	41.40	14.40
27881	Ptychadena_mascareniensis	EBD		Guinea	59.30	24.40	20.15	28.50	38.50	19.55
27835	Ptychadena_mascareniensis	EBD		Guinea	48.35	20.70	14.65	25.10	30.85	15.20
27893	Ptychadena_mascareniensis	EBD		Guinea	48.40	19.80	14.30	22.00	28.80	14.85
27888	Ptychadena_mascareniensis	EBD		Guinea	62.50	25.90	18.90	27.90	37.15	20.10
27898	Ptychadena_mascareniensis	EBD		Guinea	63.60	26.25	19.45	32.60	36.30	18.30
21647	Rana_dalmatina	EBD	Conzieu, Rhône	France	52.35	18.45	17.60	31.00	32.75	16.20
21646	Rana_dalmatina	EBD			50.60	17.15	16.75	31.55	31.80	16.70
15983	Rana_dalmatina	EBD	Castelfalfi, Pisa	Italy	47.00	15.40	15.30	25.70	29.90	16.10
15981	Rana_dalmatina	EBD	Castelfalfi, Pisa		43.60	15.40	14.80	23.95	28.50	13.70
	Rana_dalmatina	EBD		France	50.70	15.85	17.50	29.90	32.75	17.60
	Rana_dalmatina	EBD		France	49.90	17.15	16.45	29.60	32.00	17.15
7104033	Rana_dalmatina	EBD		France	53.45	17.55	16.30	30.35	32.75	17.35
	Rana_dalmatina	EBD		France	54.25	19.70	19.80	30.75	32.85	18.10
21832	Rana_dalmatina	EBD	Inoso, Alava	Spain	64.65	22.70	22.80	37.30	41.15	21.70
21830	Rana_dalmatina	EBD	Inoso, Alava	Spain	63.45	19.30	22.55	33.60	40.00	21.40
17870	Rana_iberica	EBD	Fiolleda, Lugo	Spain	47.85	16.80	16.45	23.85	28.70	14.85
700821	Rana_iberica	EBD	Incio, Lugo	Spain	46.00	16.90	15.90	25.55	29.20	15.80
680508	Rana_iberica	EBD	Xantes, Lugo	Spain	47.60	13.65	17.65	25.45	27.60	14.30
	Rana_iberica	EBD	Incio, Lugo	Spain	44.60	16.20	15.30	25.40	27.10	14.90
71040935	Rana_iberica	EBD	Incio, Lugo	Spain	58.90	19.35	20.25	26.55	34.45	17.55
71082317	Rana_iberica	EBD	Incio, Lugo	Spain	57.90	17.25	20.05	32.65	34.75	17.10
73100409	Rana_iberica	EBD	Seoane de Caurel	Spain	51.00	16.40	16.75	26.55	30.00	14.65
71040941	Rana_iberica	EBD	Incio, Lugo	Spain	52.25	18.20	16.80	27.10	31.35	16.00
7107221	Rana_iberica	EBD	Villalba, Lugo	Spain	42.05	14.65	14.05	21.75	24.25	12.10
71040938	Rana_iberica	EBD	Incio, Lugo	Spain	54.10	14.65	16.55	28.55	32.35	15.50
28472	Rana_maculata	EBD		Nicaragua	55.60	20.10	19.75	33.10	34.05	17.55
28471	Rana_maculata	EBD		Nicaragua	66.85	25.80	23.35	34.40	38.90	19.60
26971	Rana_pyrenaica	MNCN	Torla, Huesca	Spain	44.95	15.80	15.05	21.95	25.90	13.40
26970	Rana_pyrenaica	MNCN	Fanlo, Huesca	Spain	43.60	14.60	16.10	22.75	23.35	12.65
R1810	Rana_temporaria	EBD	Rilo, La Coruña	Spain	47.75	16.90	16.30	25.80	27.55	13.25
R1826	Rana_temporaria	EBD	Rilo, La Coruña	Spain	45.20	15.65	15.90	24.55	26.20	13.95
R1827	Rana_temporaria	EBD	Rilo, La Coruña	Spain	49.60	16.90	16.85	26.20	29.35	14.60

R1806	Rana_temporaria	EBD	Rilo, La Coruña	Spain	52.35	18.70	18.10	29.10	30.05	15.75
R1815	Rana_temporaria	EBD	Rilo, La Coruña	Spain	51.50	18.60	17.60	24.95	30.00	15.15
13739	Rana_temporaria	EBD	Ucieda, Santander	Spain	59.90	19.85	19.70	28.10	29.05	15.80
	Rana_temporaria	EBD	Ucieda, Santander	Spain	53.70	22.90	22.55	27.50	34.30	18.65
13743	Rana_temporaria	EBD	Ucieda, Santander	Spain	75.30	27.45	28.55	40.60	39.55	22.45
13742	Rana_temporaria	EBD	Ucieda, Santander	Spain	65.35	22.65	23.80	31.35	34.25	18.55
13741	Rana_temporaria	EBD	Ucieda, Santander	Spain	61.50	21.45	21.40	29.50	31.30	16.80
11420	Lithobates_palustris	MNCN	Powdermill, Pensilvania	USA	41.75	16.00	14.30	21.85	24.05	12.25
11419	Lithobates_palustris	MNCN	Powdermill, Pensilvania	USA	36.50	15.80	12.60	17.95	19.20	9.65
3948	Lithobates_palustris	MNCN		USA	34.00	14.10	11.00	16.45	19.15	10.05
19448	Lithobates_pipiens	MNCN	North Carolina	USA	55.35	18.20	20.25	29.95	31.35	17.20
19447	Lithobates_pipiens	MNCN	North Carolina	USA	63.85	20.95	22.25	34.75	36.85	20.80
19449	Lithobates_pipiens	MNCN	North Carolina	USA	57.00	21.15	20.20	27.90	33.35	17.75
19451	Lithobates_pipiens	MNCN	North Carolina	USA	64.20	25.25	20.15	32.65	33.90	18.75
19450	Lithobates_pipiens	MNCN	North Carolina	USA	63.05	21.45	21.10	32.15	33.95	18.70
17171	Lithobates_aureolatus	MNCN	Wawchitka, Florida	USA	64.25	25.10	25.75	26.70	30.90	17.50
3874	Fejervarya_cancrivora	MNCN	Isla de Samar	Philippines	56.10	20.50	18.70	22.60	27.45	15.55
3873	Fejervarya_cancrivora	MNCN	Isla de Samar	Philippines	64.60	24.85	22.45	27.55	32.40	18.70
3875	Fejervarya_cancrivora	MNCN	Isla de Basilan	Philippines	65.60	23.40	22.30	29.30	31.10	18.05
15610	Rana_sphenocephala	MNCN		USA	47.25	21.30	16.50	26.90	29.90	15.70
15611	Rana_sphenocephala	MNCN		USA	47.10	19.20	14.45	23.50	27.40	14.85
30806	Rana_sylvatica	MNCN	Doaktown, New Brunswick	Canada	47.80	17.90	17.30	24.10	25.45	14.40
30807	Rana_sylvatica	MNCN	Doaktown, New Brunswick	Canada	46.10	16.05	16.60	23.70	25.10	13.45
11442	Rana_sylvatica	MNCN	Westmoreland, Pensilvania	USA	56.70	21.60	19.35	27.60	30.10	16.40
11441	Rana_sylvatica	MNCN	Westmoreland, Pensilvania	USA	49.95	19.25	17.60	26.90	28.15	16.10
44076	Hylarana_galamensis	MNCN	Simenti, Tambacounda	Senegal	36.65	14.15	13.45	115.50	15.80	9.40
44075	Hylarana_galamensis	MNCN	Simenti, Tambacounda	Senegal	37.95	16.10	14.30	16.90	17.70	10.30
3996	Hylarana_chalconota	MNCN	Bulacan, Isla de Luzon	Philippines	57.20	22.00	18.65	32.15	35.75	19.10
3737	Merystogenys_jerboa	MNCN	Montes Carin,Carin Bia-Po	Myanmar	49.80	19.00	12.05	35.05	35.85	17.00
4020	Amolops_afghanus	MNCN	Montes Carin,Carin Bia-Po	Myanmar	39.50	15.35	13.10	21.50	23.40	11.90
4018	Amolops_afghanus	MNCN	Montes Carin,Carin Bia-Po	Myanmar	42.80	17.40	14.75	22.25	24.45	12.95
4017	Amolops_afghanus	MNCN	Montes Carin,Carin Bia-Po	Myanmar	40.75	16.75	13.75	22.05	25.05	12.70
4019	Amolops_afghanus	MNCN	Montes Carin,Carin Bia-Po	Myanmar	42.40	16.95	14.90	23.40	24.95	12.85
3893	Rana_arvalis	MNCN	Berlin	Germany	50.35	18.25	16.60	25.65	25.75	15.00
17174	Rana_arvalis	MNCN	Mandal	Norway	66.80	21.30	20.45	28.00	32.60	19.05
3881	Rana_arvalis	MNCN	Budapest	Hungary	42.35	16.10	15.65	23.25	27.00	13.70
3880	Rana_arvalis	MNCN	Budapest	Hungary	47.85	18.10	17.50	25.45	29.50	15.70
3879	Rana_arvalis	MNCN	Budapest	Hungary	37.10	15.10	13.20	19.05	21.85	11.45
3882	Rana_arvalis	MNCN	Budapest	Hungary	40.95	17.65	12.20	21.10	21.75	12.15
15594	Rana_arvalis	MNCN	Tonsberg	Norway	35.20	12.20	11.40	14.45	14.05	8.05
19094	Rana_macrocnemis	MNCN	Rio Samur, Samur	Azerbaijan	49.70	19.35	16.10	22.95	27.40	14.20

19093	Rana_macrocnemis	MNCN	Rio Samur, Samur	Azerbaijan	44.90	16.85	15.65	23.25	26.95	13.70
19096	Rana_macrocnemis	MNCN	Rio Rubas, Cirtich, Dagestan	Russia	42.35	16.80	13.75	20.60	22.80	12.15
19074	Rana_macrocnemis	MNCN	Rio Usuchezaj, Dagestan	Russia	47.45	19.40	15.25	23.25	26.10	13.40
19073	Rana_macrocnemis	MNCN	Rio Usuchezaj, Dagestan	Russia	46.95	18.10	15.85	25.00	26.50	14.25
17172	Rana_macrocnemis	MNCN	Montes Erciyes Dagi, Kayseri	Turkey	42.25	15.95	15.10	22.10	22.70	12.60
17173	Rana_macrocnemis	MNCN	Montes Erciyes Dagi, Kayseri	Turkey	44.10	16.75	14.70	23.70	22.90	12.95
17177	Rana_graeca	MNCN	Zelenigrad	Bulgaria	59.60	18.50	20.00	29.70	33.80	16.85
17176	Rana_graeca	MNCN	Zelenigrad	Bulgaria	69.55	21.00	27.15	41.85	43.90	22.40
17178	Rana_graeca	MNCN	Zelenigrad	Bulgaria	57.75	19.45	22.50	35.55	38.45	20.25
19177	Rana_chensinensis	MNCN	Lago Khasan	Russia	52.20	17.20	17.30	24.10	24.70	14.05
19178	Rana_chensinensis	MNCN	Lago Khasan	Russia	59.50	21.10	17.30	25.30	26.40	15.25
7007	Hylarana_nigrovittata	MNCN			51.20	18.60	15.50	24.45	26.70	14.45
7008	Hylarana_nigrovittata	MNCN			51.70	20.30	15.05	24.10	26.60	14.85

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