

# Reproduction, development and morphological plasticity in the direct-developing frog *Pristimantis rosadoi*

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**Abstract.** We report on three clutches with 13, 28 and 33 eggs, respectively, obtained from three *Pristimantis rosadoi* couples, caught in the wet Ecuadorian Chocó region. Embryos developed from two clutches and are illustrated herein. One clutch matured and 25 froglets hatched simultaneously after 21 days. The hatching of froglets, with different amounts of yolk remaining, indicates that this was a response to perceived predation risk and thus indicates plasticity in hatching time. We also observed a distinct change from rough to smooth dorsal skin in two of the three females, after disengaging from amplexus. Our observations on reproduction and morphological change are compared to data known from other species of this genus.

Key words. Amphibia, Anura, Strabomantidae, Chocó, clutch size, direct development, Ecuador, embryos, rainforest, skin texture.

**Resumen.** Reportamos tres puestas de huevos con 13, 28 y 33 huevos, obtenidos de tres parejas de *Pristimantis rosadoi* en amplexo, capturadas en el bosque húmedo de la región Chocó ecuatoriano. Los embriones de dos puestas se desarrollaron y se ilustran aquí. De una de las puestas, 25 ranas diminutas eclosionaron después de 21 días. La eclosión simultánea de ranas, con diferentes cantidades de vitelo restante, indica que esto fue una respuesta al riesgo percibido de depredación, lo que sugiere cierta plasticidad en el tiempo de eclosión. Además, observamos un cambio distintivo en la piel dorsal de dos de las tres hembras, después del amplexo la piel pasó de rugosa a lisa. Nuestras observaciones sobre reproducción y cambio morfológico se comparan con datos conocidos de otras especies de este género.

# Introduction

With about 600 valid species (FROST 2023), the strabomantid anuran genus *Pristimantis* is the most species-rich genus of vertebrates (ACEVEDO et al. 2022), and many more undescribed species are expected to be discovered (REYES-PUIG & MANCERO 2022, SZÉKELY et al. 2023). In Ecuador, this genus accounts for one third of all anuran species (RON et al. 2020, CARRIÓN-OLMEDO & RON 2021). Like all other genera in the family Strabomantidae, the members of *Pristimantis* are assumed to be direct developers, i.e., that their course of metamorphosis lacks the stage of free-living tadpoles. Instead, lecithotrophic embryos develop right into froglets that will hatch from the eggs, which are deposited in moist terrestrial or arboreal substrates (WELLS 2007, HEDGES et al. 2008, DUELLMAN & LEHR 2009). Most *Pristimantis* species seem to live in very moist habitats, predominantly tropical rain and cloud forests, where adults of many species perch at night on lower shrubs and trees (e.g., ARROYO et al. 2008, WALDEZ et al. 2011, MORALES MITE et al. 2013, FALCÓN-ESPITIA et al. 2023). These habitats are under constant threat from habitat fragmentation, degradation, and conversion, as well as from climate change (PE-RES et al. 2006, MOSANDL et al. 2008, RAY 2013, HANSEN et al. 2013, GONZALEZ-JARAMILLO et al. 2016, Global Forest Watch 2023).

Species susceptibility to environmental change may be correlated with specific trait states, such as body size or re-

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productive potential and strategies (HIRSCHFELD & RÖDEL 2017, Ruland & Jeschke 2017, Tietje & Rödel 2018, Gon-ZÁLEZ-DEL-PLIEGO et al. 2019). The knowledge of the biology of individual species may thus help judging the probability and severity of threats and aid in conservation management (BURY 2006, FODEN et al. 2013, MICHAELS et al. 2014, GALLAGHER et al. 2021, BORGELT et al. 2022). Unfortunately, surprisingly little is known about the biology of the numerous Pristimantis species, and reproductive data seem to have been published only for very few of them (see, e.g., citations in QUINTEROS-MUÑOZ & AGUAYO 2022). Recently a large study investigated the 'ecological and evolutionary trends of body size' in the genus Pristimantis (ACE-VEDO et al. 2022), and another study reported on the unexpected ability of an Ecuadorian species to quickly change its skin morphology (GUAYASAMIN et al. 2015), with both studies indicating that there remains much to learn about the biology of these frogs.

Thus, it seems advisable to gather and document biological data of the many species from which almost nothing is known. In the course of a study investigating anuran communities in the wet tropical Chocó of Ecuador (www. reassembly.de), we therefore also made notes on random observations on the biology of the various frog species encountered, including those of the genus *Pristimantis*. One of these species is *Pristimantis rosadoi* (FLORES, 1988) for which YÁNEZ-MUÑOZ et al. (2022) summarized the few known biological facts. In the following we report further reproductive details and some observations on skin morphology change.

#### Materials and methods

Species identification was based on the descriptions of this species published by FLORES (1988), LYNCH & DUELLMAN (1997) and YÁNEZ-MUÑOZ et al. (2022), as well as additional photos published in STUART et al. (2008) and ORTE-GA-ANDRADE et al. (2010). The small species (snout-vent length of males 16–19 mm, of females: 23–27 mm) has been recorded from rainforests in three northwestern Ecuadorian provinces and adjacent Colombian sites, below 800 m altitude in the foothills of the Andes, as well as off the Colombian coast on Isla Gorgona. The species seems to be rare, and currently is classified as Vulnerable in the global Red List of the IUCN, as well as in the Ecuadorian Red List of amphibians (CISNEROS-HEREDIA et al. 2004, STUART et al. 2008, ORTEGA-ANDRADE et al. 2010, 2021, YÁNEZ-MU-ÑOZ et al. 2022).

*Pristimantis rosadoi* couples (Fig. 1) were encountered during fieldwork in September 2022 and March 2023 in the Canandé Reserve, in wet tropical Chocó, close to the Chocó Lab and Lodge, Ecuador (coordinates: 0.5263, -79.2130; altitude: ca. 300 m a.s.l.), managed by the 'Fundación Jocotoco' (www.jocotoco.org.ec). This area is characterized by a mosaic of habitats ranging from pastures and plantations (often cocoa), via various stages of regenerating forests, to old-growth rainforests, in a hilly landscape with steep slopes. The annual mean temperature is 24°C, and annual precipitation sums up to about 2100 mm (BLÜTHGEN et al. 2021). Our research was not targeting this species and its reproductive biology, and the respective observations are based on random opportunities.

Upon detecting a pair in amplexus at night, we took in-situ photographs, then encouraged the couple to hop into a transparent plastic jar (height 10 cm, diameter 5 cm), filled to 1/3 with damp moss and leaf litter, and closed it with a foam plug. The frogs were then transported to the field station and kept in the dark until the next morning. Once they had deposited a clutch, we took the frogs out of the plastic jar and measured them. All adults were subsequently released at their respective original capture sites. Measures of snout–vent length (SVL) of adult frogs and egg diameter were taken with a calliper to the nearest 10<sup>th</sup> of a mm. Hatched froglets were placed on millimetre paper and their body lengths taken from photos. Weight was measured with an electronic scale (Ohaus Scout STX223, accuracy  $\pm$  0.001 g).

Because of other fieldwork, we could not monitor egg development on a daily basis, and as we tried to keep disturbances to a minimum, we mostly checked clutches for mouldy and dead eggs only, and added some water when necessary. During development (see below) we examined eggs twice under a dissecting microscope (Motic SMZ 168). Development was evaluated against the staging table provided by TOWNSEND & STEWART (1985) for Eleutherodactylus coqui (these authors also listed earlier papers concerning the embryonic development of Neotropical anurans). Otherwise the clutches remained on, and partly covered with, damp moss. The first clutch was kept in a plastic terrarium at the ambient temperature and light regime of the environment. Because this clutch did not develop, we kept the following clutches in the original plastic jars, placed in the dark within a cabinet of the Chocó Lab. Temperature there usually was around 23°C, but increased when air-conditioning was not available due to power failures, or decreased when colleagues overlooked our instruction notes and programmed the air-con differently (range not measured but presumably between 19 and 26°C). Juveniles were released at the site were we had collected their parents.

# Results

On 6 September 2022, we encountered a *P. rosadoi* couple (male 16.6 mm SVL; female 24.0 mm) at about midnight, sitting approximately at 50 cm height on a leaf (Fig. 1a). The surrounding habitat was old-growth lowland rainforest on a steep slope, about 30 m above a small torrential stream (coordinates: 0.5261, -79.2118). The couple had separated by the next morning (Figs 1c, d), and we found 13 uniform-ly yellow eggs (Fig. 2b) of 3.1 mm in diameter (three eggs measured). When taking photos of the male and female after they had separated, we observed a significant change in skin morphology, in particular in the female. While having

an extremely warty, rough skin during amplexus, particularly on the snout (Fig. 1a), the skin was much smoother the next morning (Figs 1b, d). This change in skin morphology was also visible, even if less conspicuously so, in the male (Figs 1a, c). The male's colours became lighter and more pronounced the next morning (Fig. 1c). The clutch turned mouldy after about two weeks, and no development was discernible at that time.

On 4 March 2023, at 20:30 h, we encountered another amplectant couple (Fig. 1e) close to the Chocó Lab on a steep slope in degraded rainforest above a small stream (coordinates: 0.5257, -79.2118). These frogs were again sitting on a leaf at about 50 cm above the ground. They had



Figure 1. *Pristimantis rosadoi* couples illustrating sexual size dimorphism, morphological variation and change: (a) couple encountered on 6 September 2022, the female with very rough skin in particular on the snout, photo taken in situ; the same male (c) and female (b, d) on 7 September 2022 following oviposition (see Fig. 2b) and after the couple had disengaged. The female now exhibits an almost smooth snout (b) and less rough back skin (d), with the male displaying lighter and more pronounced colours (c); (e) couple encountered on 4 March 2023 and the female's snout (f) the next morning after oviposition (see Fig. 2a) and the couple had disengaged, the female with a rough snout on both days; (g) couple encountered on 11 March 2023, the female with a comparatively smooth snout.

deposited 33 yellow eggs (Fig. 2a) by the next morning, with egg diameters of about 3 mm (4 eggs measured: 2.5, 3, 3, 3 mm). The male measured 17 mm in SVL (0.41 g), while the female measured 27 mm and weighed 1.53 g after oviposition. This couple had less rough skin than Couple 1, but the female exhibited some distinct warts on the snout during the night, which were slightly less pronounced the next morning (Figs 1e, f). No embryonic development was discernible in the eggs for the first few days after deposition (see below). When development became obvious, after about eight days following oviposition, we detected neither external gills nor a tail in the young embryos. On Day 13



Figure 2. *Pristimantis rosadoi* clutches and developing eggs. Clutches comprising 33 (a) and 13 (b) eggs, respectively; photos taken on the morning following oviposition; (c–d) embryos of Clutch 3, 14 days after egg deposition. Embryos at far advanced stages with almost fully developed extremities and regressed or absent tail (see egg on the lower left in c for hind legs and absent tail) are wrapped around large amounts of remaining yolk; (e–f) embryos 21 days after oviposition, frogs hatched from Clutch 2; (e) froglet a few seconds after hatching, six fully developed froglets shortly before hatching and an empty egg capsule; (f) three hatched froglets, one upside down, shortly after hatching, four eggs with frogs ready to hatch, and an empty egg capsule on millimetre paper to illustrate size; (g) freshly hatched froglet placed upside down to show the amount of remaining yolk (compare with f for variation in yolk amount after hatching).

after egg deposition, the clutch was checked again and egg diameters had increased to about 5 mm. The embryos were well advanced in their development now, with snout shape, limbs and eyes appearing to be almost fully developed, but with the skin still exhibiting little pigmentation. A tail was not visible, but otherwise their stage matched the stages 13 to 14 of TOWNSEND & STEWART (1985). Only the uppermost eggs of the clutch were checked, for which reason no fertilization rate could be recorded. On 25 March, 21 days after oviposition, the clutch was checked again. While taking pictures of the eggs, 25 juveniles hatched, but one remained in its egg (Figs 2e-g). The other eggs seemed to have not developed. The freshly hatched froglets measured roughly 4 mm in SVL, and their mean weight was 0.012 g (weight of five froglets: 0.011, 0.011, 0.012, 0.013, and 0.013 g). The froglets still had large, but variable amounts of yolk in their bellies (Figs 2f, g), and were extremely agile, leaping partly across distances of more than 10 cm! They were either almost entirely black dorsally or showed a light dorsolateral line extending from the snout tip to the groin, with banding being visible on their extremities (Figs 2e, f).

On 11 March 2023, at 22:00 h, we discovered a third couple, almost at the same site as Couple 1. Again the frogs were sitting on low vegetation (Fig. 1g). By the next morning they had deposited a clutch of 28 yellow eggs of 3 mm in diameter (5 eggs measured). The male measured 18 mm (weight 0.39 g), the female measured 25 mm and weighed 1.46 g after oviposition. The skin of this couple was much smoother than that of Couples 1 and 2 (Fig. 1g) on their discovery, and did not show any signs of change after the sexes had separated. During the first six days no development inside the eggs was discernible. On Day 14 after egg deposition, the embryos were far developed, with all of them showing well-shaped heads and eyes and long extremities, wrapped around a huge amount of yolk. A tail was absent and the skin showed darker pigmentation (Figs 2c, d). It appeared to us that this clutch developed slightly faster than Clutch 2. We again investigated only the uppermost eggs and thus cannot estimate the fertilization rate. Unfortunately, the entire clutch turned mouldy thereafter, and did not develop further.

#### Discussion

# Reproduction and development

The adult *Pristimantis rosadoi* observed in this study agree with the published descriptions of the species (FLORES 1988, LYNCH & DUELLMAN 1997, CISNEROS-HEREDIA et al. 2004, YÁNEZ-MUÑOZ et al. 2022). Their habitat, old-growth to slightly degraded lowland rainforest with closed canopy, as well as the species' nocturnal activity pattern, climbing on low vegetation, were also in accordance with the published data (LYNCH & DUELLMAN 1997, CISNEROS-HERE-DIA et al. 2004, MECN 2010, YÁNEZ-MUÑOZ et al. 2022). So far, the only available data concerning the reproduction of this species were based on the male and female types, which were collected while being amplectant (FLORES 1988, LYNCH & DUELLMAN 1997), a female (24.3 mm) with 52 ripe eggs collected in April, and further gravid females that were encountered in March, August and September (MECN 2010, ORTEGA-ANDRADE et al. 2010). Taking into account our new data, it seems that the species reproduces throughout most of the year, potentially with peaks in the late rainy (March) and late drier (August, September) seasons.

Different patterns of breeding phenology have been documented within *Pristimantis* before, ranging from shorter, seasonal activity to circumannual reproduction (e.g., WAL-DEZ et al. 2011, FALCÓN-ESPITIA et al. 2023). Different patterns may even exist between syntopic species, presumably as a measure to avoid or reduce competition (e.g., GRANA-DOS-PÉREZ & RAMÍREZ-PINILLA 2020). Amplectant pairs of other *Pristimantis* species are also often found at night, on vegetation, off the forest floor, during humid periods (e.g., FALCÓN-ESPITIA et al. 2023, this study, and unpubl. obs. of the authors, e.g., on numerous pairs of *P. latidiscus*).

Direct developers usually have small clutches with large eggs (e.g., DUELLMAN & TRUEB 1986, BAHIR et al. 2005, Wells 2007, Blackburn et al. 2023, Díaz et al. 2023). This also applies to P. rosadoi. We herein report egg numbers that are much lower (13-33) than the 52 reported by MECN (2010). This difference may indicate that females distribute their eggs over two (or more) clutches. Egg counts from dissected females of three Pristimantis species from a cloud forest habitat in Colombia likewise were larger, although these species were of similar size or only slightly larger than P. rosadoi (GRANADOS-PÉREZ & RAMÍREZ-PINILLA 2020). Clutch estimates from dissected females may potentially overestimate 'real' clutch size (comp. HOWARD & MAERZ 2022). We refrained from dissecting the females which we temporarily collected and checking them for further ripe eggs, and we do not know whether they had already reproduced earlier; HILL et al. (2010) observed consecutive clutches every six to eight weeks in captive P. gaigei.

Surprisingly, few clutch data exist for other species of the genus Pristimantis. The published reports indicate mostly clutch sizes of 30-38 eggs (HILL et al. 2010, ROJAS-RIVERA et al. 2011, ACEVEDO et al. 2022), but a range from 1-108 has become known from Amazonian Ecuador and from Peru, predominantly from dissected females (DUELLMAN 1978, DUELLMAN & LEHR 2009 and further literature cited therein). With an SVL of 16-27 mm P. guianensis has a body size almost identical to that of P. rosadoi. In P. guianensis three clutches of 9-13 eggs were detected amongst dead leaves, approximately 1 m above the ground (MôNICO et al. 2022). Their diameters increased from about 4 mm at oviposition to almost 6 mm when the froglets started hatching after 25 days (under lab conditions). The values of egg size and development period thus were similar to what we observed in P. rosadoi. Likewise the holotype of P. nankints (SVL 30.9 mm) deposited 20 eggs after having been captured (Ron et al. 2020). A P. attenboroughi female deposited 20 eggs of 3.5 mm in diameter. The latter clutch was guarded by the female (SVL 21.5 mm) inside a mass of moss (LEHR & VON MAY 2017). It is not known if P. rosadoi

normally would guard its eggs; however, as Clutches 1 and 3 did not develop to the end, we would not be surprised if the species usually provides parental care in one or another form to increase the chances of successful embryonic development (comp. TownsEND et al. 1984). Egg guarding was reported from, e.g., *P. colodactylus*, with a female (SVL 19 mm) guarding eight eggs in a bromeliad funnel (DU-ELLMAN & LEHR 2009); *P. achatinus*, with a female (SVL 38 mm) sitting on 38 eggs (ROJAS-RIVERA et al. 2011), *P. reichlei*, with a female (SVL 33 mm) protecting 28 eggs (QUINTEROS-MUÑOZ & AGUAYO 2022), and *P. muricatus*, number of eggs not provided (Fig. 4A in ORTEGA-AN-DRADE et al. 2010). HILL et al. (2010) reported both sexes to attend the eggs, 22–37 per clutch, in *P. gaigei*, but not together or at the same time.

From the 28 eggs protected by a P. reichlei female, 14 hatched into small frogs after 6 days, and QUIN-TEROS-MUÑOZ & AGUAYO (2022) discovered the eggs only after the female had been placed in a field collection bag without a male being present. This would indicate internal fertilization, a process know only from a very few anuran species, and usually connected with vivipartity (but compare TOWNSEND et al. 1981; see summary on internal fertilization and viviparity within Anura in the introduction by SANDBERGER et al. 2017), or – as these authors assumed - a case of parthenogenesis. In our opinion, the extremely short embryonic period, 6 days, more likely argues for the eggs being fertilized internally and deposited at a more advanced stage. In P. guianensis hatching occurred after 25 days and extended over seven days, with the emerging froglets measuring about 4.3 mm (MôNICO et al. 2022). We assume that our *P. rosadoi* froglets only hatched simultaneously because of the disturbance when handling the eggs for taking pictures, and under natural and undisturbed conditions would hatch at different moments. This assumption is supported by differences in the amount of yolk in the hatched frogs (comp. Figs 2f and g). It also indicates some plasticity in the timing of hatching, potentially connected to predation risks (or weather conditions). For instance, it has been documented for Agalychnis frogs that embryos hatch earlier when snakes or wasps start eating some eggs (WARKENTIN 1995). To our knowledge, such plastic hatching behaviour is still undocumented for direct-developing frogs and deserves more attention in the future.

The embryonic development of *Pristimantis* species is even less well documented than clutch sizes. To our knowledge, the only complete stage table for the embryonic development of a Latin American direct-developing frog (from another family) is the one for *E. coqui* by TOWNSEND & STEWART (1985). Unfortunately, we could not continuously document (and sample) the embryonic development of our frogs, thus our data consist of snapshots rather than a continuous timeline of development. Nonetheless, it seems possible that *P. rosadoi* may deviate from both *E. coqui* and other *Pristimantis* in this regard. Photos documenting the embryonic development of *P. guianensis* show tails in embryos 9–17 days old (MôNI- CO et al. 2022), a feature which is not visible in our photos of *P. rosadoi* (Figs 2c, d). However, as we could not take photos on a daily basis, it is possible that we simply missed gilled and tailed stages (compare the discussion in TOWNSEND & STEWART 1985 concerning the variation in appearance and visibility of external gills and tails in Neotropical direct-developing frogs). As anatomically specialized tails are assumed to serve as respiration organs in embryos of direct-developing frogs (e.g., TOWNSEND & STEW-ART 1985 and citations therein, NOKHBATOLFOGHAHAI et al. 2010), their potential absence in *P. rosadoi* should be further investigated (comp. SALICA et al. 2023 for individual variation in tail development and features influenced by external conditions).

# Skin texture change

Another interesting observation was the change in skin morphology of some of the collected adult frogs, two females in particular. The external morphology of the skin, i.e., skin texture, is regularly used as a diagnostic feature in taxonomic descriptions of frogs (see, e.g., DUELLMAN & LEHR 2009, RON et al. 2020), and intraspecific differences are often sex-specific (LUNA et al. 2018, PORTIK et al. 2019). Seasonal changes of skin texture and/or colour within an individual are connected to breeding activity. For instance, breeding males of many anuran species turn yellow or even blue (BELL & ZAMUDIO 2012, SZTATECSNY et al. 2012, RO-JAS 2017), develop nuptial pads and breeding glands, arm muscles increase and spines develop on fingers, arms or in the pectoral region (e.g., AMIET 1980, LUNA et al. 2018). The males of several African *Phrynobatrachus* spp. change from a warty skin in non-breeding condition to an almost smooth skin when breeding (RÖDEL 2000).

The most dramatic short-term change of the dorsal skin texture in frogs has been observed in three Pristimantis species from Ecuador and Colombia: P. mutabilis, P. sobetes and P. colomai (GUAYASAMIN et al. 2015, VALENCIA-ZULETA et al. 2016). Males and females of P. mutabilis were found to have a highly tuberculate skin in nature (and at night), but turned almost smooth within a few minutes after being captured or photographed. When placed back on a damp mossy substrate, they recovered the tuberculate skin (GUAYASAMIN et al. 2015: figs 2, 3). These authors speculated that stress, humidity/ambient moisture, and/or background may have triggered the change. They believe that this rapid, non-seasonal, non-sexual dimorphism might aid in camouflage, with the tuberculate skin mimicking mossy back- and underground. The physiological mechanisms underlying this phenomenon are unknown. The skin texture changes in P. sobetes and P. colomai were less dramatic, but they changed to smooth skin after capture as well (GUAYASAMIN et al. 2015, VALENCIA-ZULETA et al. 2016). In P. rosadoi, we particularly observed such a change in the females, i.e., from rough skin while being in amplexus (two of three females) to smoother skin after disengaging from the male. We did not observe a change back to

rough skin, but we also did not specifically check for such a change. However, based on our observation, we would not exclude the possibility that changes in skin morphology may be related to reproduction at least in *P. rosadoi*.

GUAYASAMIN et al. (2015) discussed whether this morphological change of the skin is widespread in *Pristimantis* or evolved twice independently. They already predicted that skin texture change might be observed in *Pristimantis* species other than the ones they studied. As the four species currently known to exhibit these morphological changes belong to different lineages within the genus (HEDGES et al. 2008, GUAYASAMIN et al. 2015, VALENCIA-ZULETA et al. 2016, ACEVEDO et al. 2022), it seems likely that many more *Pristimantis* species are able to change skin morphology. The mechanisms and in particular the biological meaning of that change seem to be promising topics for future research.

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