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Reproductive ecology, behaviour and development of the direct-developing frog *Pristimantis latidiscus* (Anura: Strabomantidae)

Niklas Fuchs[1,2] & Mark-Oliver Rödel[1]

[1] Museum für Naturkunde – Leibniz Institute for Evolution and Biodiversity Science, Invalidenstr. 43, 10115 Berlin, Germany
[2] Animal Population Ecology, Universität Bayreuth, Universitätsstr. 30, 95447 Bayreuth, Germany

Corresponding author: Mark-Oliver Rödel, ORCID 0000-0002-1666-195X,
e-mail: mo.roedel@mfn.berlin

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Abstract. The reproductive biology of most direct-developing frogs remains poorly understood. One such species is *Pristimantis latidiscus* from the wet Ecuadorian Chocó. We collected reproductive ecology data from seven amplectant pairs, obtained two clutches and document egg-burying behaviour and the embryonic development in this species. We left part of the eggs undisturbed, while we handled others every two days until hatching. The handled eggs hatched significantly earlier than their undisturbed siblings, suggesting phenotypic plasticity of hatching time. We assume that the trigger for early hatching most likely was our handling, probably perceived as 'predation attempts' by the embryo. We compare the embryonic development of *P. latidiscus*, to other direct-developing anurans, and provide the first description of juvenile *P. latidiscus*.

Key words. Amphibia, life history, clutch size, parental care, reproductive strategies, environmentally cued hatching, embryology, ontogeny, Ecuador.

Resumen. La biología reproductiva de la mayoría de las ranas con desarrollo directo es poco entendida. Una de estas especies es *Pristimantis latidiscus* del Chocó lluvioso ecuatoriano. Recopilamos datos sobre la ecología reproductiva de siete parejas en abrazo nupcial, obtuvimos dos puestas y documentamos el comportamiento de enterramiento de huevos y el desarrollo embrionario en esta especie. Mantuvimos parte de los huevos sin perturbaciones, mientras que manipulamos otros cada dos días hasta la eclosión. Los huevos manipulados eclosionaron significativamente más temprano que sus hermanos no manipulados, lo que sugiere plasticidad fenotípica en el tiempo de eclosión. Suponemos que el gatillante de la eclosión temprana probablemente fue nuestra manipulación, la cual pudo ser percibida como 'intentos de depredación' por el embrión. Comparamos el desarrollo embrionario de *P. latidiscus* con el de otros anuros de desarrollo directo y describimos por primera vez los juveniles de *P. latidiscus*.

Palabras clave. Amphibia, historia de vida, tamaño de la puesta, cuidado parental, estrategias reproductivas, eclosión inducida por el ambiente, embriología, ontogenia.

Introduction

Although over 25% of anuran species are direct developers (Liedtke et al. 2022), skipping a free-living larval stage and hatching as froglets (Wells 2007, Hedges et al. 2008), knowledge about their biology and development is scant, relative to frogs with biphasic life cycles (Warkentin 2011, Goldberg et al. 2020, Liedtke et al. 2022). However, information about life-history and reproduction is necessary to understand a species' ecology and evaluate potential conservation needs, e.g., in the face of global change (Bury 2006, Gallagher et al. 2021, Borgelt et al. 2022).

With over 600 species the neotropical frog genus *Pristimantis* (Jiménez de la Espada, 1870), family Strabomantidae, is the most diverse genus of all vertebrates (Acevedo et al. 2022, García-Gómez et al. 2022, Frost 2024). It is assumed that all its members are direct developers (Hedges et al. 2008). However, like in other groups of direct-developing frogs, little has been published on the reproductive biology of most *Pristimantis* species (see Rödel et al. 2024 and citations therein). New findings indicate that many interesting aspects of their reproductive biology are yet to be discovered (see e.g., Angiolani-Larrea et al. 2023). For instance, Rödel et al. (2024) argued for the possibility of environmentally cued hatching (ECH) in *P. rosadoi*. The ability of young to use cues of danger, e.g. vibrations during predation attempts, and induce early hatching to escape predation (Warkentin 2005, Warkentin & Caldwell 2009, Doody 2011), has not been documented in *Pristimantis,* and has only rarely been reported for direct developers in general (Buckley et al. 2005, Warkentin 2011). Moreover, recent work on embryonic development emphasizes how much is still to be learned about the ontogeny of direct-developing frogs, like *Pristimantis* (i.e., Salica et al. 2023).

During ecological studies in north-western Ecuador, we took notes on the biology of a locally abundant species, *P. latidiscus* (Boulenger, 1898). Our goal was to gather data on its reproductive biology, such as breeding and nest sites, clutch size, and potential parental care. We also aimed to describe its embryonic development and the hatchling phenotype, and compare the embryonic development with other neotropical direct developers. Furthermore, we tested if *P. latidiscus* does show environmentally cued hatching.

Materials and methods

Field work was conducted from March 2022 to May 2024, with an increased sampling effort between March and May 2024. The study was carried out in the Canandé Reserve, which protects some of the last remnants of Ecuador's Chocó rainforest in Esmeraldas Province. The reserve is managed by the 'Fundación Jocotoco' (www.jocotoco.org.ec) and contains a research facility, the Chocó-Lab, where all laboratory work was carried out (coordinates: 0.5263, -79.2130; altitude: ca. 300 m a.s.l.). The reserve and surroundings comprise habitats with different levels of anthropogenic influence, ranging from pastures, and active cocoa plantations, over different regeneration stages of secondary forests, to primary forest. The climate is warm-tropical, with an annual mean temperature of 24 °C, and annual precipitation above 5000 mm (Escobar et al. 2025).

We collected data on the reproduction of *P. latidiscus* opportunistically, during a study on the ecology of frog communities. Species identification was based on Yánez-Muñoz et al. (2022). We measured the perch height of amplectant *P. latidiscus* pairs, using a laser range finder (Bosch GLM 250 VF Professional, ± 1.0 mm) as well as the total leaf area of the perch leaf via the 'Easy Leaf Area' mobile app (Easlon & Bloom 2014). Those measurements were only taken for frogs found between March and May 2024. If pairs were located close to the station, and thus could easily be returned after our study, we brought them to and measured them at the Chocó-Lab. Otherwise, data were collected in the field. We measured the snout–vent length (SVL) of frogs with a caliper to the nearest mm and weighed them (in case of the egg-laying females, after oviposition) with an electronic scale (Kern TGD Max 50 g, ± 0.001 g). At the laboratory, the pairs were either placed in a small plastic container or a mesh vivarium (60 × 36.5 × 36.5 cm, length × depth × height; Fig. S1), located in a storage room, next to the Chocó-Lab. The storage room was not air-conditioned and mirrored outdoor climate, incl. daily fluctuations. The room temperatures ranged between 22 and 26 °C, with a relative humidity of 64–89%. We equipped the mesh vivaria with different nesting substrates, known to be used by other *Pristimantis* species (Lynch & Duellman 1997, Duellman & Lehr 2009, Hill et al. 2010, Rojas-Rivera et al. 2011, Martínez Dueñas 2017, Kok et al. 2018, Mebert et al. 2022, Angiolani-Larrea et al. 2023). Potential nesting structures in the mesh vivaria comprised multiple layers of leaf litter (ca. 5 cm thick), a small plastic box (18 × 11.5 × 6.5 cm, capacity 1 l) filled with moist mud, two bromeliads of different sizes, as well as an upright standing branch and a ca. 20 cm piece of rotting wood, lying on the leaf litter (Fig. S1). The plastic breeding containers, on the other hand, were equipped solely with moist moss and leaf litter.

If nesting behaviour was observed, we took pictures and behavioural notes every 30–60 min. The second obtained clutch was carefully removed from its nest with a small spoon, 17 hours after oviposition. We then placed the egg-laying box back in the vivarium, together with the female, and left them there for one more night. Two days after oviposition, the female was euthanized with 0.02 g Lidocain (Roxicaina® Atomizador Lidocaína 80 gr – Ropsohn). A liver sample was taken and stored in 73% ethanol. The frog was fixed with a 3.7% formol-solution and transferred into 75% ethanol after a few days. Tissue samples and the female voucher are deposited at the Museo de Historia Natural, Escuela Politécnica Nacional, Quito (MEPN 16418). We released all other adult specimens at their respective capture sites, three to four days after capture.

We photographed four eggs of clutch 1 and five of clutch 2 (MEPN 16418) separately on millimetre paper under a dissecting microscope (Motic SMZ 168). Clutch 2 was weighed. The eggs of clutch 2 were maintained in four round plastic cups (diameter 12.3 cm, height 6 cm) with 17–23 ventilation holes in the lid (diameter ca. 2 mm). The holes were partly covered with one layer of daily re-moistened tea filter, to increase humidity within the cups. To minimise the risk of total clutch fatality, we kept half the clutch on three layers of moistened tea filter, and the other half on 1.5 cm of mud from the original nest site (two cups each). We visually checked the eggs daily, removed rotten eggs, and added water to the substrate if necessary. The cups were placed in a drawer within the air-conditioned Chocó-Lab, together with a temperature and humidity data logger (EL-USB-2+, ± 0.5 °C, ± 3.0%). However, the temperature fluctuated due to regular power failures of the air conditioning system (mean = 23.1 ± 1.0 °C, min = 20.5 °C, max = 26 °C). The relative humidity also showed high fluctuation within the drawer (mean = 86 ± 4.4%, min = 65%, max = 94%).

We continued photographing half of clutch 2, with five eggs from one tea filter cup and one soil cup respectively, on millimetre paper, as well as on a black background every two days, until hatching. Photos were taken between 08:30 and 11:00 h. Apart from our visual daily checks (no handling of the cups), the other half of the clutch remained undisturbed. The eggs thus developed in four treatments:

(a) soil undisturbed (n = 11, -1 see below), (b) soil disturbed (n = 8), (c) tea filter undisturbed (n = 11), (d) tea filter disturbed (n = 8). Close to hatching, a single egg of the soil undisturbed group was photographed to allow a direct comparison of the developmental stage with those of embryos of the same age from the disturbance treatment. This egg was subsequently kept separate and excluded from statistical comparisons. In the final incubation period, we monitored the cups for hatchlings multiple times a day and calculated the incubation time as 24 h intervals between egg-laying and first observation of the hatchling. The incubation time was compared between the soil-treatments, using a t-test. Statistics were conducted with RStudio ver. 2024.04.2, based on R-4.4.1 (R Core Team 2024); the plots were created with ggplot2 ver. 3.5.1 (Wickham 2016).

Egg photographs were used to track the development of the embryos. We did not dissect eggs. The development of *P. latidiscus* was compared against the staging table for *Eleutherodactylus coqui* by Townsend & Stewart (1985) (subsequently termed TS), which is commonly used and adapted for describing the development of direct-developing frogs (Bahir et al. 2005, Anstis 2008, Goldberg & Candioti 2015, Schweiger et al. 2017). Hatchlings were photographed on millimetre paper. Most juveniles were released back to the collection site of their parents. Four individuals (two from soil disturbed, two from soil undisturbed) were preserved (see above), and deposited at MEPN (stored together with female, MEPN 16418). We measured the sizes of the photographed eggs, as well as the SVL of all hatchlings using ImageJ 1.58g (Schneider et al. 2012). Hatchling size was compared between the treatments using a t-test.

Results

Breeding site

Pristimantis latidiscus was commonly found in old-growth forest, as well as secondary forests of the Canandé Reserve (N. Fuchs unpubl. data). A single individual was also observed in a banana plant on a mixed cocoa-balsa plantation. During our study, we encountered seven amplectant pairs of *P. latidiscus*, four of which were brought to the lab. The first pair was observed on 6 September 2022, sitting on a large leaf close to the ground. A second amplectant pair was found on 4 March 2023 in a similar position. This pair was caught and kept in a small plastic container for four days. The third couple was caught on 2 November 2023 close to a

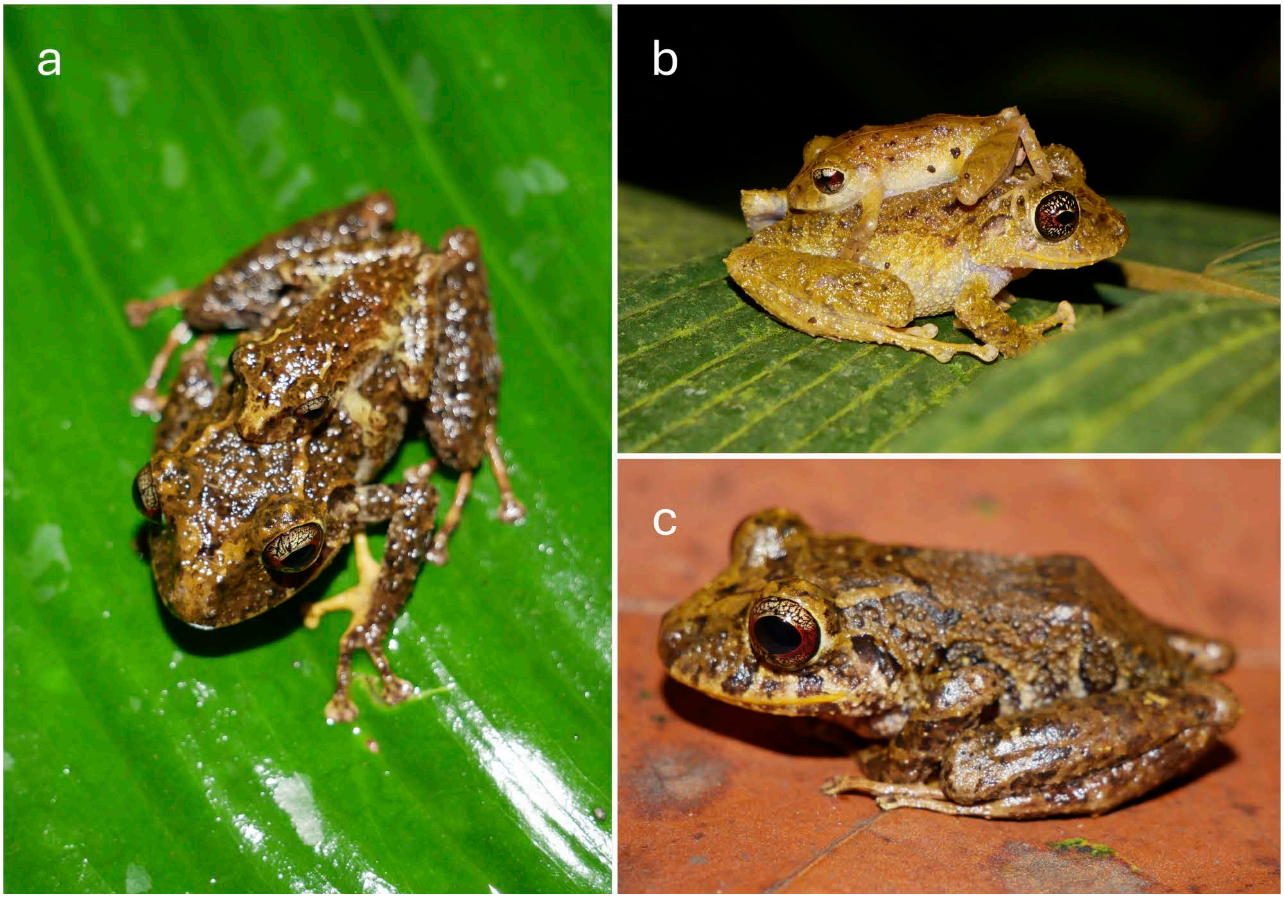

Figure 1. Adults of *Pristimantis latidiscus*. (a) Amplectant couple (couple 5); (b) couple 6 in 'inverse amplexus'; (c) female of couple 5 (MEPN 16418), this female deposited fertilized eggs (compare Fig. 2).

Table 1. Behavioural sequence during oviposition and egg burying in *Pristimantis latidiscus* (compare Fig. 2).

Date	Time	Observed behaviour.
10 April	20:26	Individuals found in amplexus in the field.
11 April	08:03	Couple still engaging in amplexus under leaf litter in the vivarium.
11 April	12:30	Couple sitting in small, excavated depression in the soil box (Fig. 2a).
11 April	13:47	No change comp. to 12:30.
11 April	14:47	Female is releasing eggs (at least 17 eggs already laid) (Fig. 2b).
11 April	15:17	Female finished oviposition and is covering clutch with its body. Male is loosely sitting on female.
11 April	15:47	Female still sitting on clutch. Male left and hid in bromeliad (Fig. 2c).
11 April	16:17	Female started burying the egg.
11 April	17:20	Clutch completely covered with soil. Female is dragging and pushing bigger particles of mud onto the clutch (Fig. 2d).
11 April	17:50	The female continues burying the eggs. It moves in circles, patting the soil under its body with its hind- and front-limbs. It regularly pauses for ca. 10 seconds.
11 April	18:20	Female is sitting on the buried clutch. Former depression is not visible anymore.
11 April	18:50	No change comp. to 18:20.
11 April	20:09	No change comp. to 18:20.
12 April	07:45	Female is still sitting in soil box, but not directly on top of the buried clutch. The nest site is smoother than on the previous evening. Eggs where now carefully excavated and removed; the female weighed and put back to the vivarium thereafter.
13 April	08:15	Female is sitting on (empty) oviposition site. Last observation.

forest stream (male 22 mm SVL; female 38 mm SVL). It was housed like the second pair. The female deposited clutch 1. A fourth pair was observed on 27 November 2023, perching on a big leaf. We caught a fifth pair (male 18 mm SVL, 0.61 g; female 34 mm SVL, 3.67 g after oviposition, MEPN 16418) during light rain on 10 April 2024 at 20:26 h on a big leaf (1389 cm^2), 121 cm from the ground (0.52599, -79.20863; Fig. 1a) and kept it in the mesh vivarium. This couple successfully produced offspring in captivity (clutch 2). We detected amplectant pair 6 (male 23 mm SVL, 0.95 g; female 37 mm SVL, 5.11 g) on 19 April 2024 at 19:03 h on a small leaf (103 cm^2), at a height of 127 cm (0.51208, -79.18904). The snout of the male was oriented towards the female's posterior (Fig. 1b). They separated immediately after being caught and did not reengage in amplexus during the three days they were kept in the mesh vivarium at the station, even though the male called during the first night after capture. The seventh pair was observed during light rain on 14 May 2024 at 19:53 h at 98 cm height, on a small leaf (not measured). While the first pair was found in old-growth forest, the others were observed in closed canopy secondary forests; all in low vegetation (< 1.5 m). In the vivaria, the animals stayed mostly hidden under leaf litter or in the bromeliads during the day. If provided with a soil box, females were also observed sitting in the mud.

Nesting in captivity and parental care

Only two of the four caught females deposited a clutch, one of which was infertile. The female of couple 3 laid unfertilized eggs two days after capture (4 November 2023), in a mix of moss and leaf litter (clutch 1). This substrate was the only option given. The female did not engage in any visible form of parental care. The female MEPN 16418 (couple 5; Fig. 1c), was placed in a vivarium, where it deposited its eggs in a depression it dug in the mud during amplexus, one day after capture (11 April 2024; Fig. 2). It covered its clutch (clutch 2) with ca. 3 cm of mud over the course of > 1.5 hours (Table 1; Fig. 2). The male left the site after oviposition. The female remained close to the buried clutch till we excavated the eggs ca. 17 hours after laying. It returned to the empty nest site, where it was found ca. 24 hours after removal of the clutch (41 hours after oviposition), before it was preserved.

Clutch maintenance and hatching

Clutch 1 consisted of 40 infertile eggs with an approximate egg diameter of 4.11 ± 0.21 mm (n = 4). Clutch 2 contained 38 eggs and had a 100% fertilization rate. It weighed 1.31 g in total (average weight of one egg = 0.04 g). Average egg diameter was 3.26 ± 0.06 mm (n = 10) ca. 18 hours after oviposition, and grew to 4.38 ± 0.04 mm (n = 5; soil disturbed eggs at 24 May 2024) close to hatching. Egg growth was more stable in eggs placed on soil compared to tea filter, which showed a strong fluctuation in size during incubation (Fig. 3). No eggs maintained on tea filter hatched. All died due to fungal infections at different points of development (mean = 15.53 ± 10.46 days; n = 19); the last one 29 days after oviposition. Two of the soil disturbed eggs also died due to a fungal infection, 7 days within development. All other eggs kept on soil (17), hatched.

The first froglet emerged from its egg in the evening of day 24 (between 16:19–21:46 h), measuring ca. 5.38 mm SVL. All eggs from the disturbed cup hatched during the next 1.5 days (mean = 25.14 ± 0.48 days), with large amounts of yolk remaining. The undisturbed animals hatched significantly later ($t = -20.30$, $df = 12.76$, $p < 0.001$), on days 29 and 30 of incubation (mean = 29.60 ± 0.32 days; Fig. 4). All but one of the hatchlings were found in the early morning of day 30. There was no significant difference in size between disturbed and undisturbed juveniles ($t = -1.434$, $df = 7.604$, $p > 0.05$; average SVL across all 17 hatchlings = 5.28 ± 0.24 mm), even though the yolk was fully absorbed in the longer incubated animals. Hatchlings remained parts of the tail (Fig. 5a, b). All juveniles were very agile. We observed leaps of over 15 cm in some cases.

Embryonic development and hatchling phenotype

Early signs of differentiation became obvious three days after oviposition (Table 2, Fig. 6a). During further development, the embryos grew a rounded tail, with a heavily vascularized fin (Fig. 6b, d, e), which stopped being visible in soil eggs on day 15 (TS12–13). The tail fins formed

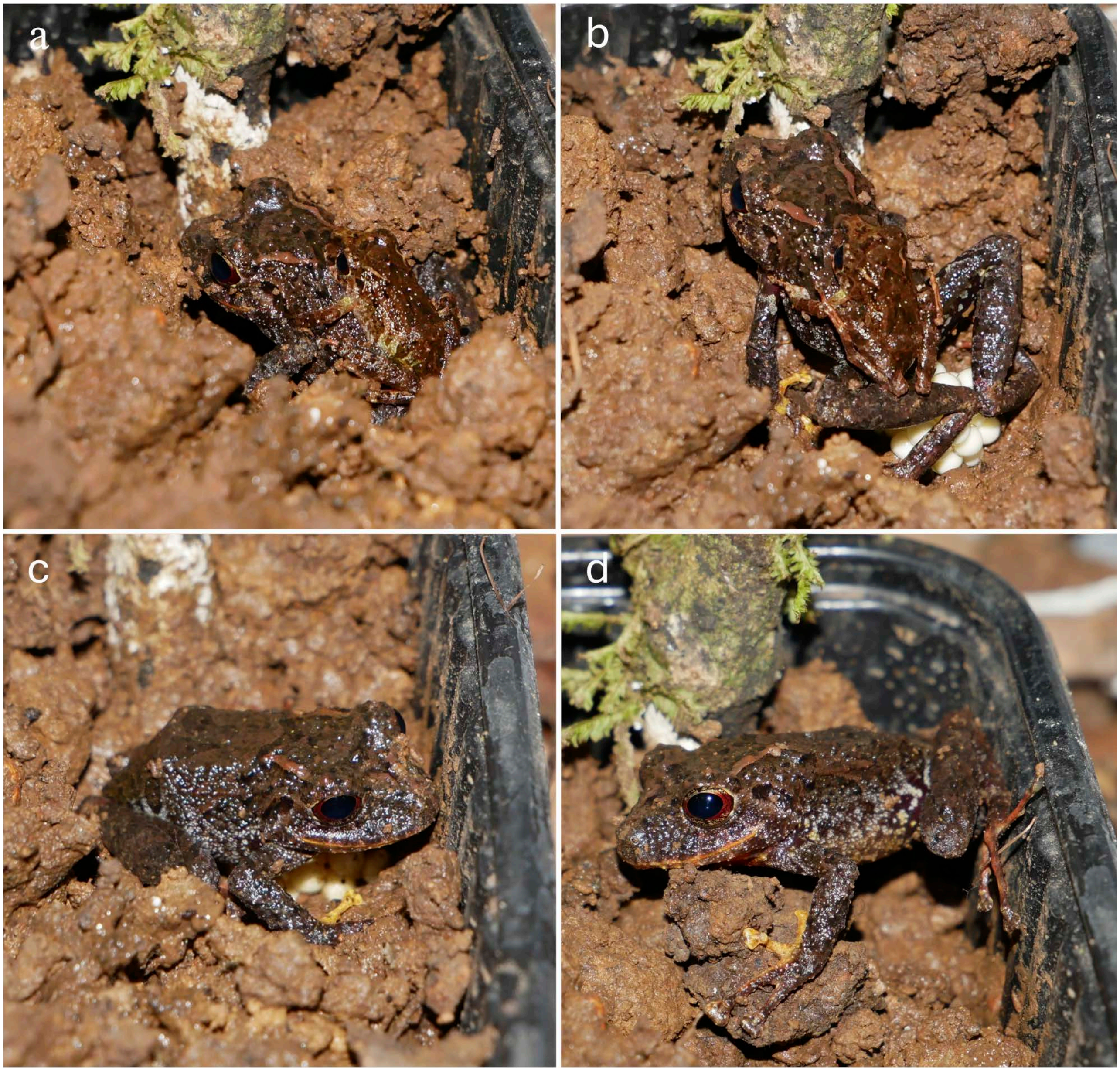

Figure 2. Selected stages during oviposition in *Pristimantis latidiscus* (compare Table 1). (a) Couple resting after excavating a depression; (b) egg-laying; (c) female resting on the clutch after oviposition; (d) female covering the eggs with soil.

lateral and caudal to the short tail axis. At its largest visible expansion, the fin encompassed almost ¼ of the yolk. Across all embryos and developmental stages, we never observed external gills. Eggs kept on tea filter fell behind in development from day 11 (Table S2). While all traits developed slower in tea filter eggs, the development of body pigmentation and embryo size was especially weak (comp. Fig. 6g). The overall development matched the description of *E. coqui* (Townsend & Stewart 1985), with some minor differences. The embryonic development is summarized in Tables 2 & S2.

Coloration of the hatched juveniles in life (Fig. 5): (1) Light greyish-brown to reddish dorsal surface and a reddish-brown coloured head, with two prominent lighter blotches posterior to the eyes, forming a '\ /'-shape. (2) Surfaces of legs and arms light brown to green, with dark transversal bands. Digits striped, but otherwise colourless. (4) Flanks behind the shoulder region of same coloration as legs and arms. (5) Venter dark grey to black with small white speckles. Ventral surfaces of the thighs lighter, more similar to upper surface of the legs. (6) Eyes solid reddish-brown, lacking the characteristic red horizontal band, present in the adults.

Discussion

Reproductive ecology and behaviour

In this study we report, for the first time, detailed information on the reproductive biology of *Pristimantis latidiscus*. Reproductive phenology differs within *Pristimantis* with some members having a seasonal reproductive period and

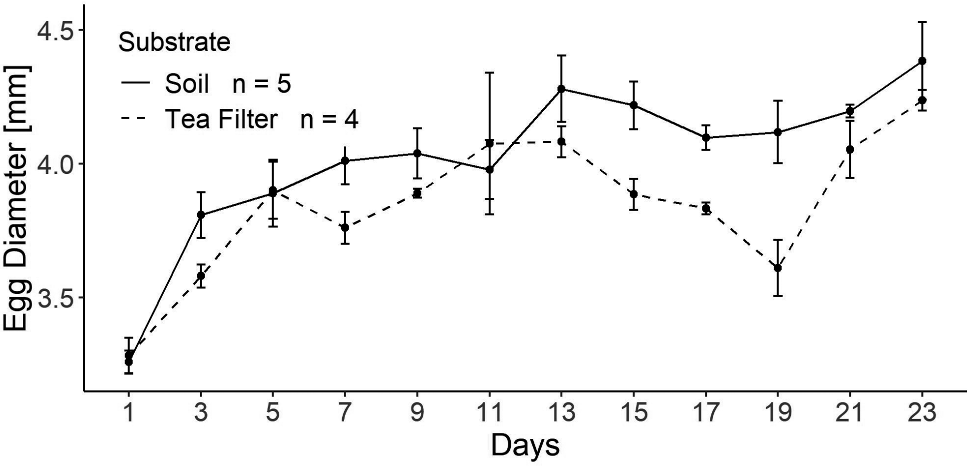

Figure 3. Development of average egg diameter of the monitored *Pristimantis latidiscus* eggs. Shown are the mean egg sizes and SD of eggs kept on soil or tea filter (compare method section and Tables 2 and S2).

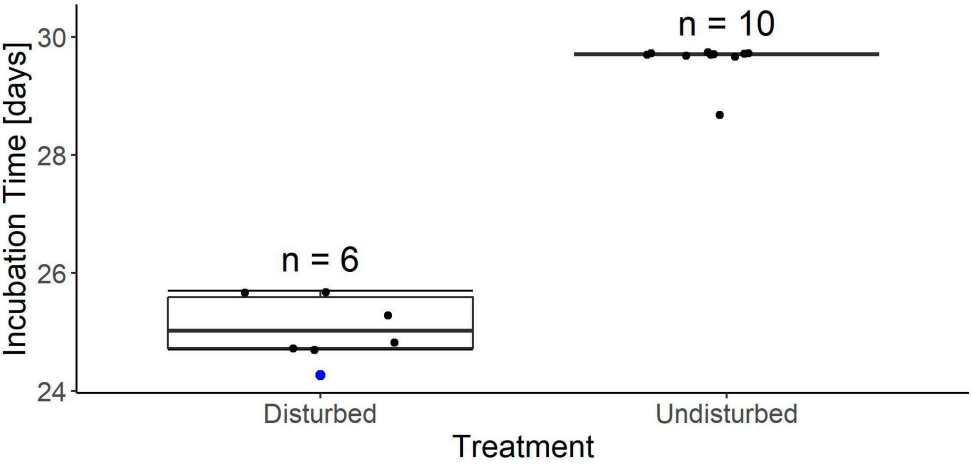

Figure 4. Incubation time of disturbed and undisturbed *Pristimantis latidiscus* eggs, kept on soil (compare method section). Incubation time (time between oviposition and first observation of the hatchling) is given in days. An additional blue point marks the time of hatching of one egg, which was only disturbed once on day 23 (compare text). This data point was not included in box-plot calculation and not counted in sample size.

others reproducing all year (Waldez et al. 2011, Granados-Pérez & Ramírez-Pinilla 2020, Falcón-Espitia et al. 2023), or at least have an extended reproductive period. *Pristimantis latidiscus* likely belongs to the latter, with records of amplectant pairs available from the months of September (this study), November (Duarte-Marín & de Plecker 2020, this study), December (Lynch & Duellman 1997), March, April and May (this study). However, all these months fall within the more pronounced rainy season.

Amplectant pairs of *P. latidiscus* are, like many of its congeners, typically found at night, sitting on low to medium vegetation (e.g. Lynch & Duellman 1997, Duellman & Lehr 2009, Yánez-Muñoz et al. 2022, Rödel et al. 2024). While there is arguably an observer-bias towards animals perching under head-height, it makes sense that these frogs use low vegetation for mating, as *P. latidiscus* and many other *Pristimantis* species deposit their eggs on the forest floor (e.g. Lynch & Duellman 1997, Duellman & Lehr 2009, Rojas-Rivera et al. 2011, Martínez Dueñas 2017, Acosta-Ortiz & Sierra-Rueda 2021, Angiolani-Larrea et al. 2023, this study). Even some species, which lead a primarily arboreal lifestyle as adults, are known to use nest sites on the ground, potentially because of a higher abundance of small prey items for the hatchlings in leaf-litter compared to bushes or trees (Lynch & Duellman 1997). Another, non-exclusive explanation might be that the microclimate on the forest floor is more constant and humid than in any more elevated parts of the forest.

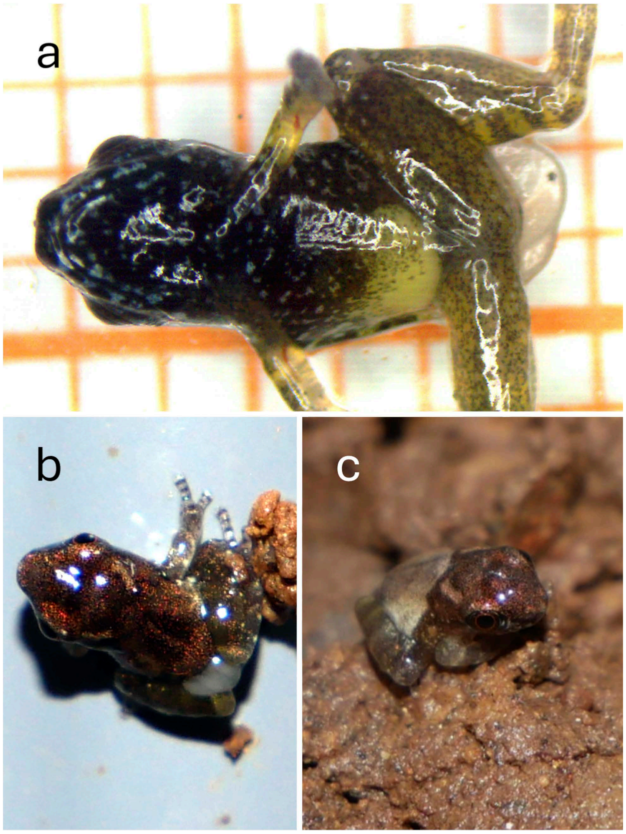

Figure 5. *Pristimantis latidiscus* hatchlings. (a) Ventral view of froglet right after hatching (~ 29 days of incubation); (b, c) dorsal view of two hatchlings (~ 24 days of incubation). Note the visible tail remnants in a & b.

Due to those nest site preferences, there is a high density of juvenile *Pristimantis* in leaf litter (Lynch & Duellman 1997). This was also observed in the Canandé Reserve (authors pers. observations). Species composition of juvenile leaf-litter *Pristimantis* assemblages are still largely unknown, because froglets of this genus are difficult to identify. They are not necessarily 'miniature versions' of the adults (Duellman & Lehr 2009) but may show a coloration and pattern different to the adults (comp. Rödel et al. 2024, Ron et al. 2024, this study). Ecology and habitat use of juveniles thus remains obscure. Further reports of juvenile phenotypes and the collection of voucher specimens are crucial to enable future works on their ecology.

Like other direct developers, *P. latidiscus* has small clutches with large eggs (Wells 2007, Furness et al. 2022). The herein reported clutch sizes of 38 and 40 eggs are in line with the only other published data on egg numbers in *P. latidiscus* by Lynch & Duellman (1997). These authors caught a female that deposited 40 eggs. Egg size seems to correlate with female size in this species, with larger females laying bigger eggs (clutch 2: egg ca. 3.26 mm, female 34 mm SVL; clutch 1: egg ca. 4.11 mm, female 38 mm SVL; Lynch & Duellman 1997: egg ca. 4.5 mm, female 42.4 mm SVL).

The egg-attendance which we observed is a simple kind of parental care, common amongst direct-developing frogs, in which one parent stays with the eggs for some time after oviposition (Furness & Capellini 2019, 2022). Females and males of over a dozen *Pristimantis* species are known to engage in this form of care (females: Duellman & Lehr 2009, Ortega-Andrade et al. 2010, Rojas-Rivera et al. 2011, Lehr & von May 2017, Martínez Dueñas 2017; males: Hill et al. 2010, Chinchilla-Lemus & Meneses-Pelayo 2016). We do not know how long the observed female would have attended its eggs. However, we assume it kept a strong connection to the nest site at least for the first days, as it returned to the nest site after we collected the clutch. As most observations of egg-attendance in *Pristimantis* are brief, the duration of it remains unclear in most cases. In *P. chocoensis* females seem to leave within one day after oviposition (Angiolani-Larrea et al. 2023), yet in some members of the genus, e.g. *P. medemi* and *P. gretathunbergae*, females have been observed sitting on far developed eggs (Acosta-Ortiz & Sierra-Rueda 2021, Mebert et al. 2022), implying they engage in prolonged egg-attendance.

Egg-burying behaviour is another form of parental care in *Pristimantis* that has only recently been described for *P. chocoensis*; also from within the Canandé Reserve (Angiolani-Larrea et al. 2023). Our study is the second report of this behaviour within the genus. Different taxa of terrestrially breeding frogs are already known to deposit their

Table 2. Development of *Pristimantis latidiscus* embryos in eggs kept on soil and partial development of eggs maintained on tea filter (compare method section and Figs 3 and 6) up to day 11. Key changes, traits and observations are highlighted in bold. Day = days after oviposition. TS = stage after Townsend & Stewart (1985). If key traits don't unanimously indicate one TS-stage, the respective TS is indicated by arrows behind the conflicting traits.

Day	TS	Comments on development
1	1	No development visible.
3	2	Early stages of differentiation visible within the egg (Fig. 6a).
5	4	Head shape outlined **Eye bulges** visible, unpigmented **Limb-buds** present, forelimb-buds more defined and closer to trunk than hindlimb-buds **No gills** visible during the whole development.
7	5–6	Head more prominent and separated into distinct parts (optic region, mouth and auditory region) Limb-buds more pronounced; hindlimb-buds round to ovoid; no constrictions (joints) visible yet ►TS5 **Tail-bud** pronounced and round (Fig. 6b) Endolymphatic calcium deposits (**ECD**) already clearly **visible** (Fig. 6c); round ►TS6 Fast rotation of the embryos.
9	6–7	**Eyes** more pronounced and **pigmented** **Limb-joints** first evident **Future body-wall** (small disc around the trunk) first observed ►TS7 **Tail** elongated with small **fin**, formed lateral-caudal from the axis (Fig. 6d) ►TS6 **Melanophores** scattered across body ECD still prominent and round Strong **heartbeat** Embryo rolls over when turned (yolk facing upwards) ►TS7.
11	8	Limbs elongated; joints more pronounced Small disc enlarged **Tail fin** bigger and heavily **vascularized** (Fig. 6e) Melanophores denser; especially in the paravertebral region; body wall only lightly pigmented (Fig. 6f) **ECD triangular**, with extension towards the eye **Tea filter eggs** start to **lack behind** soil eggs in growth and pigmentation; further development of tea filter eggs in Supplementary Table S2.
13	11	**Digits** on front limbs **visible** hindlimbs far developed; toes not visible due to positioning of the embryo Body wall encompasses over 2/3 of the yolk Tail fin bigger; strong blood flow; **largest observed size of the tail** Melanophores dense on trunk; less pigmentation on hind-legs and body wall; no pigmentation of tail fin **ECD** longitudinally **elongated**, but barely visible through pigmentation.
15	12–13	Toes still not visible due to positioning of the embryo Body wall encompasses almost all the yolk ►TS12 Tail no longer visible Trunk and head heavily pigmented ►TS13 **ECD** completely **masked by pigmentation** (Fig. 6g).
17	13	Toes visible and fully developed Body wall encompasses all the yolk.
19	13–14	Characteristics used to differentiate further TS-stages (e.g. eyelids and presence of toepads) not visible through outer egg-layer due to size and dark coloration of the embryo.
21	13–14	No key structures visible.
23	14	**Fully developed eye** (Fig. 6h) One day before first animal hatched.

clutches in small depressions and cover them with soil, like the Asian Shrub Frogs *Philautus* spp. (Bahir et al. 2005, Schulte 2020), the Pumpkin Toadlet *Brachycephalus ephippium* (Pombal et al. 1994), or the African Squeaker Frogs *Arthroleptis* spp. (Guibé & Lamotte 1958, Lamotte & Perret 1963, Wager 1986, Schweiger et al. 2017). However, the combination of egg-burying and egg-attendance is rare (Schulte et al. 2020, Liedtke et al. 2022, Angiolani-Larrea et al. 2023). Both, *P. chocoensis* and *P. latidiscus*, are placed within the subgenus *Hypodictyon* (Cope, 1885, Hedges et al. 2008, Crawford et al. 2010, Reyes-Puig et al. 2020), and in this regard are distinct from most sympatric *Pristimantis* species in the Chocó (comp. García-Gómez et al. 2022). It will be interesting to see how widespread the egg-burying behaviour is within and amongst the different sub-clades of *Pristimantis*.

Developmental time and ECH

The fluctuation in size of eggs kept on tea filter (Fig. 3) was likely due to the unstable humidity within the drawer. Eggs on soil were probably less affected, as the thick layer of soil guaranteed a more stable moisture content. Under natural conditions, desiccation can be one of the main causes of mortality in terrestrial amphibian eggs (Byrne & Keogh 2009). Sufficient moisture is crucial for the survival and health of the embryos of terrestrial breeding frogs and an important parameter by which adults determine the quality of a nest site (O'Brien et al. 2020, Mitchell 2022). *Pristimantis latidiscus* eggs seem to be highly dependent on stable, humid conditions during incubation, possibly contributing to the species' preferences for old secondary and primary forest as habitat. In addition to the higher water availability in soil cups, the higher survival rates of eggs kept on forest mud compared to tea filter could also have been influenced by the soil microbiome suppressing fungal pathogens (Schlatter et al. 2017, Vecchioni et

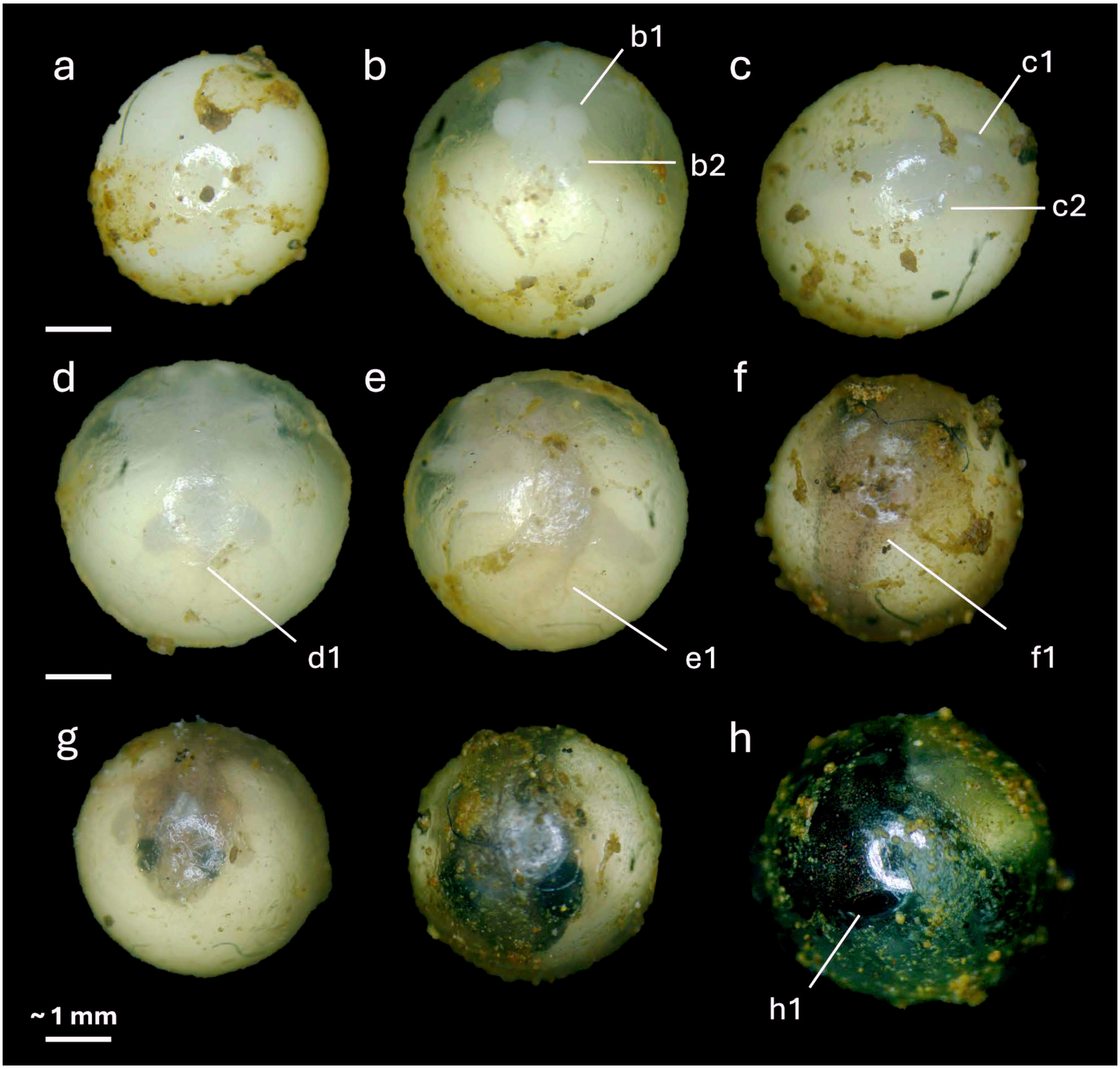

Figure 6. Selected stages of embryonic development in *Pristimantis latidiscus*. (a) Egg kept on soil 2 days after oviposition; (b) visible hindlimb-buds (b1) and tail-bud (b2) (tea filter; day 7); (c) visible ECD (c1) and fore-limb-buds (c2)(soil; day 7); (d) elongated tail with small fin (d1)(tea filter; day 9); (e) heavily vascularized fin (e1)(tea filter; day 9); (f) melanophores (f1)(soil; day 11); (g) tea filter (left) and soil placed egg (right) of equal age (day 15); (h) fully formed eye (h1)(soil; day 23). The maintenance substrate (compare methods), and age of the egg is given in brackets (compare Tables 2 and S2).

al. 2022), which were responsible for egg mortality in our study.

Our study gives the first report on incubation time in *P. latidiscus*. Length of incubation varies greatly in *Pristimantis* species, with at least some, like *P. reichlei* hatching less than a week after oviposition (Quinteros-Muñoz & Aguayo 2022). The incubation of *P. latidiscus* is longer (24–30 days at 20.5–26 °C), and comparable with most congeners with available data, e.g. *P. gaigei* (28–39 days at 21–26 °C), *P. guianensis* (25–33 days at 24.8–28.3 °C), *P. rosadoi* (21 days at ~ 23 °C), and *P. urichi* (~ 28 days at 25 °C; Hill et al. 2010, Nokhbatolfoghahai et al. 2010, Mônico et al. 2022, Rödel et al. 2024). In our study, disturbed eggs hatched significantly earlier than undisturbed ones, which indicate phenotypic plasticity in hatching time. However, the exact environmental cue that triggered hatching is not as clear as in other reported observations of ECH in direct developers, where eggs hatched immediately after mechanical disturbance (Buckley et al. 2005, Rödel et al. 2024). In cases where hatching is induced by vibration as a predation-cue, animals emerge from the egg within seconds after the disturbance (Warkentin 1995, 2005, Warkentin et al. 2007, Doody & Paull 2013). Hatching 24 hours after handling, as we observed, does not allow sufficient reaction to such an emergency.

We see two potential hypotheses to explain our observation. First, ECH could have been triggered by pathogens instead of vibrations. Early hatching to escape a spreading infection is known in amphibians (Warkentin et al. 2001, Warkentin 2011). As two eggs from the disturbance-treatment died due to a fungal infection, it is likely that pathogens spread in the egg cup, even though obviously infected eggs had been removed. However, we never observed signs of an infection on remaining eggs during our bi-daily checks, thus pathogen load must have been low. This hypothesis would also not explain why the only handled egg from the undisturbed cup (in which no obvious cases of infection were observed), hatched earlier than others from the same cup. Another explanation thus seems more likely. Hatching might have been triggered by handling, but an immediate response was not possible due to constraints of hatching mechanism or development (Warkentin 2011). The capacity of early hatching differs greatly between frog taxa. Some species of leaf breeding frogs can hatch up to 36% earlier than normal if under threat (Gomez-Mestre et al. 2008). In direct-developing frogs such shortening of the egg phase might not be possible, as hatchlings need to survive in a terrestrial habitat instead of water (Warkentin 2011, Gomez-Mestre et al. 2008, Delia et al. 2019). In *E. coqui*, froglets are competent to hatch 16% earlier than typical if mechanically disturbed (13 instead of 16 days; Townsend & Stewart 1985, Buckley et al. 2005). This is similar in scale to our observations in *P. latidiscus* (ca. 18%). However, Buckley et al. (2005) observed early hatchlings of *E. coqui* to leave their eggs underdeveloped, significantly smaller and less agile than normally incubated hatchlings. In our study, early hatched *P. latidiscus* were neither smaller than froglets from undisturbed eggs nor did they hatch significantly underdeveloped. High amounts of unabsorbed yolk in the earlier hatchlings were the only obvious difference. *Pristimantis latidiscus* might thus have an even more conservative hatching strategy, possibly affected by its chosen nest site underground. Hatchlings presumably would need a good constitution to be able to dig through mud, as compared to leaving those more accessible semi-enclosed nest sites used by *E. coqui*, like palm petioles, rolled dead leaves or bamboo (Townsend & Stewart 1986, Elinson et al. 1990, Westrick et al. 2022). Hatching too early could render *P. latidiscus* juveniles incapable of digging themselves from their nests (Warkentin 2011), leading them to hatch only after reaching a certain developmental threshold, even when faced with a potential threat (vibration during handling). Finally, underground eggs may face less predation risk and thus lower selection pressure on ECH. While predation pressure on buried clutches is presumably low, apart from the periods of oviposition and hatching (Riley & Litzgus 2014), the effectiveness of egg-burying to reduce predation risk is poorly studied. Documented cases of predation on eggs of direct-developing frogs are rare. In Central America, ants, flies, and snakes have been reported to prey on *Eleutherodactylus* eggs (Townsend et al. 1984, Stewart & Woolbright 1996, Savage 2002). Snakes in particular may be able to locate buried anuran clutches, as indicated by species preying on reptile eggs (Broadley 1979, Huang et al. 2011, Griesbaum et al. 2023).

Without more experimental data, best including species which differ in their egg deposition sites, the questions raised about hatching strategy and mechanism cannot be reliably answered. Nevertheless, our observations in *P. latidiscus* indicate that ECH is likely more widespread in direct developers than represented in literature. Further investigation into potential constraints on early hatching seems promising when trying to unravel ECH in frogs with direct development.

Embryonic development

We have presented here a first description of the embryonic development of *P. latidiscus*. The absence of external gills observed during development seems typical for strabomantids (Nokhbatolfoghahai et al. 2010, Goldberg & Vera Candioti 2015, and literature cited therein, Rödel et al. 2024, this study). A specialized tail is thought to take over the respiratory function (Nokhbatolfoghahai et al. 2010, Salica et al. 2023). The tail of *P. latidiscus* shows adaptations towards gas exchange (expansive and thin structure with high blood flow) and is similar to the tail of *P. urichi* (Nokhbatolfoghahai et al. 2010), which is to the best of our knowledge the only other *Pristimantis* species with a detailed description of its embryonic development. Their tail structures differ from known eleutherodactylids and brachycephalids by fins forming lateral and caudal to the axis instead of dorso–ventral, and a short tail axis that does not expand through most of the fin (Nokhbatolfoghahai et al. 2010; Goldberg et al. 2012, 2020; Salica et al. 2023; this study). The fins we observed in *P. latidiscus* were smaller than in *P. urichi*. They never encompassed over ¼ of the yolk, while almost fully encompassing it in *P. urichi* (Nokhbatolfoghahai et al. 2010). Furthermore, regression of the fins might begin earlier in *P. latidiscus* (comp. Table 2 and Nokhbatolfoghahai et al. 2010). Early and rapid tail regression in some *Pristimantis* species could explain why tails are not always seen when looking at embryos in later stages of development through the egg membrane (Rödel et al. 2024). While we only observed development from outside the egg and thus had restricted visibility of fine structures of the embryo, working with living specimens allowed us to simultaneously follow development, observe incubation time, and test for ECH. Our study has thus greatly increased overall knowledge of the reproductive biology of *P. latidiscus* but also shows how much remains to be learned concerning the biology of the largest genus of all vertebrates.

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Supplementary data

The following data are available online:

Supplementary Figure S1. Mesh vivarium used for maintaining pairs of *Pristimantis latidiscus*.

Supplementary Table S2. Development of *Pristimantis latidiscus* embryos in eggs kept on tea filter after day 11.