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Taste organ growth and development in the direct developing frog *Eleutherodactylus coqui* (Lissamphibia: Eleutherodactylidae)

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Abstract

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Previous research on amphibian taste organs concerned amphibians with a biphasic life history, that is, with larval period and metamorphosis. Direct developing frog species, such as *Eleutherodactylus coqui*, undergo a cryptic metamorphosis before hatching, and many larval-specific features are vestigial or have been lost entirely from their ontogeny. Taste buds are present in larval stages of biphasically developing anurans and are replaced by taste discs during metamorphosis. One goal of this study was to characterize the ontogeny of taste buds and/or discs in *E. coqui*. The other goal was to examine correlations between body size and taste organ density and size in different regions of oral epithelium. The research reveals the presence of only one type of taste organ, characteristic of metamorphs of biphasic amphibians, namely taste disc. In addition, taste disc density and the area of the taste disc sensory zone change dramatically during growth.

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Introduction

Previous studies of taste organs in Anura and Urodela were carried out on species with aquatic larvae, which feed and grow until they metamorphose into a terrestrial adult (indirect developers with a biphasic life history). In these species, the presence of two generations of taste organs was demonstrated: taste buds (TBs) in larvae (Nomura *et al.* 1979a; Żuwała and Jakubowski 1991) and taste discs (TDs) in metamorphs (De Han and Graziadei 1971; Graziadei and De Han 1971; Jasiński 1979; Richter *et al.* 1988; Żuwała and Jakubowski 1991, 1997, 2001; Witt 1993; Osculati and Sbarbati 1995; Żuwała *et al.* 2002). TBs do not give rise to TDs, such that TDs have a distinct developmental origin (Nomura *et al.* 1979b; Shiba *et al.* 1980; Żuwała and Jakubowski 1991; Takeuchi *et al.* 1997). The main difference between TBs and TDs is the presence in the latter of the differentiated non-sensory supporting cells: mucous cells, wing cells and glial-like cells (Żuwała and Jakubowski 2001).

The distribution of TDs is similar in the species investigated. They are located in the epithelium of the palate and the dorsal surface of the tongue. The size of the TD sensory zone (expressed as its diameter) varies in a large range from 45 to 90 μm in *Salamandra salamandra* (Żuwała and Jakubowski 2001), from 60 to 90 μm in *Hyla arborea* (Żuwała and Jakubowski 2004), from 130 to 137 μm in *Pelobates fuscus* (Żuwała 2002) to even from 200 to 250 μm in *Rana catesbeiana* (Jaeger and Hillman 1976). The literature lacks quantitative data to determine changes in the number and size of taste organs during individual life history. Such relationships concerning TBs have been demonstrated in some actinopterygians species, such as Channel catfish *Ictalurus punctatus* (Finger *et al.* 1991), Japanese sardine *Sardinops melanostictus* (Matsuoka 2001), African sharptooth catfish *Clarias gariepinus* (Mukai *et al.* 2008) and Iridescent shark *Pangasianodon hypophthalmus* (Mukai *et al.* 2010). The research indicates an increase in TB number during the growth of the individual, at least for fish.

In direct developing frogs, which have eliminated the free-swimming larvae, the developmental period before hatching is comprised of features that typically occur after hatching during the larval period and metamorphosis in indirect developers (Wake 1989). Members of the genus *Eleutherodactylus* exhibit the most extreme form of direct development and do not possess some larval characters, including lateral line organs, cement gland, larval mouthparts and a coiled gut (Sampson 1904; Lynn 1942; Townsend and Stewart 1985; Elinson 1990). To analyse how the evolution of direct development has affected the taste system, we have examined the species regarded as the classical example of a direct developer – the Puerto Rican coqui *Eleutherodactylus coqui* Thomas, 1966 (Townsend and Stewart 1985, 1994; Kerney and Hanken 2008; Kerney *et al.* 2010; Elinson and del Pino 2012; Elinson 2013).

The aims of the current study were to: (i) describe tongue and taste organ development in ontogeny of *E. coqui*; (ii) identify what type of taste organs are present in ontogeny of *E. coqui*; (iii) examine whether the size of the individual affects the density and size of its taste organs; and (iv) examine whether there are individual differences in density and size of taste organs in different regions of oral epithelium.

Materials and Methods

Various stages of *E. coqui* development were selected for the study: embryos (TS 7, TS 11, TS 13, TS 15) – according to Townsend and Stewart tables (1985), hatchlings (a few days after hatching) and adults. The adults were collected on the Big Island of Hawaii, under Injurious Wildlife Export Permits issued by the Department of Land and Natural Resources, Hawaii, to Richard P. Elinson, and housed in a reproductive colony at the Department of Biological Sciences, Duquesne University, Pittsburgh, PA, USA. The embryos and hatchlings were derived from the colony, and the hatchlings were within a few days after hatching, and had not yet started feeding. Two or three individuals representing each ontogenetic stage were used for qualitative investigation under light microscopy (LM) and scanning electron microscopy (SEM), and hatchling and adult samples were investigated under transmission electron microscopy (TEM). Additionally, six hatchlings and five adults were used in a quantitative study in SEM, and snout–vent length (SVL) was used as a measure of the overall size of the individual. The hatchlings were homogeneous in this respect (6 mm each), while in the adult group, there were three males and two females (Table 1).

The individuals were anesthetized in 0.1% tricaine (MS-222), and the cranial areas were processed. For LM, the heads were fixed in Bouin's fixative and decalcificated in solution of formic acid and formaldehyde (1:1) or EDTA solution. Then, the material was dehydrated in an increasing ethanol gradient, permeated by toluene and embedded in paraffin (60 °C). Paraffin sections (7 μm thick) were stained with haematoxylin and eosin. For SEM, the material was split into the palate and the oral floor with the tongue. Then, the epithelium was washed in 0.1 M cacodylate buffer (pH 7.2) and fixed immediately in Karnovsky's fixative for amphibians (pH 7.2) based on 0.1 M cacodylate buffer (see Karnowsky 1965). After dehydration in an increasing ethanol gradient and 100% acetone, the material was dried at the critical point of CO_2, and sputter coated with gold. The preparations were studied under a JSM-5410 (JEOL) scanning electron microscope in the Laboratory of Scanning Electron Microscopy and Microanalysis, Jagiellonian University in Kraków, Poland. For TEM, fragments of the palate and the tongue epithelium were fixed in Karnovsky's fixative for amphibians (pH 7.2) (see Karnowsky 1965) and a 1.0% osmium tetroxide solution was used as a second fixative. The preparations were dehydrated in ethanol and embedded in epon 812. Ultrathin epon sections after uranyl acetate and lead citrate contrasting were analysed under a Jeol – 2100HT microscope ((JEOL) in the Department of Cell Biology and Imaging, Jagiellonian University in Kraków, Poland.

Table 1 Mean density of taste discs/1 mm^2 of oral epithelium. Each value in the table is the average of four randomly selected fields of view

	Tongue		Palate	
SVL (mm)	Anterior	Posterior	Anterior	Posterior
6	875	300	0	25
6	725	725	0	25
6	550	825	0	25
6	625	675	0	25
6	500	575	0	25
6	625	525	0	25
Mean	650	604	0	25
♂ 34	46	40	5	13
♂ 35	46	44	4	11
♂ 37	36	34	2	10
♀ 39	30	29	13	11
♀ 44	19	21	12	8
Mean	35	34	7	11

Density of taste organs in the oral epithelium (calculated per 1 mm^2 of the surface) and size of taste organs expressed as an area of TD sensory zone were examined for all eleven hatchling and adult *E. coqui*. The study was performed on digital photographs of the SEM in four areas of the oral epithelium: palate – anterior and posterior (towards the throat) regions and dorsal surface of the tongue – anterior and posterior (towards the throat) regions. The boundary between the regions was established as a line dividing the tongue and the palate into two equal parts. Additionally, a buffer zone, where no measurements were performed, was arbitrarily determined. Within each region, four fragments of the epithelium (to determine TD density) and 20 TD sensory zones (to determine the area of TD sensory zones) were randomly selected. Furthermore, the diameter (the longest distance across an oval) of TD sensory zones of the tongue (without

anterior-posterior division) on the basis of 10 randomly selected TD sensory zones was measured. Measurements were performed using ImageJ (Schneider *et al.* 2012).

Spearman correlation (using all individuals, $N = 11$ and using only adults, $N = 5$) was used to investigate the association between SVL and TD density and the areas of TD sensory zones. Using TD density and the area of TD sensory zone as means of each oral epithelium region, one-way *t*-test comparing two means was used.

Results

Tongue and taste organ development

At stage TS 7 of *E. coqui* embryo development, the mouth aperture remains unperforated, and there is also no tongue fold nor any oral epithelial appendices (in sagittal section through the head in LM). In the oral floor epithelium of *E. coqui,* the tongue fold begins to shape in 11-day-old embryos (TS 11). In sagittal sections through the embryo oral cavity under LM, characteristic elevation in the front part of the oral floor is visible (Fig. 1B), while under SEM, the dorsal surface of the tongue fold is heart-shaped (Fig. 1A). In the central part of the tongue, there are two small hills that are not present in other regions or subsequent stages (Fig. 2A). On top of the hill, there was no sensory epithelium, which was confirmed by observations in LM. In addition, in sagittal sections through the tongue in the hills, there were no structures histologically distinguishing them from neighbouring areas. In the next stage (TS 13), the tongue fold gets flattened and begins to grow in the throat direction (Fig. 1C,D). At stage TS 15 in hatchlings, elongated tongue fold extends 1/2 to 2/3 the length of the oral floor. In the antero-central part of the tongue, a characteristic nodular elevation is visible (Fig. 1E,F). The tongue of the adult is roundish and attached to the oral floor in its front part. The posterior edge of the tongue is rounded, and the tongue does not cover the entire surface of the oral floor epithelium.

In the oral epithelium of *E. coqui* embryo from stage TS 7 onwards, no taste-bud-type taste organs were found. In the stages TS 11 and TS 13, the cells of the dorsal tongue epithelium form characteristic rosettes (Fig. 2B). While in the stage TS 15 on the dorsal surface of the tongue, there are numerous growing fungiform dermal papillae, whose upper surfaces are covered with a layer clearly distinguished by the epithelium (Fig. 2C) or even with sensory epithelium in some places (Fig. 2D). At this time, the palate epithelium remains smooth. In the hatchlings on the dorsal surface of the papillae, there are sensory zones of the taste organs (Figs 2D and 3A, C). A few sensory zones were also found in the smoothly outstretched palate epithelium (Fig. 3D).

In the adult, numerous taste organs are located in the epithelium of the tongue (Fig. 3B) and the palate (Fig. 3E), as well as on the inner surfaces of the mandible and maxilla. On cross-sections through the epithelium of oral cavity, TDs are found on the fungiform dermal papillae (Fig. 4A),

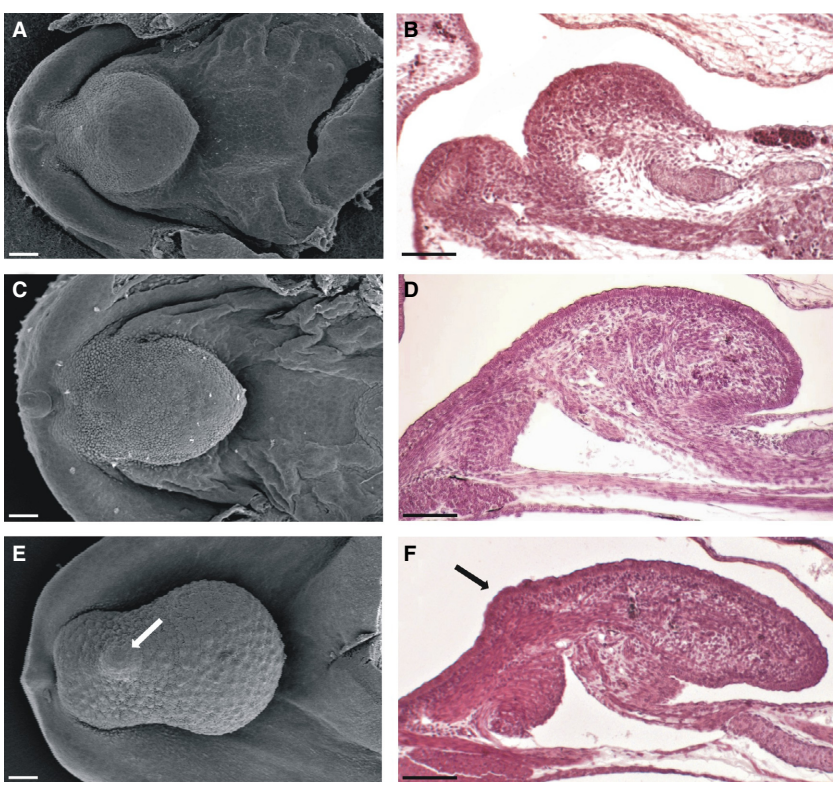

Fig. 1—Dorsal view (SEM) and sagittal section (LM) through the tongue fold of *Eleutherodactylus coqui* at embryo stage TS 11 (—**A**, —**B**), TS 13 (—**C**, —**D**) and TS 15 (—**E**, —**F**) with characteristic nodular elevation (arrow). Scale bar: 100 μm.

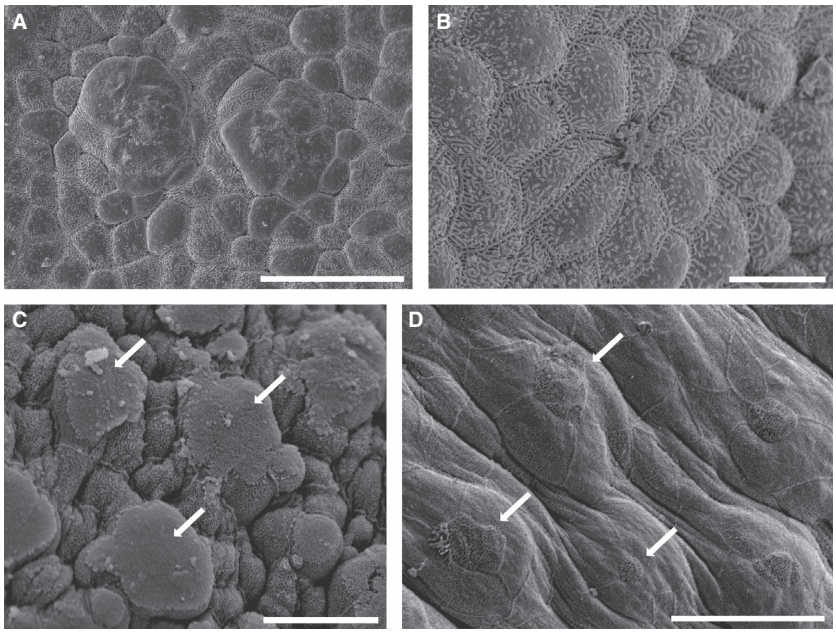

Fig. 2—Two small hills (—**A.**) and characteristic rosettes (—**B.**) on the dorsal surface of the tongue epithelium in TS 11 of *Eleutherodactylus coqui* embryo. Numerous growing dermal fungiform papillae covered with a layer of the epithelium (arrows) in TS 15 (—**C.**) and clearly visible sensory zones of taste discs (arrows) in hatchlings (—**D.**). Scale bar: A, D - 30 μm, B - 10 μm, C - 18 μm.

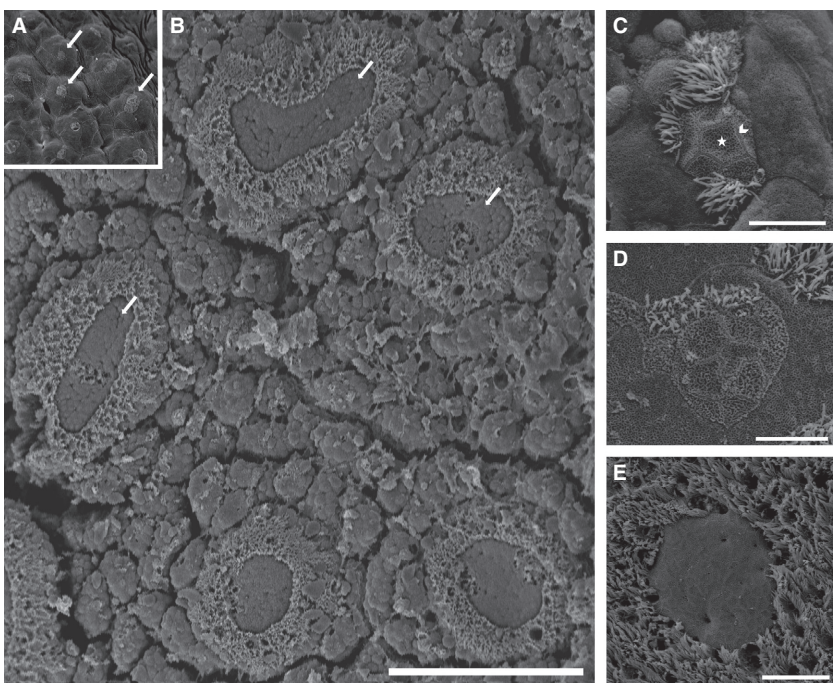

Fig. 3—Fragment of the dorsal surface of the tongue of hatchling (—**A.**) and adult (—**B.**) *Eleutherodactylus coqui* with visible taste disc sensory zones (arrows). Sensory zone of taste disc in the hatchling's tongue (—**C.**) and the palate epithelium of the hatchling (—**D.**) and the adult (—**E.**). Apical projections of mucous cell (asterisk) and wing cell (arrow head). Scale bar: A, B - 130 μm, C, D - 8 μm, E - 40 μm.

compared to TDs on the palate, which are found lying in the epithelium on the relatively smooth surface (Fig. 4B). TD sensory zones of the tongue are round and usually surrounded by a thick ring of ciliated epithelium (Fig. 3B). On the palate, TD sensory zones have a more oval shape and are surrounded by epithelial cells mostly covered with microvilli (Fig. 3E).

Within the TD sensory zone, there are visible apical projections of wing cells, mucous cells and taste receptor cells (Fig. 4C). The wing cells separate the mucous cells from each other. In contrast, between the apical parts of three wing cells, apical projections of taste receptor cells are visible: as a bundle of short microvilli and/or less frequently as a single apical projection (Fig. 4C,D). Differentiation of these associated (non-sensory) cells, characteristic to TD, is already visible under TEM in hatchling. Longitudinal sections of the apex part of the TD show mucous cells surrounded by narrow strips of cytoplasm of wing cells (with meandering cell membrane), as well as apical projections of taste receptor cells (Fig. 4D).

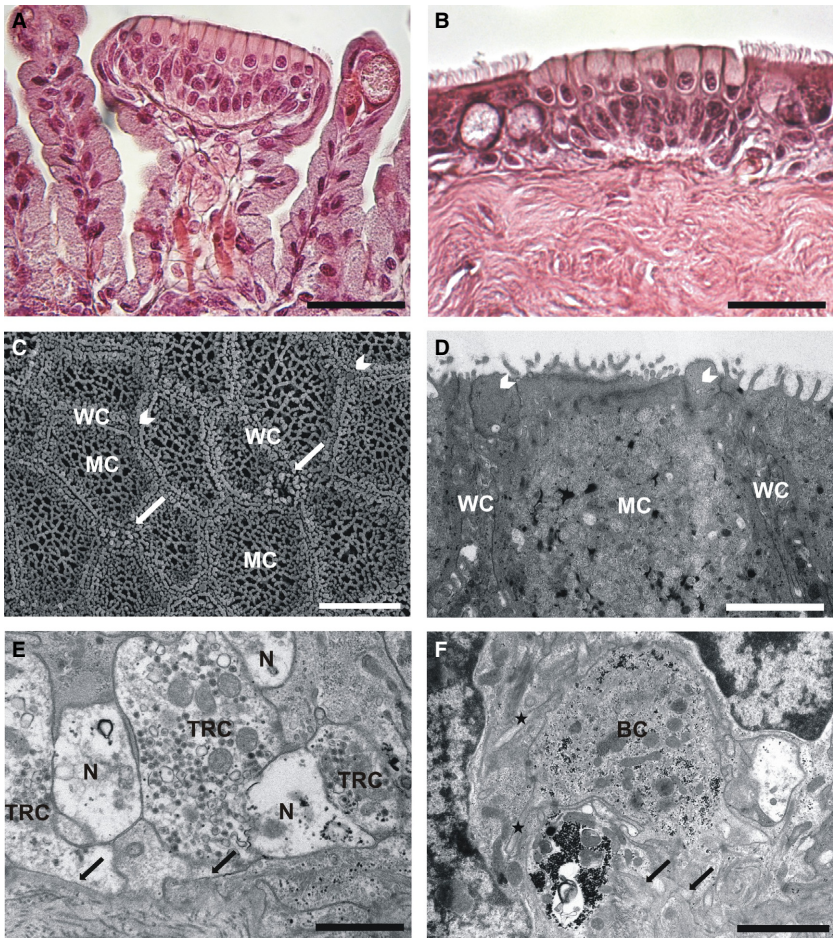

Fig. 4—Longitudinal section through a lingual (—**A.**) and palatal (—**B.**) taste disc (TD) from the oral cavity lining in adult *Eleutherodactylus coqui*. Fragment of TD sensory zone (—**C.**) with clearly visible apical projections of taste receptor cell as a bundle of short microvilli (arrow) and as a single apical projection (arrow head), wing cells (WC) and mucous cells (MC). Longitudinal section through the apical part of taste organ with visible apical projections of taste receptor cell as a single apical projection (arrow head), WC and MC (—**D.**). Taste receptor cell with numerous synaptic-like vesicles and nerve endings (N) in the basal part of the organ, above the basement membrane (arrows) (—**E.**). Fragment of the basal cell with two finger-like microvilli (asterisk) directed towards the basement membrane (arrows) (—**F.**). Scale bar: A, B - 50 μm, C - 5 μm, D, F - 2 μm, E - 1 μm.

Below, there are taste receptor cell bodies, whose cytoplasmic projections form a tangle with the nerve endings above the basement membrane (see Fig. 4E). The synaptic vesicles (electron-dense granules), of about 80 nm (N = 6) diameter, were observed in the cytoplasm of the basal part of the taste receptor cells. Their average diameter in the adult was about 100 nm (N = 12). Additionally, in the adult, the presence of basal cells (Fig. 4F) lying on the basement membrane and numerous afferent synaptic connections between taste receptor cells and nerve endings was found. The ultrastructure of cells forming TDs is similar to those of previously described species (see e.g. Osculati and Sbarbati 1995).

Quantitative comparison of hatchlings and adults

Randomly selected photographs of the anterior region of the palate of hatchlings did not reveal the presence of TD sensory zones, and therefore, TD density of the region was defined as zero (Table 1). Only systematic searching throughout the epithelium in the area resulted in finding a few TD sensory zones in each of the individuals. Due to the different methodology of their detection, they were not included in the results of Table 1. Additionally, in the posterior region of the palate in hatchlings, there were very few TD sensory zones, which made the sample size smaller (see Table 2, results with a letter s). Diameters of TD sensory zones of the tongue increase in size like the area of TD sensory zone, such that in hatchlings, the diameter ranges from 6.7 μm to 14.6 μm (mean=11.34 μm, N = 60), and in adults, it ranges from 37.9 μm to 103.1 μm (mean=66.76 μm, N = 50).

The mean TD density on the tongue was significantly higher in hatchlings than in adults (Fig. 3A,B), except for the anterior region of the palate (Table 1). A strong negative correlation between SVL and TD density in all tested regions of the epithelium was detected (Table 3). However, within adults, such a correlation was significant only in the tongue (Table 4). Mean area of TD sensory zones was many times larger (over 50 times higher on the tongue) in adults than in hatchlings (Table 2), and in each of the studied regions, a positive correlation between SVL and the area of TDs sensory zones was found (Table 3). In the case of adults alone, such a correlation was significant only in the anterior palate (Table 4).

In both hatchlings and adults, TD density was much higher in the epithelium of the tongue than of the palate (Table 1). However, the area of TD sensory zones in

Table 2 Mean area of taste disc (TD) sensory zone (μm^2). Each value not marked by an s is the average of 20 randomly chosen TDs. Each value marked with a letter s (small) is the average of 2–5 TDs that could be found on the hatchling palate

	Tongue		Palate	
SVL (mm)	Anterior	Posterior	Anterior	Posterior
6	46.9	31.7	106.7^s	105.6^s
6	63.9	73.7	115.4^s	129.4^s
6	49.8	57.5	79.4^s	135.1^s
6	81.2	57.6	153.3^s	119.7^s
6	60.6	62.2	142.3^s	153.7^s
6	79.8	73.7	83.9^s	117.6^s
Mean	63.7	59.4	113.5	126.8
♂ 34	3598.8	2524.5	2112.8	2911.9
♂ 35	2874.4	2319.7	1924.3	1889.4
♂ 37	2922.5	2875.7	2750.1	2536.5
♀ 39	3169.1	4335.7	4191.2	2587.3
♀ 44	3523.4	3844.8	4213.8	3248.4
Mean	3217.6	3180.1	3038.4	2634.7

Table 3 The *r* value of the Spearman test investigating correlation between snout–vent length (SVL) and taste disc (TD) density and between SVL and area of TD sensory zone within each of the investigated epithelium area in hatchlings and adults together. $N = 11$, $^*P < 0.05$

		Density	Area
Tongue	Anterior	−0.916*	0.818*
	Posterior	−0.907*	0.899*
Palate	Anterior	0.946*	0.907*
	Posterior	−0.657*	0.858*

Table 4 The *r* value of the Spearman test investigating correlation between snout–vent length (SVL) and taste disc (TD) density and between SVL and area of TD sensory zone within each of the investigated epithelium area in adults. $N = 5$, $^*P < 0.05$, the remaining: $P > 0.05$

		Density	Area
Tongue	Anterior	−0.975*	0
	Posterior	−0.900*	0.800
Palate	Anterior	0.500	0.900*
	Posterior	−0.820	0.400

hatchlings was almost twice as high on the palate as on the tongue. The area of TD sensory zones on the tongue was higher in adults, but not significantly (Table 2).

In hatchlings, the difference in TD density and the area of TD sensory zones between the anterior and posterior regions of the palate was significant (T-value = 5.0, $P < 0.05$).

However, in adults, there was no statistically significant difference in TD density or the area of TD sensory zones between the anterior and posterior region of the tongue and palate (Table 5). When analysing the above differences for each individual separately, in two of six hatchlings, the TD density was significantly higher in the anterior region of the tongue than in the posterior (T-value = 3.715, T-value = 3.051, $P < 0.05$). There were also numerous significant differences found in the group of adults. For example, in all three males examined, TD densities were significantly higher in the posterior region of the palate (respectively: T-value = 2.333 T-value = 2.216 and T-value = 2.324, $P < 0.05$). In the case of the area of TD sensory zone, significant differences were also noted.

Table 5 The *t*-value of the *T*-test comparing two means (anterior and posterior areas) of the tongue and palate in terms of taste discs (TDs) density and area of TD sensory zone in hatchling and adult group. $^*P < 0.05$, the remaining: $P > 0.05$

	Density		Area	
	Tongue	Palate	Tongue	Palate
Hatchling	0.494	5.0*	0.497	0.953
Adult	0.276	1.436	0.090	0.742

Discussion

Tongue and taste organs development

One of the first investigations concerning oral epithelium structure of *Eleutherodactylus* sp. was made on only one specimen (embryo) by Wassersug and Heyer (1988). The current study is detailed description of tongue and taste organ development in the ontogeny of a direct developing anuran.

Tongue development of *E. coqui* starts in the anterior region of the oral floor, similar to that so far examined in other anuran tadpoles (Nomura *et al.* 1979a; Żuwała 1991, 2002, 2005; Żuwała and Jakubowski 1997). The heart-shaped tongue fold in stage TS 11 of *E. coqui* can be compared in appearance with tongue fold at larval stage 39 (according to Gosner 1960) in *R. temporaria* (Żuwała 1991). The two small hills in *E. coqui* are located in a similar position as are found fingerlike papillae (with TBs in the apical part) of anuran tadpoles before metamorphosis (Nomura *et al.* 1979b; Viertel 1982; Żuwała 1991, 2002; Toyoshima *et al.* 1999). At stage TS 15 and in hatchlings, the tongue fold of *E. coqui* is comparable to that of Gosner stage 44 in *R. temporaria* (Żuwała 1991). Additionally, the oral epithelium of prehatching *E. coqui* does not form papillae, characteristic to nidicolous larvae of indirect developing anurans (Wassersug 1980; Viertel 1982; Wassersug and Heyer 1988; Żuwała 1991, 2002, 2005; Toyoshima *et al.* 1999). The lack of these papillae can be treated as an example of a lost larval character in *E. coqui* along side larval mouthparts (Elinson 1990), where endotrophy or

sufficient yolk supply obviates the need for larval feeding (Salthe and Mecham 1974; Elinson 1990).

The tongue of adult *E. coqui* is attached to the anterior part of the oral floor, as in the case of some previously examined Anura (Żuwała 1991, 2002, 2005; Toyoshima *et al.* 1999), while its posterior edge remains free, similar to *P. fuscus* (Żuwała 2002), *Hyla arborea* and *Rana esculenta* (Żuwała and Jakubowski 2004). Furthermore, the posterior edge is rounded as in *P. fuscus* (Żuwała 2002), *Bombina variegata* (Żuwała 1997) and *Bufo bufo* (Żuwała 1991, 2005; Żuwała and Jakubowski 1997).

In the ontogeny of *E. coqui,* TBs are lacking (absence in the embryo from stage TS 7 onwards), which contrasts to the presence of TBs in larval forms of indirect developing Anura (Nomura *et al.* 1979a; Żuwała 1991, 2002, 2005; Toyoshima *et al.* 1999). TD-type taste organs – characteristic to metamorphs of indirect developing amphibians (e.g. Jasiński 1979; Richter *et al.* 1988; Witt 1993; Osculati and Sbarbati 1995; Żuwała *et al.* 2002) – begin to form on the tongue fold between Gosner stage 32 and 33 in the form of small peaks (Żuwała 2005). At stage TS 15 of *E. coqui,* numerous peaks on the tongue epithelium become clearly visible under SEM. However, the surfaces of TD sensory zones are revealed from the mucous layer after hatching, while TD sensory zones of indirect developing species become visible at Gosner stage 41 (Żuwała 1991). Because *E. coqui* lacks a free-living larval form and acquires nutrition solely from yolk reserves prior to hatching and achieving the juvenile form, larval taste organs, namely TBs, likely have no application in the prehatching period. The presence of adult taste organs, namely TDs, is required presumably only when the individual starts to acquire food independently as a juvenile.

Eleutherodactylus coqui TD morphology and ultrastructure is similar to those of previously studied anurans (Żuwała and Jakubowski 1991; Osculati and Sbarbati 1995; Żuwała 2002). The results from TEM show that after hatching, TDs are still being formed and the first responses to stimuli are predicted based on the presence of synaptic vesicles, which could be compared to Gosner stage 41 of *R. temporaria* and *P. fuscus* (Żuwała 2002). However, the diameter of synaptic vesicles present in the cytoplasm of taste cells of hatchling *E. coqui* is similar to those of *R. temporaria* at Gosner stage 45 (Żuwała and Jakubowski 1991). In adults, taste organs are fully developed (presence of basal cells) and active (presence of full, numerous synapses).

Quantitative comparison of hatchlings and adults

The present study is the first quantitative analysis of TD density and size with respect to SVL in an amphibian. Previous research on taste organs (in indirect developing amphibians) usually included information only about the diameter of the sensory zone of TBs (in larvae) and/or TDs (in metamorphs) (e.g. Iwasaki and Kobayashi 1988; Żuwała *et al.* 2002). The diameter of TD sensory zones of adult *E. coqui* is similar to those of *H. arborea* (60–90 μm) and much smaller than in *R. esculenta* (100–150 μm) (Żuwała and Jakubowski 2004), *P. fuscus* (130–137 μm) (Żuwała 2002) and *R. catesbeiana* (200–250 μm) (Jaeger and Hillman 1976). This trend in TD sensory zone diameters among species may be related to body size where *E. coqui* and *H. arborea* are smaller frogs than *R. esculenta, P. fuscus* and *R. catesbeiana.*

Our results clearly show that hatchlings have higher TD density and smaller TD sensory zone area than adults. A significant correlation between TD density and TD sensory zone area with SVL could arise from individual growth, but also from the fact that only two very different stages were taken into account. Statistical analysis performed on the adults alone shows not so ubiquitous correlation, but it may be due to the fact that the sample size is low. Additionally, it may be affected by the differences of the measured parameters of taste organs between males and females, but this requires detailed research using a larger sample size. TD density in the epithelium of the palate, as well as of the tongue, probably decreases with the individual growth (which is also associated with the age), while the area of TD sensory zone increases. The results of some previous studies on amphibian taste organs also suggested the increase in the area of TD sensory zones, which was a larger diameter in older individuals. For example, after metamorphosis of *Hynobius dunni*, the diameter of TD sensory zones on the tongue ranges from 26 μm to 36 μm, while in older animals, which are presumably larger, it may reach as much as 56–71 μm (Żuwała *et al.* 2002). In fish, which have only TBs and not TDs throughout ontogeny, the range of taste organ location is much broader than in amphibians (Reutter and Witt 1993), and an increase of TB number on the surface of the head and trunk with the individual growth was observed (e.g. Finger *et al.* 1991; Matsuoka 2001; Mukai *et al.* 2008). However, Finger *et al.* (1991) investigating taste organs in *I. punctatus* at two size classes of individuals (small and large) achieved results similar to those obtained here for *E. coqui*. He showed that TB density on fins and on the surface of the body in small fish was slightly higher than in large. In addition, the average number of cells contained in the apical part of the TB was higher for large fish (Finger *et al.* 1991), which may indicate a growth of TB sensory zone with the size of the individual. Amphibians have indeterminate growth even after sexual maturity (Perrin and Sibly 1993); therefore, it can be assumed that their taste organs expand in size throughout life, but this finding needs to be confirmed in subsequent studies.

In hatchlings, small TD sensory zones first appear in high density in the dorsal surface of the tongue. TD sensory zones of the palate emerge much later in development, but they are greater in size than those of the tongue. The apical projections of the cells on the surface of the TD sensory zone form a characteristic honeycomb-like structure in the anuran metamorphs that have been examined (Iwasaki and Sakata 1985;

Iwasaki and Wanichanon 1993), and this structure was used as a criterion in their identification. The period after hatching in *E. coqui* seems to be a time of intensive TDs development (see Takeuchi *et al.* 1997), which may explain the different results in the measurements of TD density and the area of TD sensory zone. Furthermore, low number of TD sensory zones found in the epithelium of the palate (compared to those found in the adults) indicates the ongoing process of morphological differentiation in this stage of ontogeny. Within adults, the area of TD sensory zone on the tongue is slightly larger than that on the palate, but the differences are not statistically significant.

We determined the division into anterior and posterior region of the palate and the tongue with the aim of checking whether development of taste organs takes place in a uniform manner. We expected some differences particularly in hatchlings, which could reflect ongoing growth process at this stage. The differences in TD density and the area of TD sensory zones between anterior and posterior regions of the tongue and palate obtained for adults individually suggest the presence of some certain dependence; however, higher sample size is necessary to formulate reliable conclusions on this basis. Much greater TDs density on the tongue than on the palate is persistently significant, which is consistent with its dominant role in receiving taste stimuli.

Conclusions

The current results indicate that throughout the ontogeny of the direct developing frog *E. coqui*, there is only one type of taste organ, namely TD. The lack of oral papillae and TBs within the oral epithelium contribute to the distinctions between direct developers and indirect developing anurans. After hatching in *E. coqui*, hatchling and adults significantly differ in terms of TD density and area of the TD sensory zone. TD density is decreased in the epithelium both on the palate and on the tongue between hatchling and adult, which is at least partly compensated for by the increased area of the TD sensory zones. This developmental tendency may be more widely distributed within amphibians, as evidenced by results of research on other amphibians, both direct developing (*Plethodon cinereus*), as well as indirect developing (*Incilius alvarius*) species. Amphibians may possess a limited pool of TDs, which does not change during growth. To check this, further and broader investigations on direct and indirect developing species are warranted.

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