FRAGMENTA FAUNISTICA 67 (2): 85–92, 2024
PL ISSN 0015-9301 © MUSEUM AND INSTITUTE OF ZOOLOGY PAS
DOI 10.3161/00159301FF2024.67.2.085

A case report on a male common toad, *Bufo bufo*, with reduced size of the parotoid macroglands

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Abstract: Anurans secrete a wide diversity of toxins from their skin glands to defend themselves against predators, parasites and pathogens. Bufonid toads, both males and females, produce potent poison in their parotoid macroglands to avoid predation. Here, I provide a case report on a male common toad, *Bufo bufo* (Linnaeus, 1758), with the reduced size of parotoids. The parotoids' surface was reduced by 60% and 32% in the left and right macrogland, respectively, when compared to the mean surface of macroglands measured in 176 randomly selected males inhabiting the Barbarka woodland settlement in Toruń (central Poland). Such reduced-size parotoids might produce and store a lower amount of toxic poison that may affect predation avoidance, male-male rivalry during breeding season, and defence against microorganisms. It is the first observation of a male toad with such reduced parotoids in the studied area. Although variation in gland morphology among different anuran species have been discussed in the herpetological literature, it is still unclear if parotoid macroglands with reduced size occur more frequently in toads or if it is a rare and local phenomenon.

Key words: Anura, Bufonidae, chemical defence, male-male rivalry, predation avoidance, skin glands, toad poison

INTRODUCTION

Amphibian skin plays an important role in respiration, water balance maintaining, reproduction and defence against parasites, microorganisms and predators (Toledo et al. 1992, Toledo & Jared 1993, Clarke 1997, Tempone et al. 2008, Pinto et al. 2009). Two morphological types of exocrine glands are recognized in the amphibian skin: acinar glands (mucous or mixed glands) and syncytial glands (also known as serous, granular, or poison glands) with a lumen full of secretion (Jared et al. 2009, Regueira et al. 2016, 2017, O'Donohoe et al. 2019, 2021). Both gland types can be homogenously distributed throughout the body (called the ordinary glands) or grouped in limited skin regions, forming macroglands such as mental glands, ventrolateral glands, lumbar glands and parotoids (O'Donohoe et al. 2019, 2021).

Parotoid macroglands are located in the postorbital-supratympanic region (Fig. 1A and B) (Toledo et al. 1992, Clarke 1997) and act as efficient means of passive chemical defence (Jared et al. 2014). They are paired multiglandular protuberances formed by a group of large syncytial glands that comprise a pore, a secretory duct, a neck, and a secretory portion (also called the adenomere), surrounded by a layer of myoepithelial cells (Toledo & Jared 1995, O'Donohoe et al. 2019, 2021). The neck and secretory portion are embedded in the dermis, while the duct breaks through the epidermis onto the skin surface (Jared et al. 2009, Regueira et al. 2016, 2017, O'Donohoe et al. 2019, 2021). Parotoids evolved independently in different families of amphibians, including bufonid toads and salamanders, and present high variability among different species regarding their morphology, toxin content, and mechanisms of secretion release (Toledo & Jared 1995, Arntzen et al. 2013, O'Donohoe et al. 2019, 2021). Here, I report on a male common toad, *Bufo bufo* (Linnaeus, 1758), with parotoids presenting reduced size of surface, captured in the Barbarka woodland settlement in Toruń (central Poland) in early spring 2024, during migration of toads to the water reservoirs for breeding.

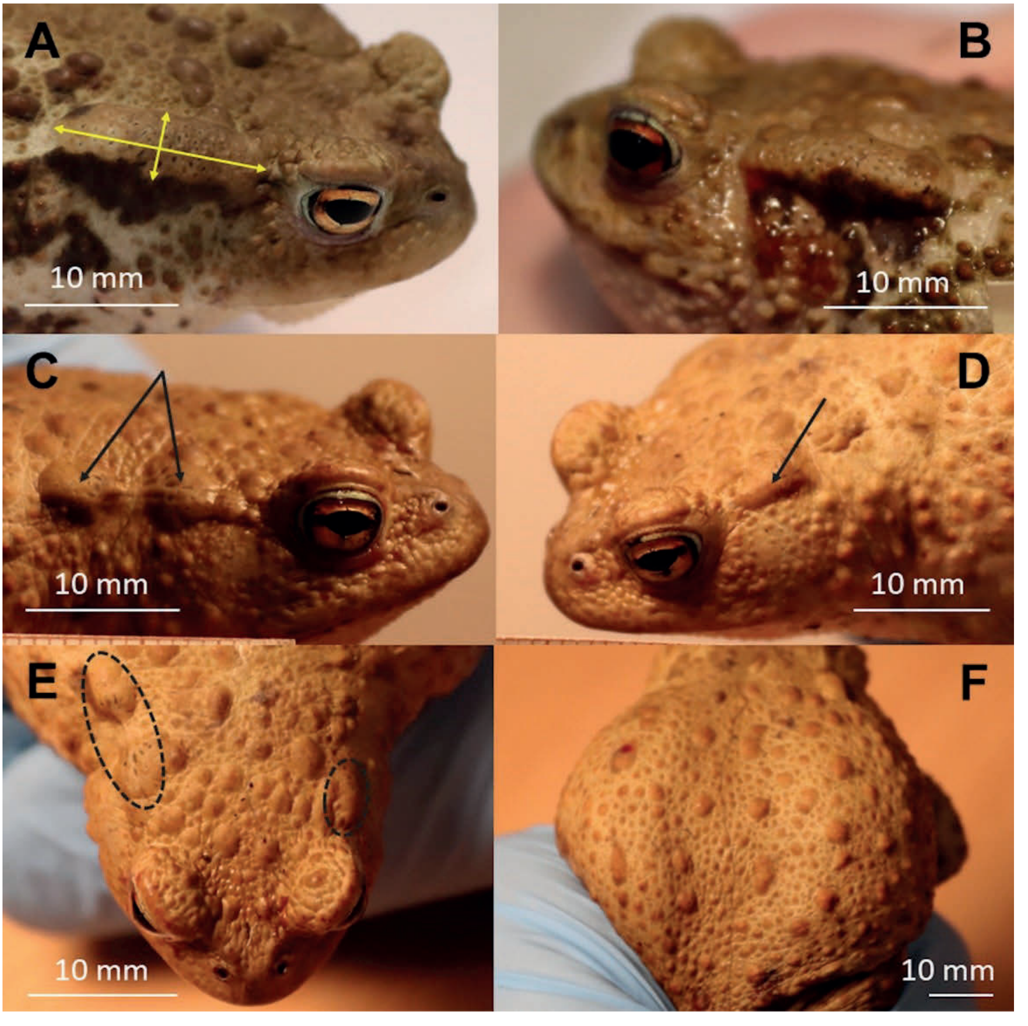

Fig. 1. Parotoid macroglands of the common toad, *Bufo bufo*. Parotoids of female (A) and male (B) toad. Right (C) and left (D) parotoid of a male common toad presenting the reduced size of gland surface. Right macrogland consists of two smaller glands separated by a gap (C), while left parotoid is a single triangle-shaped macrogland (D). Dorsal view of head with reduced-size parotoids (E) and the ordinary syncytial glands covering dorsal skin (F) of a male toad. Black arrows (C and D) and dashed ellipses (E) indicate parotoids. Yellow arrows (A) indicate the length and width of the macrogland. Photos by Anna Kowalczewska.

Material and Methods

Animal trapping and housing

Animals were trapped from March to April in 2021 (28 toads), 2022 (20 toads) and 2023 (42 toads), and from late February to March in 2024 (87 toads) in the Barbarka woodland settlement in Toruń, central Poland (53°00′ N, 18°35′ E), during the toad migration to water reservoirs. A male with parotoids presenting reduced size of surface was captured on 3rd March 2024. Toads were captured using 100 plastic buckets (10 L each) dug into the ground, interspaced with a 50 cm high plastic mesh fence. At the peak of migration season the buckets were checked twice a day: early in the morning and after dusk. Captured animals were identified and transported in boxes to laboratory, where their snout-to-vent length (SVL), body mass (m_b) and length and width of both (left and right) parotoid macroglands were measured. SVL, length and width of each macrogland were measured to the nearest 0.1 mm with an electronic caliper (digiMax type 2001; Wiha, Switzerland), and m_b to the nearest 0.1 g with an electronic balance (SPU402; Ohaus, USA). Toads were placed individually in aqua-terraria (46 × 30 × 28 cm, V = 39 L) with a mixture of peat and sand covering the bottom. Terraria were regularly irrigated to maintain high humidity and each terrarium contained a water tank to allow the toads to submerge in water, and a flower pot as a shelter. Animals had unlimited access to water and food (mealworm larvae). Toads were kept in the animal room under controlled conditions (air temperature: 20±2 °C; humidity: 50±10%; photoperiod: 12L:12D; lights on at 08:00 am) for a month. After completion of measurements, animals were released at the place of capture.

Morphological measurements of parotoid macroglands

To estimate the parotoids' size, I measured the length and width of each parotoid macrogland using an electronic caliper (digiMax type 2001, Wiha, Switzerland) (Bókony et al. 2019). Following earlier studies (Regueira et al. 2017, Bókony et al. 2019), I measured the parotoid length as the longest straight line between the anterior and posterior end of the gland, and the parotoid width as the longest straight line perpendicular to parotoid length, measured roughly at the middle of the gland (Fig. 1A). I did not measured the height of parotoids as that would have required histological procedures and killing the animals (Regueira et al. 2017). Then, I calculated the parotoid size as the upper oval area of a gland. I calculated the area of the oval as $\pi \times r1 \times r2$, where r1 = the longest straight line between the anterior and posterior portion of the oval divided by 2, and r2 = oval width measured as the longest straight line perpendicular to parotoid length, divided by 2 (Fig. 1A).

Data analysis

Data regarding a male common toad with reduced parotoids are presented as single values, while those provided for other 176 males with non-reduced parotoid macroglands as the mean value ± SD (standard deviation of the mean) of the indicated number of replicates (n). Before the statistical analysis, the normality of the data distribution was checked using the Shapiro-Wilk test. All variables (SVL, parotoid length and width, oval area) were normally distributed ($P > 0.05$). To indicate statistically significant differences in the mean parotoid's surface (oval area) between 176 randomly selected males with non-reduced macroglands and a male toad with reduced surface of parotoids, the Goodness of Fit Test was performed. The statistical analyses were carried out using R software (R Core Team 2018). Significant results were considered those with a P-value of $P < 0.05$.

RESULTS

Snout-to-vent length of a male common toad with reduced parotoids was similar to the mean SVL of other 176 males with non-reduced macroglands captured in the same localisation (65.8 mm and 64.0 ± 3.36 mm, respectively; Table 1), but its parotoids were considerably smaller than other males (Fig. 1C and D) and consisted of two smaller macroglands separated by a gap on the right side of head (Fig. 1C), and one triangle-shaped macrogland on the left side (Fig. 1D). These macroglands were still distinct from the ordinary syncytial glands covering the dorsal surface of the body (Fig. 1E and F).

The length of the left parotoid macrogland of the male with reduced parotoids was equal to 6.4 mm, while the width to 4.6 mm. The parotoid size was 23.11 mm^2 (Table 1). The length of the first right macrogland (located closer to the eye) was 4.8 mm, while the width was 5.1 mm.

The length of the second right macrogland (located behind the first gland) was 5.8 mm, while the width was 4.5 mm. The oval area of the two right macroglands was 19.22 mm^2 and 20.49 mm^2, respectively. The total size of the right parotoid was equal to 39.70 mm^2 (Table 1).

Table 1. Comparison of the snout-to-vent length (SVL), length, width and oval area of parotoid macroglands between the male common toad (*Bufo bufo*) with reduced parotoids and other males (N = 176) with non-reduced macroglands inhabiting the Barbarka woodland settlement in Toruń (central Poland). The values calculated for males with non-reduced glands are presented as the mean value ± SD.

		Left macrogland			Right macrogland		
	SVL [mm]	length [mm]	width [mm]	oval area [mm^2]	length [mm]	width [mm]	oval area [mm^2]
male with reduced glands	65.8	6.4	4.6	23.11	4.8[a] 5.8[a]	5.1[a] 4.5[a]	39.70[b]
males with non-reduced glands	64.0±3.36	13.2±1.13	5.5±0.60	57.70±8.15	13.3±1.16	5.5±0.58	57.96±7.90

[a]Two values are provided for a male with reduced parotoids, because its right parotoid macrogland consisted of two smaller glands separated by a gap (for details see the Results and Fig. 1C).
[b]The value obtained by summing the oval areas of two smaller glands forming the right parotoid macrogland (for details see the Results and Fig. 1C).

The mean length of the left parotoid macrogland of other males with non-reduced parotoids was equal to 13.2 ± 1.13 mm, while the width to 5.5 ± 0.60 mm. The mean length of the right macrogland was 13.3 ± 1.16 mm, while the width was 5.5 ± 0.58 mm. The oval area of the left parotoid macrogland was 57.70 ± 8.15 mm^2, while the oval area of the right macrogland was equal to 57.96 ± 7.90 mm^2 (Table 1). Thus, the surface of the left parotoid macrogland of the male with reduced parotoids was reduced by 60% (Goodness of Fit Test: $\chi^2 = 15.29$, $df = 1$, $P < 0.001$), while the surface of the right parotoid by 32% ($\chi^2 = 3.45$, $df = 1$, $P < 0.05$) when compared to the mean parotoids' surface measured in 176 randomly selected males with non-reduced macroglands inhabiting the studied area. It is the first observation of a male toad with such reduced parotoids in the Barbarka woodland settlement. Because this terrain is inhabited by around 600 individuals of this species, it may be assumed that reduced parotoids occur in ca. 0.5% of common toads from this population.

Discussion

Bufonid toads produce potent poison in their parotoid macroglands to defend themselves against parasites, microorganisms and predators (e.g., Abdel-Rahman et al. 2010, Gao et al. 2010, Banfi et al. 2016). Both, female and male toads possess parotoids (Fig. 1A and B) (Chen et al. 2017), and larger toads have larger parotoids in which they produce and store larger amounts of toxic secretions (Chen et al. 2017, Blennerhassett et al. 2019). In female toads, concentration of toxins in parotoids is expected to enhance the effectiveness of frontal displays to deter predators and facilitate the transfer of stored poison to eggs (called maternal transfer). In males, toxins stored in parotoids and widely dispersed across any part of the body may help to avoid predation and may be involved in male-male rivalry during mating (Chen et al. 2017). It raises an interesting question, if the reduced size of parotoids may have any negative consequences for male toads, especially in the context of predator avoidance, male-male rivalry during breeding season, and defence against microorganisms?

Adult male and female toads use the available habitats differently. Females often occupy sites with dense vegetation cover far from water, whereas males congregate around breeding ponds waiting for females (González-Bernal et al. 2015). Males that call females from exposed sites near water bodies may be more vulnerable to the attacks of avian predators (Beckmann &

Shine 2011). Therefore, they may benefit from a wide distribution of toxins across the dorsal surface of the body which is most likely to be contacted by an aerial predator (Chen et al. 2017). However, it must be emphasized that in the common toads the breeding season last from a few days to several weeks and then both males and females leave the pond and spend most of the year on the land (Davies & Halliday 1979, Höglund 1989). Also, during mating some single males leave the pond on sorties, possibly to find unpaired females (Reading & Clarke 1983). During these sorties from the pond and after breeding season male toads may be more vulnerable to the attacks of terrestrial predators, such as the grass snake (*Natrix natrix* Linnaeus, 1758), mustelids, hedgehogs or owls (Brodie 1977, Ewert & Traud 1979, Nilsson 1984, Sidorovich & Pikulik 1997). It has been shown that toxic secretions produced by skin glands may help to avoid predation by terrestrial predators. Poison stored in parotoid macroglands may be useful in deterrence of the grass snake which often attacks the toad's head and first of all comes into contact with the toxic secretions of parotoids. Similar hunting behaviour has been observed in hedgehogs preyed upon *B. bufo* (Kowalski et al. 2018). Thus, reduced parotoids' size and diminished chemical defence may also have negative consequences for male toads during encounter with a terrestrial predator.

Secondly, during breeding season, male toads compete vigorously for access to females, with rival males often usurping an already-amplexed male. In the cane toad, *Rhinella marina* (Linnaeus, 1758), the dorsal skin of male becomes spinose during the breeding season, and thus, together with toxic secretions, is expected to assist males to deter other male from displacing them during amplexus (Chen et al. 2017). A newly-arriving male usually ends up on top of the first male. In this position the spinose skin of the first male pushes directly against the thin ventral skin (including pelvic region) of the newly-arrived rival. Toxic secretions released from granular glands in that area could further repel the rival (Chen et al. 2017).

In the common toad, males usually gather at breeding ponds earlier and throughout the breeding season stay at the pond longer than females (Davies & Halliday 1979, Reading & Clarke 1983, Höglund 1989). Females arrive the pond either in amplexus or alone a few days later than most males. Spawning does not occur immediately on the arrival at the pond, but usually after a period of a few days to several weeks. During this period males compete vigorously for females. Male-male competition is manifested by active searching for females and intensive struggles for mates (Davies & Halliday 1979). Then, communal spawning takes place and males fertilize the eggs (Höglund 1989).

Males may obtain females in two ways, either by encountering and clasping onto the back of an unspawned female, gripping her pectoral girdles with his forearms and forming amplexus or by fighting and dislodging an already paired rival, thereby achieving a takeover (Davies & Halliday 1979). While encountering a pair in amplexus, a single unpaired male usually launches a vigorous attack and attempts to dislodge his rival from the female's back by holding onto the front of the paired female and pushing the paired male of her backwards with his hind legs or by squeezing in between the paired male and female from behind and pushing forward to force the paired rival out of amplexus (Davies & Halliday 1979). It has been shown that large males are more successful at obtaining a female than small ones. A large male is more likely to displace a small male from his female than vice versa (Davies & Halliday 1977, 1979). Small males that formed amplexus with large females are very easily dislodged, because they may be unable to grasp a large female properly. However, in the case of prolonged competition, a small male is likely to push himself in between a pair in amplexus and thus displace a large paired rival from the back of a large female (Davies & Halliday 1979).

As in the case of *Rhinella marina*, also toxic secretions stored in parotoids and dorsal skin glands of the common toads could possibly help males to displace a rival from the female's back. When a single unpaired male tries to squeeze in between the pair in amplexus, his dorsal skin comes into contact with the ventral skin of the rival, and may be irritating or even noxious to the already-amplexed male. Especially secretions of the dorsal ordinary glands seem to be useful in repelling the paired-rivals. It is likely that secretions of these glands may, at least partially, compensate lower amounts of poison produced and stored in smaller parotoids, and thus provide access to the females during breeding season.

Finally, secretions of dorsal and ventral skin glands of the striped toad, *Rhinella crucifer* (Wied-Neuwied, 1821), have been recently confirmed to have antimicrobial activity against *Enterococcus faecalis* and *Escherichia coli*, indicating an important role of the ordinary syncytial glands in protection against microorganisms (Gomes Barbosa et al. 2024). Thus, reduced size of parotoids does not necessarily mean a weaker chemical defence against pathogens, because the poison stored in the ordinary glands in dorsal and ventral skin may provide protection against them.

It has recently been shown that the morphology of toad parotoids may differ among populations of different *Bufo* species as well as within the same species. Arntzen et al. (2013) reported a great variation in parotoids' size and shape among populations of the common toad (*B. bufo*) and the spiny toad (*B. spinosus* Daudin, 1803), and showed that the length of parotoids of the common toad may be asymmetric. Future comparative studies should explain if there is a similar variation in parotoid's histology. Also, it cannot be excluded that the unusual morphology of parotoids observed in the male common toad described in this work is a result of some malfunction during ontogenetic development of glands. Histological analyses should help to answer this question. However, because histological procedures require killing the animal, in this work I performed only the morphological investigation of parotoid macroglands.

The Barbarka woodland settlement, where the male with reduced parotoids was captured, is inhabited by around 600 individuals of the common toad. Because the reduction of parotoids' size has been observed only in this one male so far, it may be assumed that reduced parotoids occur in ca. 0.5% of toads from this population. These results suggest that this anomaly is rather scarce within *B. bufo* population. Further observations should explain whether parotoids with reduced size occur more frequently in common toads or if it is a rare and local phenomenon.

Acknowledgements

I am grateful to the Director and employees of the "Barbarka" Forest School in Toruń for help in catching toads in the Barbarka woodland settlement; to Natalia Kalwasińska, Anna Kowalczewska and Anna Nowak from the Department of Vertebrate Zoology and Ecology (Faculty of Biological and Veterinary Sciences NCU, Toruń), and to members of the Student Research Association "Tetrapoda" and other volunteers for help in the field work, animal care and taking photos of toads. Trapping procedure, handling, housing of animals and all measurements were done in accordance with relevant guidelines and regulations. All experimental protocols were approved by licensing committees: the Local Ethical Committee for Ethics in Animal Research in Bydgoszcz, Poland (decisions # 15/2021, # 8/2022 and # 13/2023) and the Regional Directorate for Environmental Protection in Bydgoszcz, Poland (decisions # WOP.6401.4.30.2021.MO, WOP.6401.4.16.2022.MO and # WOP.6401.4.23.2023.MO).

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STRESZCZENIE

[Opis przypadku samca ropuchy szarej, *Bufo bufo*, ze zredukowanymi przyusznymi gruczołami jadowymi]

Płazy bezogonowe (Anura) produkują w skórze toksyczne wydzieliny wykorzystywane jako obrona chemiczna przeciwko pasożytom, bakteriom i drapieżnikom. Ropuchy (Bufonidae) aby uniknąć ataku drapieżnika produkują silne toksyny w przyusznych gruczołach jadowych, tzw. parotoidach. W niniejszej pracy opisuję przykład samca ropuchy szarej, *Bufo bufo* (Linneusz, 1758), ze zredukowanymi gruczołami zausznymi. W porównaniu do średniej powierzchni parotoidów, wyliczonej dla 176 losowo wybranych samców ropuchy szarej odłowionych na terenie Osady Leśnej na Barbarce w Toruniu (centralna Polska), powierzchnia lewego gruczołu zredukowana była o 60%, zaś prawego gruczołu o 32%. Parotoidy o tak zredukowanej wielkości mogą produkować i gromadzić mniej toksycznych wydzielin, co może wpływać na obronę przed drapieżnikami i patogenami oraz na konkurencję o samice pomiędzy samcami podczas okresu rozrodczego. Jest to pierwsza obserwacja samca ropuchy szarej z tak znacząco zredukowanymi gruczołami zausznymi na terenie Osady Leśnej na Barbarce. Choć zróżnicowanie w morfologii gruczołów skórnych płazów było dyskutowane w literaturze, nadal nie jest jasne, czy występowanie tak silnie zredukowanych parotoidów jest zjawiskiem powszechnie występującym u ropuch, czy ma charakter sporadyczny i lokalny.

Accepted: 7 October 2024