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Diverse anatomy of the tongue and taste organs in five species of caecilian (Amphibia: Gymnophiona)

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ABSTRACT

Limited previous studies on caecilian taste organs have demonstrated the presence of very few taste buds in the oral epithelium, while providing somewhat contradictory reports of their distribution within the oropharynx and across taxa. Here we report on the gross morphology of the tongue and explore the distribution, number and morphology of taste organs of five caecilian species representing five families, focusing upon variation within the group and investigating whether larvae and adults have the same type of taste organs. We find that taste buds are widespread in the oropharynx of caecilians and that they occur both in adults and larvae of a species with a biphasic life history. Thus Gymnophiona differ substantially from Batriachia, which have distinct larval and adult taste organs.

1. Introduction

Caecilian amphibians (Gymnophiona) are one of the three modern amphibian orders, currently comprising 204 named species. Adults of most caecilian species are soil-dwelling burrowers although some are active on the surface at least some of the time and a few have a derived aquatic lifestyle (e.g., Gower and Wilkinson, 2008; Taylor, 1968; Wilkinson, 2012). Roth et al. (1992) argued that the evolutionary diversification of modern amphibians is associated with many secondary simplifications of their sense organs and nervous system. In the case of caecilians, a soil-dwelling lifestyle led to the reduction of hearing (Maddin et al., 2012; Maddin and Sherratt, 2014; Roth et al., 1992; Wever, 1975) and vision (Badenhorst, 1978; Himstedt, 1996; Roth et al., 1992). Within caecilians, reduced vision relates, inter alia, to the covering of eyes by skin or by skin and bone, loss of intrinsic muscles, and reduction of extrinsic musculature, retina, lens and visual pigments (e.g., Mohun et al., 2010; Wake, 1985; Wilkinson, 1997). The least reduced eyes of adult caecilians occur in the Rhinatrematidae (Mohun and Wilkinson, 2015), the sister group of all other caecilians. Highly reduced eyes under bone are found in all (Chikilidae, Herpelidae) or some (Caeciliidae, Dermophiidae, Indotyphlidae, Siphonopidae) members of six of the 10 currently recognised families (Kamei et al., 2012; Wilkinson et al., 2011), indicating substantial convergent evolution of eyes and perhaps also of other sensory systems (see also Wilkinson, 1997).

Wake (1992) considered that the regressive evolution of some sensory organs in caecilians provided an opportunity for the development of new sensory organs: specifically, the paired tentacles that are unique among vertebrates and which are most likely related to touch and the chemosensory functions of Jacobson's (vomeronasal) organ to which the tentacular ducts connect (Badenhorst, 1978; Billo and Wake, 1987; Duenker, 1997; Himstedt and Simon, 1995; Schmidt and Wake, 1990; Wake, 1993). Caecilians are thought to have a well-developed sense of smell, and their olfactory organs extend about one-fourth of the entire length of the head (Lessa and Wake, 1992; Wake and Hanken, 1982). Chemoreception is considered likely to be one of the best developed sensory systems in caecilians (Jared et al., 1999) reflecting their soil-dwelling habitus and ecology and reduced eyes and ears. Past research of caecilian chemoreception has mainly addressed, and demonstrated considerable variation in, the morphology of olfactory organs (e.g., Schmidt and Wake, 1990), Jacobson's organ, and tentacles (Badenhorst, 1978; Billo and Wake, 1987), with very

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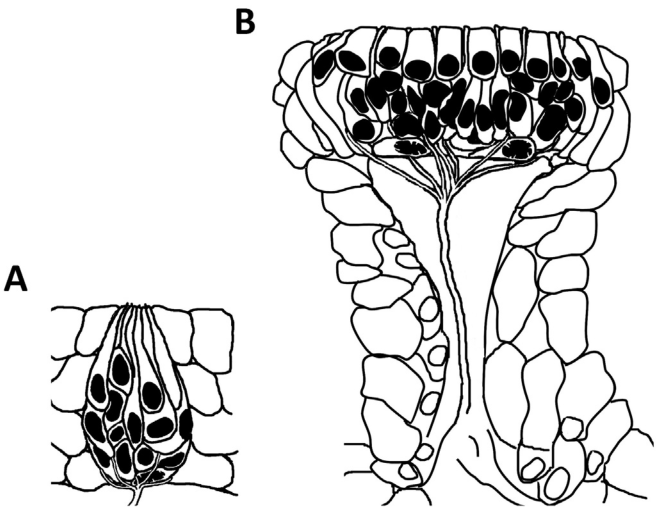

Fig. 1. Schematic diagram showing the typical structure of (A) taste buds of larval amphibians and adult caecilians and (B) taste discs on fungiform papilla of adult batrachians.

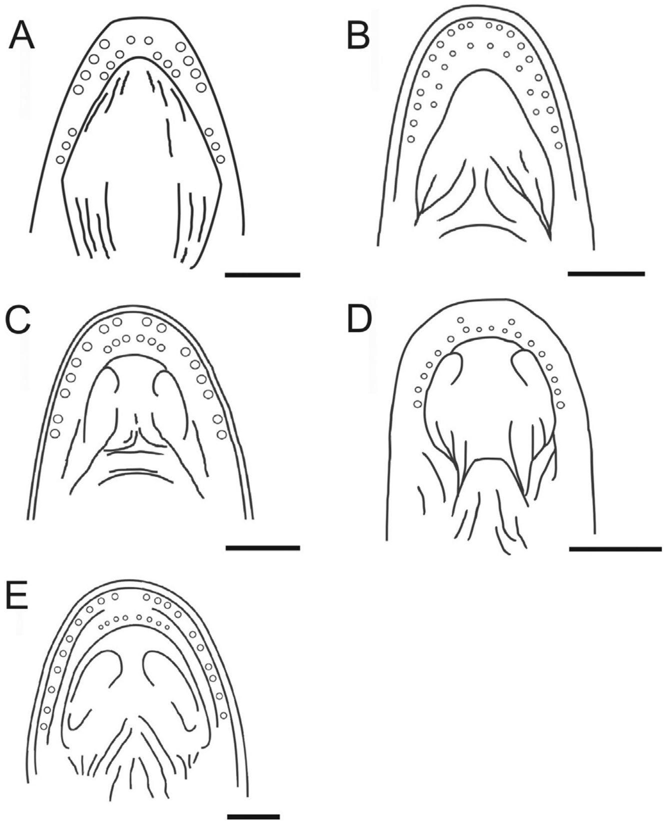

Fig. 2. Differentiation of oral floor structure in the studied adult caecilians: *Rhinatrema bivittatum* (A), *Ichthyophis* cf. *kohtaoensis* (B), *Herpele squalostoma* (C), *Idiocranium* sp. (D), *Typhlonectes natans* (E). Scale bar: 1.8 mm.

limited study of their function in prey detection (Himstedt and Simon, 1995).

Limited previous research on caecilian taste organs has agreed on there being very few taste buds (TBs) but has otherwise produced somewhat contradictory reports of their physical distribution within the oropharynx and of their taxonomic distribution (Budzik et al., 2013; Junqueira et al., 1999; Wake and Schwenk, 1986). In Batrachia (= Anura and Urodela) studied thus far there are, uniquely among vertebrates, two generations of taste organs (Fig. 1), with TBs in the lining of the oral cavity of larvae replaced by taste discs (characterized by associated nonsensory mucous, wing, and glial-like cells) at metamorphosis (Żuwała and Jakubowski, 1991, 1997, 2001, 2007). In contrast, TBs have been reported from both adult and larval caecilians, and taste discs are not known to occur within the group. Here we report on the distribution, number and morphology of taste organs of five caecilian species representing five families, focussing upon variation within the group and investigating whether larvae and adults have the same type of taste organs. Caecilian family-level taxonomy follows Wilkinson et al. (2011) and Kamei et al. (2012), and we follow Wilkinson and Nussbaum (2006) in using Teresomata to denote the group including all extant caecilians except for rhinatrematids and ichthyophiids.

2. Material and methods

We studied adults of five species of caecilians, and larvae of one of these (see Table 1) using scanning electron microscopy (SEM) and light microscopy (LM). *Typhlonectes natans* individuals were obtained from a colony maintained at the Municipal Zoological Garden, Łódź (Poland) and fresh materials prepared after lethal anaesthesia (0.1% tricaine mesylate, MS222). Other materials were dissected from previously fixed whole specimens. Where material was available from a single individual only, the oral floor (with pharynx) was cut longitudinally into two halves for separate SEM and LM study.

For SEM, material was dehydrated in ethanol and then acetone, dried at the critical point of CO_2, and sputter coated with coal and gold. Preparations were examined under JSM-5410 (JEOL, Tokyo, Japan) and HITACHI S-4700 (CISE, Hitachi, Japan) scanning electron microscopes in the Laboratory of Scanning Electron Microscopy and Microanalysis, Jagiellonian University, Poland. For LM, material was decalcified in a solution of formic acid and formaldehyde (1:1), dehydrated through an increasing ethanol gradient, permeated with toluene and embedded in paraffin at 60 °C. Paraffin sections were cut at 7 μm and stained with hematoxylin and eosin.

Total numbers of TBs were counted in three contiguous areas: anterior to the tongue near the teeth (teeth), the surface of the tongue (tongue), and the mucosa of the pharynx (pharynx). These numbers were determined for three species (*Ichthyophis* cf. *kohtaoensis, Idiocranium* sp., *T. natans*) from counts by a single observer (KAB) using serial sections of half of the oral floor (with pharynx) multiplied by two. The diameter and the area of TB sensory zones photographed during SEM were measured directly using ImageJ (Schneider et al., 2012).

Null hypotheses of no differences in numbers of TBs between the studied species or between different areas of oral mucosa were evaluated using chi-square tests. Due to the short length (on the LM sections) of the ('teeth') area anterior to the tongue, the expected value for each area (teeth, tongue, pharynx) was arbitrarily defined in a 1:2:2 ratio.

3. Results

All adults of the studied species have a muscular tongue, which is seemingly universal among adult caecilians (Taylor, 1968). In contrast, larvae of *Rhinatrema bivittatum* have a much less muscular, primary tongue (sensu Edgeworth, 1935) with a free anterior edge formed by the basibranchial copula that projects into the buccal cavity, as in larval *Uraeotyphlus* (Wilkinson, 1992). In the adult of *R. bivittatum* and in *Ichthyophis* cf. *kohtaoensis* the tongue is relatively flat, attached to the oral floor and has longitudinal ridges and grooves over most of the lingual surface (*R. bivittatum*) or only more posteriorly (*Ichthyophis* cf. *kohtaoensis*) (Fig. 2A and B). In contrast, the tongues of *Herpele squalostoma, Idiocranium* sp. and *T. natans* are