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Variation in the glossal skeleton arrangement of *Rhinatrema ron* (Gymnophiona: Rhinatrematidae) and its systematic implications

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The glossal skeleton in caecilians has been related to respiratory and feeding functions (e.g., Bemis et al. 1983, O'Reilly 1990, Carrier & Wake 1995), although its function is still not fully understood (Wake 2003). The arrangement of the glossal skeleton, particularly the number and fusions of arches, has been used in taxonomic and phylogenetic studies of caecilians (e.g., Nussbaum 1977, 1979, Wilkinson & Nussbaum 1996, Wilkinson 1997). Nussbaum (1977) described the Rhinatrematidae recognizing the uniqueness of its glossal skeleton among the Gymnophiona with 19 unique character states used to justify the familial status of this group. Differentiation of the two rhinatrematid genera *Rhinatrema* and *Epicrionops* is based on tail size and the number of ceratobranchial arches in the glossal skeleton of adults (Wilkinson & Nussbaum 2006). Herein, we describe the variation of the glossal skeleton arrangement in the recently described *Rhinatrema ron* Wilkinson & Gower, 2010 and compare it with the descriptions of the glossal skeleton of *Rhinatrema bivittatum* and *Epicrionops* spp. provided by Nussbaum (1977) and Wake (2003).

The six specimens studied are mature adults (fully developed gonads) collected in the Municipality of Oriximiná, Pará, Brazil, in the herpetological collection of the Museu Paraense Emilio Goeldi (Coleção Herpetológica Osvaldo Rodrigues da Cunha, MPEG), Belém, Pará, Brazil. We dissected five formalin-fixed specimens of *Rhinatrema ron* (MPEG 16975, female, 233 mm body length; MPEG 17435, female, 167 mm; MPEG 20168, male, 173 mm; MPEG 20170, male, 182 mm; MPEG 20173, female, 213 mm) by completely removing the glossal skeleton of the last four. One specimen (MPEG 27864, female) was cleared and double-stained following the protocol of Taylor & Van Dyke (1985).

Our examination revealed remarkable variation in the glossal skeleton arrangement of *Rhinatrema ron* (Fig. 1A–D). The hyobranchium of all specimens had the following character states in common, namely: (a) medially fused pairs of ceratohyals and first ceratobranchials; (b) ceratohyals and first ceratobranchials connected by the first basibranchial; (c) a second ceratobranchial not connected to the first ceratobranchial, considerably more slender and smaller than the first; (d) arytenoid cartilages positioned posteriorly of the hyobranchial arches. Two specimens (MPEG 16975 and 27864) do not have third ceratobranchials. MPEG 17435 has two pieces of cartilage (Fig. 1A) where a third ceratobranchial is usually expected to occur. In specimens MPEG 20168, 20170 and 20173, a different kind of arrangement was observed. The third ceratobranchial is present and linked medially to the second ceratobranchial arch (Fig. 1B-D). In all specimens, both the second and third ceratobranchials are shorter than the first. MPEG 20168, 20170 and 20173 show variation in shape and relative size of the second and third ceratobranchials. MPEG 20168 has a third ceratobranchial, which is smaller than the second ceratobranchial, with a U-shaped medial anterior indentation (Fig. 1B). MPEG 20170 has a posteriorly bifurcated second ceratobranchial on the left side (Fig. 1C), which probably represents a partial fusion of the second and third ceratobranchials; on the right side, the third ceratobranchial is well defined and slightly longer than the second. MPEG 20173 has well defined second and third ceratobranchial arches, with the second being larger than the third (Fig. 1D). In all specimens, the arytenoid cartilages lie posteriorly of the hyobranchial arches (Fig. 1).

Since the work of Nussbaum (1977), two new species of *Rhinatrema* were described, *Rhinatrema shiv* and *R. ron* (Gower et al. 2010, Wilkinson & Gower 2010). Recently, Maciel & Hoogmoed (2011), working on the description of a new species of *Rhinatrema* based on a series of 31 specimens, noted a resemblance of those specimens to

species of the genus *Epicrionops* in having a longitudinal vent in most specimens (except for one specimen with a transverse vent) instead of the transverse condition known for *Rhinatrema*. Prior to Maciel & Hoogmoed (2011), Wilkinson & Gower (2010) described a single specimen originating from Amazonas, Brasil, of this taxon under the name *Rhinatrema ron*. Wilkinson & Gower (2010) noted an additional feature by which *R. ron* resembles species of *Epicrionops*, namely a plicate palatal mucosa. Thus, only two synapomorphies are known for *Rhinatrema*: (1) a shorter tail in relation to *Epicrionops* and (2) the absence of a third ceratobranchial arch. Surprisingly, Wilkinson et al. (2011) did not discuss the state of the glossal skeleton in the diagnosis of either *Epicrionops* or *Rhinatrema*, and relied on the number of postcloacal annuli as the characteristic to differentiate these two genera (more or fewer than 10 postcloacal annuli). Here we demonstrate, even in our relatively small sample, that the retention of the third ceratobranchial in adults occurs in *Rhinatrema* as well as

in *Epicrionops* and even varies within a population of the same species.

Our observations of the glossal skeleton condition in *R. ron* agree with the statements of Nussbaum (1977) and Wake (2003) that there is a gradual reduction in size of the posterior ceratobranchials, and that the arytenoid cartilages lie posteriorly of the hyobranchial arches in the Rhinatrematidae. However, our analysis suggests that the glossal skeleton arrangement is not a synapomorphy of *Rhinatrema*. Wilkinson et al. (2011) remark that the reciprocal monophyly of *Epicrionops* and *Rhinatrema* is uncertain, and recently, Pyron & Wiens (2011) presented a molecular phylogeny of amphibians in which *Rhinatrema bivittatum* is the sister taxon to *Epicrionops niger,* rendering *Epicrionops* paraphyletic. Pyron & Wiens' (2011) hypothesis relies, however, on very limited taxon sampling (only two out of eight species of *Epicrionops,* and one out of three *Rhinatrema* species were sampled). The above evidence suggests that a systematic review of the family Rhinatrematidae as
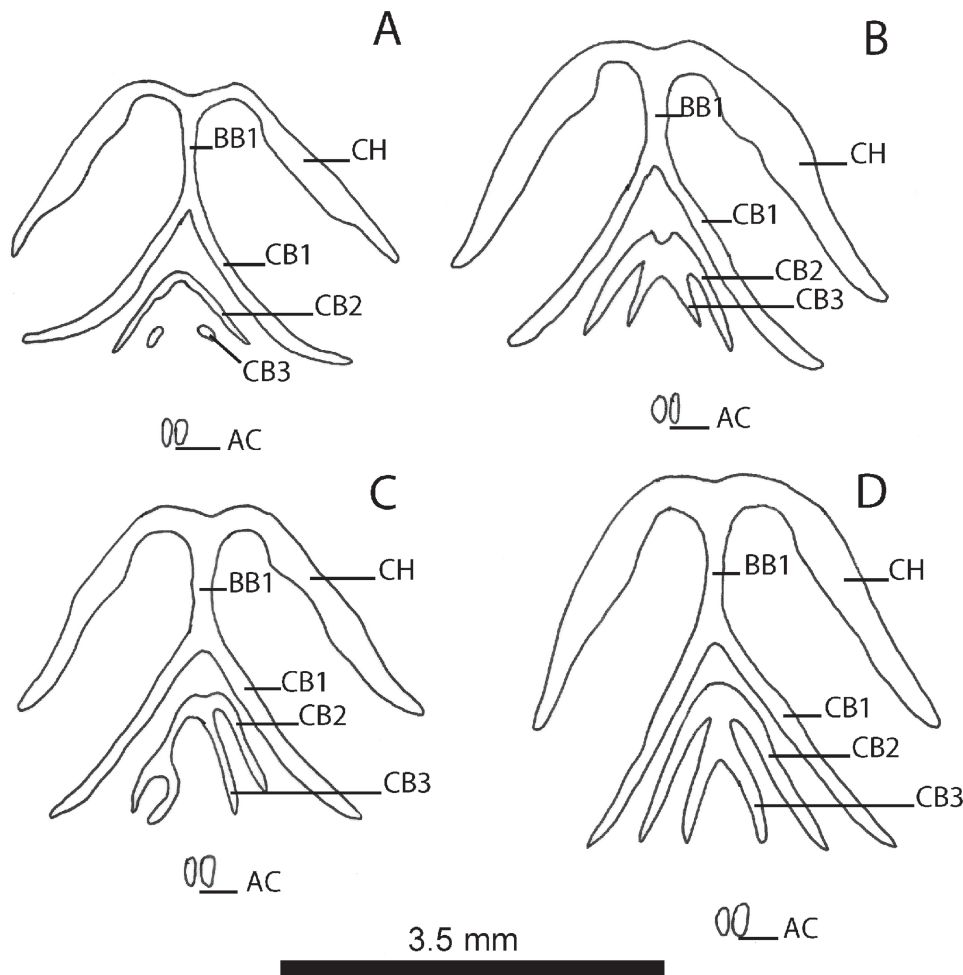

Figure 1. Glossal skeleton arrangement variation in *Rhinatrema ron* in ventral view. (A) MPEG 17435; (B) MPEG 20168; (C) MPEG 20170; (D) MPEG 20173. BB1 – first basibranchial arch; CH – ceratohyal; CB1, CB2, and CB3 – first, second and third ceratobranchials, respectively; AC – arytenoid cartilages.

a whole is warranted. However, larger taxon sampling of both genera is indispensable before any conclusive statement about their relationships and taxonomy can be made.

There is no report on variation in the hyobranchial arrangement of adult caecilians, as we have presented here. It appears that, at least in the studied population, metamorphosis might be occurring in different ways, regarding the fusion of paired larval elements. Wake (1982) reported from an adult specimen of *Epicrionops bicolor*, an anomalous partially ossified CB3 instead of the cartilaginous condition expected for adult specimens, but no specimen in her large series exhibited an asymmetrical pattern of remaining elements as we found here in one case. Additional specimens and more comprehensive studies on the development of the hyobranchium are necessary to elucidate whether the reported variations also occur in different populations of *R. ron*, as well as in other caecilian species.

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