1998

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Recommended Citation
de Sá, Rafael O., and Shannon Hill. "Chondrocranial Anatomy and Skeletogenesis in *Dendrobates auratus*." *Journal of Herpetology* 32, no. 2 (1998): 205-10. doi:10.2307/1565298.

Journal of Herpetology, Vol. 32, No. 2, pp. 205–210, 1998

Chondrocranial Anatomy and Skeletogenesis in *Dendrobates auratus*

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ABSTRACT.—**The larval chondrocranium and visceral skeleton of *Dendrobates auratus* is described and compared with those of other dendrobatids. Four characters, i.e., lack of fusion between orbital cartilages and otic capsules, wide processus muscularis palatoquadrati, lack of processus pseudopterygoideus, and lack of processus anterolateralis hyalis, represent derived conditions for *Dendrobates* within Dendrobatidae. Cranial and postcranial ossification sequences are reported for *D. auratus* and *Epipedobates tricolor*. Skeletogenesis is earlier in *E. tricolor*, but the overall pattern of ossification is similar in the two species.**

RESUMEN.—**Se describe el condrocráneo y esqueleto visceral de *Dendrobates auratus* comparándolo con el de otros dendrobatidos Cuatro características—no fusión de cartílagos orbitales con cápsulas óticas, proceso muscular del palatocuadrado ancho, ausencia de proceso pseudopterigoideo, y ausencia de proceso anterolateral hyalis—representan estados derivados para *Dendrobates*. Se reportan la secuencia de osificación cranial y post-cranial para *D. auratus* y *Epipedobates tricolor*. La osificación es más temprano en *E. tricolor*, pero el patrón general de osificación es similar en las dos especies.**

The family Dendrobatidae consists of approximately 157 recognized species clustered in six genera (Frost, 1985; Ford, 1993). The genus *Dendrobates* comprises 47 recognized species whose combined distribution extends from southern Nicaragua throughout South America, reaching Bolivia and Peru. *Dendrobates auratus* (Girard, 1855) is found from southern Nicaragua to Colombia at elevations of 0–800 m.

Chondrocranial anatomy has been reported for only seven species of Dendrobatidae (Haas, 1995), representing about 4% of known species of dendrobatids. Data on skeletogenesis has not been reported for any species in the Dendrobatidae. Herein, we describe the chondrocranial anatomy and ossification sequence of *Dendrobates auratus*. Skeletal development of *D. auratus* is compared with that of *Epipedobates tricolor*.

MATERIALS AND METHODS

Tadpoles were staged following Gosner's table (1960). Specimens were cleared and double-stained for bone and cartilage using Alizarin Red S and Alcian blue respectively, following the technique of Dingerkus and Uhler (1977). A total of 26 tadpoles of *Dendrobates auratus* (stages 29–44, and one juvenile) and 28 tadpoles of *Epipedobates tricolor* (stages 29–46) were examined. Specimens are deposited in the National Museum of Natural History, Washington D.C. (*Dendrobates auratus* USNM 509456–509482; *Epipedobates tricolor* USNM 509483–509510).

The chondrocranium of *Dendrobates auratus* is described based on a stage 32 tadpole, this corresponds to the latest larval stage before cranial ossification was observed. Terminology follows that of de Sá (1988) and Haas (1995). Observations and illustrations were made using a Wild MC3 stereomicroscope with the aid of a camera lucida attachment.

RESULTS

The lower horny beak of *Dendrobates auratus* larvae is supported by two cartilagines infra-

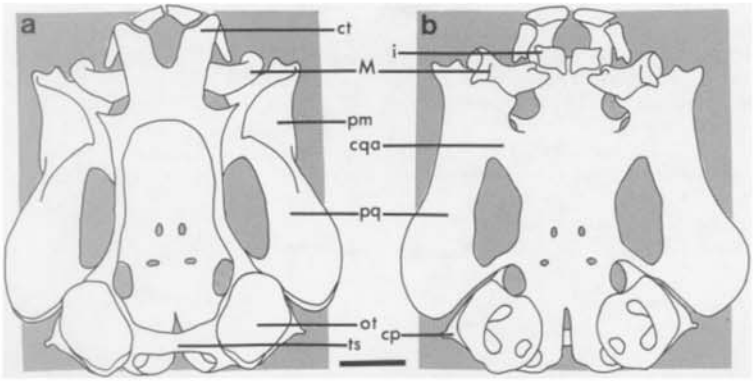

FIG. 1. Chondrocranium of *Dendrobates auratus* (USNM 509465), Gosner stage 32. a) dorsal view and b) ventral view. Bar = 1.0 mm. ct = cornua trabeculae, cqa = commissura quadratocranialis anterior, cp = crista parotica, i = infrarostral, M = Meckel's cartilage, ot = otic capsules, pm = processus muscularis, pq = palatoquadrate, ts = tectum sinoticum.

rostrales. These cartilages are small and square shaped. They are medial and anterior to the cartilago Meckeli and ventral to the cornua trabeculae. The upper horny beak is supported by a quadripartite cartilago suprarostralis. The cartilago suprarostralis consists of two medial elements, the pars corporis, and two lateral and slightly concave cartilaginous plates, the pars alaris. All four elements articulate with the anterior tip of the cornua trabeculae. The cornua trabeculae are short, representing approximately one-ninth of the total chondrocranial length (Fig. 1a). The cornua trabeculae are interconnected posteriorly by the planum ethmoidale with which they are continuous; the cornua trabeculae have a small processus lateralis trabeculae. The planum trabeculare anticum forms the anterior portion of the chondrocranial floor and meets the planum ethmoidale forming the anterior wall of the braincase.

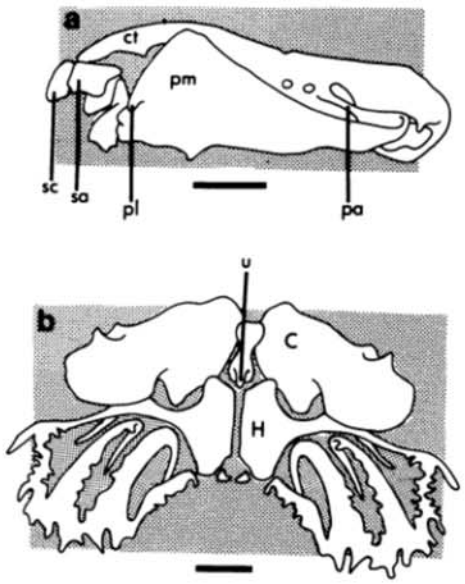

FIG. 2. *Dendrobates auratus* (USNM 509465), Gosner stage 32. a) lateral view of chondrocranium, b) ventral view of hypobranchial apparatus. Bar = 1.0 mm. C = ceratohyalia, ct = cornua trabeculae, H = hypobranchial plate, pa = processus ascendens, pl = processus lateralis of the muscular process, pm = processus muscularis, sa = pars alaris of suprarostral, sc = pars corporis of suprarostral, u = urobranchial process.

The cavum cranii is enclosed laterally by the cartilago orbitales, forming the lateral walls of the braincase, and ventrally by the chondrocranial floor. During early larval stages, the midsection of the chondrocranial floor possesses a thin sheet of cartilage, the planum intertrabeculare, with a large fenestra basicranialis. In later development, the planum intertrabeculare chondrifies completely and only two pairs of foramina remain open. The most posterior pair corresponds to the foramen caroticum primarium, while the anterior ones are the foramina craniopalatinum (Fig. 1b).

In lateral view (Fig. 2a), the cartilago orbitales are low throughout development; consequently the brain is visible and extends past the dorsal margin of the cartilago orbitales. The cartilago orbitales forms the lateral edges of a large and wide fenestra frontoparietalis. At stage 32, *Dendrobates auratus* lacks taenia tecti transversalis and taenia tecti medialis; however, the latter will form later. The cartilago orbitales are continuous with the planum ethmoidale anteriorly and dorsally, and with the trabecula cranii ventrally. Later in development the cartilago orbitales are also continuous with the tectum anterius. Posteriorly, the cartilago orbitales fuse with the capsulae auditivae. The lateral walls of the cartilago orbitales have several foramina. The foramen

opticum (anterior) and the foramen oculomotorium (posterior) are small and about equal in size. These foramina are separated by the pila antotica and found anteriorly to the point of attachment of the processus ascendens palatoquadrati to the braincase. The foramen trochleare is anterodorsal to the foramen opticum and partially visible as a small indentation on the dorsal margin of the cartilago orbitales.

Two large foramina, the foramina prooticum, on the right and left sides of the posterior region of the braincase are defined anteriorly and dorsally by the cartilago orbitales; ventrally, by the planum basale (posterior part of the chondrocranial floor); and posteriorly, by the capsulae auditivae. The capsulae auditivae have a larval crista parotica represented by a lateral and slightly expanded edge of cartilage that projects away from the chondrocranium. The cristae parotica have a small lateral process, the processus anterolateralis of crista parotica. This process does not reach the posterior curvature of the palatoquadrate and no processus oticus is present in this species. The otic capsules are interconnected dorsally by a bridge of cartilage, the tectum synoticum; which is anteriorly continuous with the cartilago orbitales. During stage 39, an anterior medial growth of the tectum synoticum forms a short and blunt taenia tectii medialis. The fusion of the tectum synoticum and the posterior edge of the otic capsules curves ventrally forming the arcus occipitalis and gives rise to the occipital condyles. The arcus occipitales are continuous with the planum basale. The occipital condyles and the planum basale enclose the foramen jugulare. In ventral view, the foramina jugulare appear at about the same level, but dorsal to, the foramina perilymphaticum inferior; the latter are located on the ventral side of the otic capsules. In ventral and lateral views a large fenestra ovalis is clearly visible on the otic capsules. A deep chordal notch divides the planum basale along the midline.

The palatoquadrate attaches to the lateral walls of the braincase anteriorly by the commissura quadratocranialis anterior and posteriorly by the processus ascendens. The commissura quadratocranialis anterior is broad and projects slightly dorsally (in an about 30° angle with the frontal plane) connecting the palatoquadrate with the planum trabeculare anticum. A well developed processus antorbitalis is found on the anterior edge of the commissura quadratocranialis anterior, at the point of contact with the planum trabeculare anticum. However, the commissura quadratocranialis anterior lacks a processus quadratoethmoidale and a processus pseudopterygoideous. Later in development (stage 42), the planum ethmoidale extends laterally on each side of the anterior braincase and above the processus antorbitalis to form the tectum nasi. Furthermore during stage 43, the planum ethmoidale lengthens anteriorly forming the tectum anterius, which in turn becomes posteriorly confluent with the cartilago orbitales. The processus ascendens attaches to the pila antotica at the level of and posterior to the foramen oculomotorium, and dorsal to the trabecula cranii. The processus ascendens forms the posterior curvature of the palatoquadrate; its most posterior edge reaches the midlevel of the fenestra ovalis (Figs. 1, 2a). Anteriorly, the palatoquadrate possesses a wide and well-developed processus muscularis palatoquadrati. The processus muscularis palatoquadrati tapers posteriorly and uniformly; consequently the arcus subocularis appears wide in lateral view. The dorsal tip of the processus muscularis palatoquadrati lies lateral and slightly ventral to the tectum nasi; it lacks a chondrified commisura quadratoorbitalis. The pars articularis quadrati, the most anterior end of the palatoquadrate, is short and articulates with Meckel's cartilage by three blunt processes. At the transition between the anterior margin of processus muscularis palatoquadrati and the pars articularis quadrati a small process projects laterally. We refer to this process as the processus lateralis of the processus muscularis.

Meckel's cartilage is sigmoid shaped and has three processes: the processus dorsomedialis, projecting dorsally towards the cornua trabeculae; the processus retroarticularis, which curves posteriorly and ventrally to articulate with the pars articularis quadrati; and the processus ventromedialis, the anterior process that articulates with the infrarostral.

The hypobranchial apparatus articulates through the condylus with the ventral area of the processus muscularis palatoquadrati, the facies articularis hyalis of the palatoquadrate. The ceratohyalia consists of two clearly identifiable portions: a medial, horizontal portion and a lateral portion, the latter projects posteroventrally from the medial portion (Fig. 2b). The medial portion has a short and poorly defined process on its anterior edge, the processus anterior hyalis, and a well developed processus posterior hyalis, on its posterior edge. The processus anterolateralis hyalis is found on the anterior edge of the medial portion of the ceratohyal, whereas the condylus is on the anterior edge of the lateral portion of the ceratohyal. A poorly chondrified pars reuniens overlaps ventromedially, but does not fuse with, the ceratohyals. The pars reuniens is continuous with the copula posterior which possess a well defined processus urobranchialis. The copula posterior is free from the plana hypobranchiales.

TABLE 1. Skeletogenesis in *Dendrobates auratus* and *Epipedobates tricolor*. Endochondral elements are in boldface; elements associated with a single stage appeared simultaneously in the specimens examined.

Gosner stage	*D. auratus*		*E. tricolor*	
31			Frontoparietal	Parasphenoid
			Exoccipital	**Vertebral centra**
			Neural arches	
33	Frontoparietal	Parasphenoid		
34	**Exoccipital**	**Vertebral centra**		
	Neural arches			
36			**Prootic**	
37	**Femur**	**Tibiofibula**	**Femur**	**Humerus**
38	**Transverse processes**		**Transverse processes**	
	Humerus	**Scapula**	**Tibiofibula**	**Scapula**
	Radioulna	**Tibiale-fibulare**	**Hypochord**	Cleithrum
			Ilium	
39	**Coracoid**	**Clavicle**	**Radioulna**	**Tibiale-fibulare**
	Hypochord			
40	**Prootic**	**Ilium**		
	Proximal **Metacarpals**			
	Proximal **Metatarsals**			
41	Cleithrum		**Operculum**	**Clavicle**
	Medial **Metatarsals**		**Coracoid**	
	Medial **Metacarpals**		Proximal **Metacarpals**	
			Proximal **Metatarsals**	
42	Alary process of Premaxilla		Alary process of Premaxilla	
	Septomaxilla		**Ischium**	
	Distal **Metatarsals**		Medial **Metatarsals**	
	Distal **Metacarpals**		Medial **Metacarpals**	
43	Nasals	Maxilla		
	Dentigerous process of Premaxilla			
	Palatine process of Premaxilla			
44	Ventral ramus of Squamosal		Palatine process of Premaxilla	
	Ischium	Dentary	Angulosplenial	
			Distal **Metatarsals**	
			Distal **Metacarpals**	
45			Nasals	Maxilla
			Pterygoid	**Columella**
Post metamorphic	**Columella**	**Mentomeckelian**	Dentary	Septomaxilla
	Vomer	**Carpals**	Vomer	Quadratojugal
	Pterygoid	Other **Tarsals**	Squamosal	**Carpals**
	Quadratojugal	**Operculum**	Dentigerous process of Premaxilla	
	Posterior and anterior rami of Squamosal		**Mentomeckelian**	
	Angulosplenial		Other **Tarsals**	

The hypobranchial plates remain independent from each other (not fused medially). The anterior portion of each of planum hypobranchialis is thick medially and becomes thinner as they extend laterally and become continuous with Ceratobranchalia I (Ct I). A sharp process, the processus anterior branchialis, is dorsally visible on Ct I, but in ventral view it is hidden by the lateral portion of the ceratohyal. Ct II, III, and IV are free from the plana hypobranchiales but posteriorly connected with each other by the commisurae terminales. A short and wide process projects from Ct II towards Ct III, this is the processus branchialis. However, this process does not fuse with Ct III consequently a commisura proximalis is absent. The processus branchialis is absent in other cerabranchials. All ceratobranchials, except for Ct IV, bear spicula dorsally. In *D. auratus*, the spiculum of Ct I is fused to the plana hypobranchiales, whereas the spicula of Ct II and III are not fused with the plana hypobranchiales. Near the posterior tip of the plana hypobranchiales there are two small, unidentified plates of cartilage.

Summary of results of the analysis of cranial and postcranial ossification sequence for *Dendrobates auratus* and *Epipedobates tricolor* are given in Table 1. Initiation of cranial ossification pre-

cedes post-cranial ossification and it begins earlier in *E. tricolor.* Ossification of the appendages and pectoral girdle takes place in both species around stage 38; however, ossification of the pelvic girdle is earlier in *E. tricolor* than in *D. auratus*. The squamosals, septomaxillae, and dentaries are postmetamorphic ossifications in *E. tricolor,* whereas these elements are either partially or completely pre-metamorphic ossifications in *D. auratus.* Similarly, the pterygoids and angulosplenials appeared postmetamorphically in *D. auratus* whereas they are premetamorphic ossifications in *E. tricolor.* The premaxillae begin to ossify during stage 42 in both species; the alary, dentigerous, and palatine processes of the premaxillae are present by stage 43 in *D. auratus,* but in *E. tricolor* their ossification is completed postmetamorphically.

Discussion

The classification and monophyly of the Dendrobatidae has been supported using adult characteristics (Myers, 1987; Myers et al, 1991; Ford, 1993). Haas (1995) examined the chondrocranial anatomy of six species of dendrobatids, including one species of *Dendrobates* (*D. tinctorius*). Furthermore, he provided a combination of 26 shared larval characters to diagnose the family.

Dendrobates auratus exhibits some of the characters reported by Haas (1995) for dendrobatids. It has a quadripartite cartilago suprarostralis. The pars corporis and pars alaris articulate with short cornua trabeculae that have a processus lateralis trabeculae. The cartilages orbitales are low and the brain extends past their dorsal margin; the foramen trochleare is anterodorsal to the foramen opticum. The crista parotica has a small processus anterolateralis and lacks a larval processus oticus. The processus muscularis palatoquadrati lacks a chondrified commissura quadratoorbitalis. The hypobranchial structure of *Dendrobates auratus* is also consistent with other described dendrobatids. It has Ceratobranchalia II and III free from the plana hypobranchiales and a processus branchialis is present on Ceratobranchial II.

Some characteristics found in *Dendrobates auratus* differ or require special notice from those reported for other dendrobatids. The cartilages orbitales of *D. auratus* is fused with the otic capsule, a characteristic previously reported only for *D. tinctorius* (Haas, 1995). The lack of connection between the cartilages orbitales and the otic capsules was considered a paedomorphic character for dendrobatids (Haas, 1995). Also *D. auratus* and *D. tinctorius* uniquely share having a wide processus muscularis palatoquadrati. The fusion of the cartilages orbitales and a processus muscularis palatoquadrati are unique characteristics of *Dendrobates* among the chondrocrania of known dendrobratids; consequently, these characters can be considered derived conditions for the genus within the family. *Dendrobates auratus* lacks a processus pseudopterygoideus and a processus quadratoethmoidalis, however a small processus quadratoethmoidalis is present in *D. tinctorius.* Among known dendrobatids, the hypobranchial apparatus of *D. auratus* and *D. tinctorius* share two unique features: the absence of a processus anterolateralis hyalis and the presence of a pair of small cartilaginous plates behind the plana hypobranchiales. Haas (1995) suggested that these cartilaginous plates may correspond to a spiculum IV. However, in *D. auratus* ceratobranchial IV has a small process that curves ventrally on its anterior end; herein this process is interpreted as a reduced spiculum IV. This seems a possible homology considering that spicula II and III are also fused to their corresponding ceratobranchial but are free from the plana hypobranchiales. If our interpretation is correct, then the two cartilages posterior to the plana hypobranchiales are of unknown homologies. In addition, *D. auratus* differs from *D. tinctorius* by the lack of a commisura proximalis between Ceratobranchalia II and III.

Skeletal development begins later in *Dendrobates auratus* than in *Epipedobates tricolor* (Table 1). Postcranial ossification begins with the development of the vertebral centra and neural arches at stage 31 in *E. tricolor* and at stage 34 in *D. auratus.* In both species the early cranial ossifications correspond to the frontoparietals, parasphenoid, and exoccipitals as reported for other anurans (Hanken and Hall, 1988). Development of these first three cranial elements in *D. auratus* is initiated in stages 33–34. These data agrees with previous reports for *D. tinctorius* that indicated these cranial elements are absent at stage 31 and present by stage 37 (Haas, 1995). However, for *E. tricolor* the present study showed an earlier ossification (stage 31) of these three cranial elements than previously reported for the species (stage, 34–36, as *E. anthonyi,* Haas, 1995; *E. anthonyi* = *E. tricolor,* Henle, 1992). Cranial development in *E. tricolor* continues with the ossification of the prootic at stage 36; however, in *D. auratus* this ossification does not appear until stage 40. An ossified prootic was reported at stage 39 for *D. tinctorius* (Haas, 1995). During stages 37–38, both species show ossification of long bones of anterior and posterior limbs. The pattern of ossification of the carpalia and tarsalia is similar and takes place before metamorphosis is completed in both species, but it occurs earlier in *D. auratus.* Ossification of the pectoral girdle begins earlier in *D. auratus* (stage 39) than in *E. tricolor* (stage 41).

The ventral ramus of the squamosal and the dentary appear at stage 44 in *D. auratus*, whereas in *E. tricolor* these elements are completely post-metamorphic additions.

Acknowledgments.—Specimens for this project were provided by the National Aquarium in Baltimore. Support for S. Hill and R. de Sá was provided by NSF grant BIR-9510228.

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Accepted: 15 January 1998.