**UNIVERSIDADE ESTADUAL PAULISTA**
**“JÚLIO DE MESQUITA FILHO”**
**Instituto de Biociências**
**Câmpus do Litoral Paulista**

# SEQUÊNCIA DE OSSIFICAÇÃO ESQUELÉTICA DE *THOROPA TAOPHORA* (MIRANDA-RIBEIRO, 1923) E SUAS IMPLICAÇÕES ECOLÓGICAS

**GUILHERME REY DE FRANÇA SICHIERI**

**SÃO VICENTE – SP**
**2021**

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**Discente: Guilherme Rey de França Sichieri**
**Docente orientador: Prof. Dr. Ivan Sergio Nunes Silva Filho**

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*Um dia... Pronto!... Me acabo.*
*Pois seja o que tem de ser.*
*Morrer: Que me importa?*
*O diabo é deixar de viver***.**
(Mário Quintana)

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**Resumo:** Os anfíbios anuros estão distribuídos em todos os continentes, exceto na Antártica, e compõem 87% de todas as espécies de anfíbios. A metamorfose – processo na qual as larvas tornam-se adultos – é característica dos anfíbios, sendo que as larvas dos anuros são popularmente conhecidas como girinos. Girinos são tipicamente aquáticos, porém em algumas espécies há certo grau de terrestrialidade como em *Thoropa taophora* em que os girinos habitam afloramentos rochosos úmidos. São conhecidas algumas adaptações externas para esse estilo de vida semiterrestre, entretanto a anatomia interna destes girinos continua inexplorada. Assim, a presente dissertação busca descrever o condrocrânio larval, a sequência de ossificação craniana e as mudanças que ocorrem no condrocrânio desta espécie durante a metamorfose. Para visualização destas estruturas, foi utilizada a técnica de diafanização em 33 girinos em diversos estágios de desenvolvimento e sete espécimes pós-metamórficos. O condrocrânio larval de *T. taophora* não apresenta nenhuma especialização evidente para o estilo semiterrestre e assemelha-se aos condrocrânios de larvas aquáticas. A ossificação cranial inicia com formação do exocipital enquanto que no pós-crânio os ossos dos membros posteriores apresentam início de mineralização. Apesar da ossificação pós-cranial e cranial começarem simultaneamente, ao final da metamorfose o pós-crânio já está quase inteiramente ossificado enquanto o crânio ainda possui vários elementos cartilaginosos. Pouca ossificação cranial também é encontrada em indivíduos metamórficos de *Eupsophus calcaratus* (Alsodidae) e *E. emiliopugini*.

**Palavras-chave:** Anatomia, metamorfose, morfologia, girinos.

**Abstract:** The anuran amphibians are a diverse and widespread taxa worldwide, except Antarctica, which comprise up to 87% of all amphibians. The metamorphosis (i.e., the process through which larvae become adults) is typical of amphibians, and anuran larvae are commonly known as tadpoles. These latter are typically aquatic dwellers, although some species have evolved differently towards an independence level from the aquatic environment as *Thoropa taophora*, which lives in wet rocky outcrops. Some already known external morphological specializations for living in such environment are demanded, and yet the internal anatomy of these tadpoles has remained unexplored. This dissertation aimed at describing the larval chondrocranium, the cranial ossification sequence and subsequent anatomical changes in the chondrocranium of *T. taophora* during metamorphosis. The clearing and double-staining method was used in 33 tadpoles in different developmental stages, as well as in seven post-metamorphic specimens. The larval chondrocranium of *T. taophora* seems not to present any specialization for semiterrestriality and resembles that of free-living larvae. Once formed, the exoccipital marks the onset of cranial ossification, while the mineralization of hindlimb bones the onset of post-cranium ossification. Despite both processes start at the same stage, the post-cranium is almost entirely ossified by the end of metamorphosis, whereas the cranium still bears several cartilaginous elements. Few ossifications on metamorphic specimens are also found in *Eupsophus calcaratus* (Alsodidae) and *E. emiliopugini*.

**Keywords:** Anatomy, metamorphosis, morphology, tadpoles.

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# SKELETAL OSSIFICATION SEQUENCE OF *THOROPA TAOPHORA* (MIRANDA-RIBEIRO, 1923) AND ITS ECOLOGICAL IMPLICATIONS

GUILHERME SICHIERI[1]*& IVAN NUNES[1]

[1]Universidade Estadual Paulista (UNESP), Instituto de Biociências, Laboratório de Herpetologia (LHERP), Campus do Litoral Paulista. Praça Infante Dom Henrique, s/n, Parque Bitaru. 11330-900, São Vicente-SP, Brasil. E-mail: ivan.nunes@unesp.br.

*Corresponding author. E-mail: guilherme.rey@unesp.br

## INTRODUCTION

### Anuran larvae and metamorphosis

Amphibians are a diverse class of vertebrates with more than 8100 described species distributed in every continent, except Antarctica (Frost 2021). The class Amphibia is subdivided in three orders: Anura, represented by frogs and toads; Caudata, represented by newts and salamanders; and Gymnophiona represented by caecilians. Anura is a monophyletic group (de Saint-Aubain 1981; Ford & Cannatella 1993) and by far the most speciose order, harboring almost 87% of all species of amphibians (Frost 2021).

One of the most remarkable features of anurans is their biphasic life cycle, which in most species consists of a free-living larva known as tadpole that will later metamorphose into a frog or toad (Altig & McDiarmid 1999). Tadpoles usually explore aquatic resources whereas adults explore terrestrial resources, thus preventing niche overlap (Wassersug 1975). However, to perform such drastic shift in habitat the tadpole must necessarily undergo metamorphosis in order to attain reproductive organs and the general adult *bauplan* of anurans (Wassersug 1975). Throughout this metamorphosis many structures in the axial and cranial skeleton are rebuilt or appear *de novo* (Larson & Reilly 2003; Vera & Ponssa 2014). The chondrocranium of tadpoles protects the brain, supports the jaws and sense organs and is made up of cartilage (Cannatella 1999). However, the cranium of adults is made up mostly of bony tissue, so during metamorphosis there is an intense restructuring and an equally intense ossification of cranium, axial and appendicular elements. The hyobranchial apparatus is unique of tadpoles and serves to trap food particles and irrigate the respiratory surfaces of the gill

(Cannatella 1999). During metamorphosis this structure is remodeled and gives rise to the hyoid apparatus of adults (Trewavas 1933).

Towards the end of metamorphosis, the adult is more independent from the aquatic environment and its *bauplan* is substantially different from that of the larva (Altig & McDiarmid 1999; Handrigan & Wassersug 2007; Roelants et al. 2011). The diversity of niches occupied by anuran larvae has intrigued scientists for decades. Most tadpoles are exotrophic and live in waterbodies, consuming algal matter or detritus, although some species are obligate carnivorous (e.g. *Lepidobatrachus laevis*; Ruibal & Thomas 1988), whereas others are endotrophic and do not feed at all, relying entirely on yolk to complete their development (e.g. *Cycloramphus stejnegeri*; Lavilla 1991). Although tadpoles were overlooked for long, their enormous diversity has led scientists to group them either based on their ecology or morphology, in an effort to search for an evolutionary pattern (Orton 1953; Starrett 1973; Altig & Jonhston 1989). Additionally, more recent and comprehensive works using phylogenetic and morphometric methods found that ecological factors exert selective pressure on morphological variation in tadpoles, meaning that tadpole *bauplan* may be strongly linked to its ecology (Bossuyt & Milinkovitch 2000; Sherratt et al. 2017; Sherratt et al. 2018).

Despite more attention is being given to larval descriptions in anurans, tadpole internal anatomy is still a largely unexplored field, even for many well-known families. A handful of works have focused on correlating musculature (e.g. Satel & Wassersug 1981; Vera Candioti 2005), chondrocranium (e.g. Hall & Larsen 1998; Alcalde & Rosset 2003; Vera Candioti 2005) or internal buccal anatomy (e.g. Seale & Wassersug 1979; Wassersug 1980) of tadpoles with their feeding habits, however investigations of correlations between larval internal anatomy and microhabitat is still a fruitful and unexplored field (Noble 1929; Haas & Richards 1998; Hall et al. 2002; Vera Candioti 2006). For instance, Haas & Richards (1998) investigated musculature and chondrocranial anatomy in suctorial tadpoles and found several specializations, especially on the chondrocranium: 1) anteriorly expanded or fused *cornua trabeculae*; 2) robust lower jaw cartilages; 3) chondrocranium larger at the mandible articulation level; 4) upper jaw with partial or totally fused cartilages; 5) robust palatoquadrate connected to the braincase anteriorly by a wide *commissura quadratocranialis anterior*; 6) palatoquadrate bearing three processes connecting it to the braincase posteriorly: otic process, basal process and *processus ascendens*. However, similar works investigating internal anatomy of tadpoles that live in other unusual habitat, such as semiterrestrial larvae, were not done yet for any species.

## Semiterrestrial tadpoles

The concept of reproductive mode for amphibians, involving oviposition and development sites, was originally defined by Salthe & Duellman (1973). The commonest reproductive mode in anurans is the oviposition in ponds or ephemeral bodies of water, followed by the hatching of an exotrophic aquatic larva (Haddad & Prado 2005). There are many other reproductive modes however, such as the direct development, in which a fully formed miniature adult hatches the egg, thus “skipping” the free-living larval phase (Lutz 1947). This remarkable diversity is especially seen in the Neotropical region where 21 modes are described from which eight are exclusive to that region (Hödl 1990; Duellman & Trueb 1994). Many classifications for anuran reproductive modes were proposed over time. The first was the work of Boulenger (1886) in which he divided anurans in ten groups based on how parents deposit or protect their offspring. Almost a century later, Duellman & Trueb (1994) recognized 29 reproductive modes and currently there are 42 recognized reproductive modes for anurans (Malagoli et al. 2021)

The semiterrestrial mode (Mode 19 of Haddad & Prado 2005) is characterized by the oviposition in humid rocks followed by the hatching of an exotrophic larva that feed and develop upon these rocky outcrops. In the Neotropical region, this mode is found in the genus *Thoropa* and in several species of *Cycloramphus*; both belonging to the family Cycloramphidae (Jetz & Pyron 2018). Two other species also have this reproductive mode in the neotropics: *Leptodactylus rugosus* (Altig & Johnston 1989) and *L. lithonaetes* (Heyer 1995). Semiterrestrial frogs are also found in other biogeographical regions across the world. For instance, the afrotropical *Arthroleptides martiensseni* (Drewes et al. 1989) and some species of the Eastern African genera *Petropedetes* (Barej et al. 2010) and *Notophryne* (Bittencout-Silva et al. 2016) have semiterrestrial larvae. In the oriental region, the semiterrestrial mode is found in *Indirana beddomii* and *I. semipalmata*, both from the Western Ghats mountain range (Boulenger 1920; Kirtisinghe 1958) and also in *Nannophrys ceylonensis* from Sri Lanka

(Wickramasinghe et al. 2004). Due to the high degree of endemism in the Western Ghats and Sri Lanka, this region is classified as a biodiversity "hot-spot" (Myers et al. 2000). Given the great biodiversity of the region, and the pace at which new species are described (even an entirely new subfamily of frogs was recently described by Vijayakumar et al. 2019) possibly several other species also share this reproductive mode (Nair et al. 2012). Curiously, semiterrestriality evolved only in tropical and subtropical regions, possibly due to higher humidity levels (da Silva et al. 2012) and its representative species all share several morphological characters: Big dorsolaterally positioned eyes, large posterior limbs, long tail with reduced or absent fins, brown cryptic color, buccal apparatus with horny beaks densely keratinized to grasp on the rocks and flattened bodies to increase surface area for adhesion to substrate (Wassersug & Heyer 1983; Altig & Johnston 1989; Drewes et al. 1989; Haddad & Prado 2005). The presence of all these characters in distant related species reveals that semiterrestriality evolved independently a couple of times.

Literature dealing with tadpoles of semiterrestrial species is extremely scarce and virtually nothing is known on basic ecology, natural history or internal anatomy from African semiterrestrial species. Wassersug & Heyer (1983) described the internal buccal anatomy of *Cycloramphus duseni* and *Thoropa taophora* (*T. miliaris* back then). Physiology of adults of *Thoropa taophora* to tolerate salinity — since many populations live very close to seashores — was explored by Abe & Bicudo (1991) and physiology of tadpoles was explored more recently by Gallo et al. (2020). More recently, Verdade et al. (2019) provided data on breeding biology of *Cycloramphus bandeirensis* and Dias et al. (*In press*) provided data on anatomy of several species of Cycloramphidae tadpoles. Regarding Asian species, data on natural history and breeding of *Nannophrys ceylonensis* were provided by Wickramasinghe et al. (2004), whereas Wickramasinghe et al. (2005) reported on feeding ecology of tadpoles of this same species raised under laboratory conditions. Two years later, the same work group studied ontogenetic changes that occur in the intestinal morphology of tadpoles of *N. ceylonensis* and found a major shift in diet, from herbivory to carnivory, to be well-correlated with decreasing in gut length (Wickramasinghe et al. 2007). Predation on tadpoles of *Thoropa taophora* by trap-jaw ants was reported by Facure & Giaretta (2009). Jumping behavior and kinematics of tadpoles of *Indirana beddomii* was reported by Veeranagoudar et al.

(2009). Breeding ecology data on egg clutches and mortality of tadpoles of *Indirana* sp. were provided by Gaitonde & Giri (2014).

### The genus *Thoropa*

The family Cycloramphidae Bonaparte, 1850 harbors three genera: *Cycloramphus* Tschudi, 1838 with 30 species and *Thoropa* Cope, 1865 with seven species (Frost 2021). All species of *Thoropa* have semiterrestrial larvae, however only some few species of *Cycloramphus* share this reproductive mode (de Sá et al. 2020). As all other semiterrestrial species across the globe, tadpoles of *Thoropa* have several morphological specializations to live in humid rocky outcrops, such as flattened bodies, very reduced or absent tail fins and cryptic coloration that matches the rocks they live upon (Lutz 1947, 1948; Barth 1956; Wassersug & Heyer 1983; Cocroft & Heyer 1988; Altig & Johnston 1989; Haddad & Prado 2005; Feio et al. 2006), which may even be 90º inclined (Bokermann 1965).

The genus *Thoropa* Cope, 1865 comprises seven species distributed in Eastern Brazil, all of which are endemic to the Atlantic Rainforest (Frost 2021): *Thoropa bryomantis* Assis, Lacerda, Guimarães, Peixoto, Luna & Feio, 2021; *Thoropa lutzi* Cochran, 1938; *Thoropa megatympanum* Caramaschi & Sazima, 1984; *Thoropa miliaris* (Spix, 1824); *Thoropa petropolitana* (Wandolleck, 1907); *Thoropa saxatilis* Cocroft & Heyer, 1988 e *Thoropa taophora* (Miranda-Ribeiro, 1923). Females of *Thoropa* mate and deposit their eggs in rocky gneissic outcrops (Lutz 1954; Bokermann 1965) or even rocky shores very close to the sea (Cochran 1955; Sazima 1971; Brasileiro et al. 2010). Some populations are also found in plateaus 1500 above sea level (Feio et al. 2006). The name *Thoropa taophora* (Miranda-Ribeiro, 1923) was re-attributed by Feio et al. (2006) only for the populations from São Paulo state, Brazil, that were previously identified as *T. miliaris*. Its southern limits are Estação Ecológica Jureia-Itatins in municipality of Iguape, and its northern limits are municipality of Ubatuba. The species can also be found in some continental islands as Ilha Anchieta, Ilha Vitória e Ilha dos Búzios (Feio et al. 2006).

The most recent phylogenies that sampled cycloramphids (Sabbag et al. 2018; Jetz & Pyron 2018; Hime et al. 2021) retrieved similar results as Pyron & Wiens (2011) did, with *Thoropa* and *Cycloramphus* nested within Cycloramphidae, together with families Hylodidae, Alsodidae and in some (Jetz & Pyron 2018; Streicher et al. 2018; Hime et al. 2021) also Batrachylidae as sister family of Cycloramphidae. It is worthy noting that Streicher et al. (2018) took a step further and proposed the name Neoaustrarana for the monophyletic group composed by the families Alsodidae, Batrachylidae, Cycloramphidae and Hylodidae. Due to the low taxon sampling performed by Feng et al. (2017) — only one species of Cycloramphidae and two species of Odontophrynidae — I chose to follow the hypotheses of Pyron & Wiens (2011), Blotto et al. (2013), Sabbag et al. (2018), Jetz & Pyron (2018), Streicher et al. (2018) and Hime et al. (2021) in considering *Thoropa* as a genus of the family Cycloramphidae, which is sister-taxon to the families Alsodidae (genera *Alsodes, Eupsophus, Limnomedusa*), Batrachylidae (genera *Atelognathus, Batrachyla, Chaltenobatrachus*, *Hylorina*) Hylodidae (genera *Crossodactylus*, *Hylodes*, *Megaelosia*, *Phantasmarana*).

## OBJECTIVES

Herein we describe the chondrocranium of larvae of *Thoropa taophora* in various developmental stages and its associated metamorphic changes as well as basic ontogeny aspects. Furthermore, the skeletal ossification sequence and adult skull are also described and compared to other skulls from close-related families available on the scientific literature.

## MATERIALS AND METHODS

Specimens of *Thoropa taophora* were collected in the municipality of São Vicente, São Paulo state, Brazil (23º58’44”S – 46º22’18”O, 12m a.s.l.; WGS 84 datum) (Fig. 1) during the summers of 2017–2019. Individuals were euthanized and fixed in buffered formalin 10% and are currently housed at Amphibian Collection of Herpetology Lab (HCLP – A) of São Paulo State University, coastal campus (UNESP–IB/CLP). Tadpoles were staged according to the table of Gosner (1960) and clustered in three groups (modified from Trueb 1985): pre-metamorphic (Until Gosner stage 41; GS41) with 22 specimens, metamorphic (GS42–45) with 11 specimens and post-metamorphic (GS46), including juveniles, with seven specimens. Two measurements were taken on tadpoles following recommendations of Altig & McDiarmid (1999):

body length (BL) and tail length (TAL). In post-metamorphic specimens (GS46) only body length measurement was taken in order to characterize specimen maturity, since those specimens lack a tail. Nomenclature for bones followed Trueb (1973), for larval chondrocranium and hyobranchial apparatus it was followed the nomenclature of Larson & de Sá (1998), Púgener & Maglia (2007) for nasal capsules and Trewavas (1933) for the adult hyolaryngeal apparatus. For ligaments, the nomenclature of Haas (1995) was followed.

Ossification tables are presented as the sheer series of ossification events instead of using Gosner Stages as landmarks, following the recommendations of Smith (2001). Ossification indices were calculated for cranial and postcranial bones as the number of bones present at a given stage divided by the total number of bones present at GS46 (Haas 1999). Timing of ossification was defined as the earliest developmental stage at which a bone retained alizarin, even if that bone was colored only unilaterally, similar to the criterion adopted by Haas (1999). For instance, if the right humerus of a GS40 specimen retained alizarin but the left one did not, I considered GS40 as the ossification onset of the humerus. Plots were made using ggplot2 package for RStudio sotware version 3.5.0 (R Core Team 2018). Prior to staining, specimens were skinned as much as possible to allow maximum penetration of staining solutions. All specimens were cleared and double-stained for bone and cartilage following the protocol of Taylor & Van Dyke (1985) with few modifications:

- **Dehydration**: Specimens were dehydrated for 1 hour in ethanol 90% and then in ethanol 99% for another 1 hour in order to prevent the acetic acid in the next step demineralize cartilages;
- **Cartilage staining:** Specimens were transferred to a solution containing 20 – 25 mg Alcian Blue + 80 ml ethanol 99% + 20 ml glacial acetic acid during 5 or 6 hours depending on the size of the specimen. Post-metamorphic specimens were left overnight to ensure good staining;
- **Neutralization**: After the cartilages were stained, specimens were transferred to a super-satured solution of Sodium Borate for almost 24 hours. The solution was renewed after 12 hours;

- **Depigmentation**: Following neutralization, specimens were submerged in a solution containing 80 ml KOH 0.5% and 20 ml $H_2O_2$ 3% for approximately 15 minutes. After depigmentation specimens were sunk in a solution of Sodium Borate to eliminate any hydrogen peroxide residue;
- **Enzymatic digestion of soft tissue**: After proper depigmentation, specimens were immersed in a solution of 30% concentrated Sodium Borate and 70% distilled water containing 0.5 – 1g of pancreatin. After 24 hours the solution was renewed. For smaller specimens, 36 hours of digestion was enough, however for bigger specimens 48 hours yielded better results;
- **Enzyme neutralization**: Afterwards, specimens were transferred to a solution of KOH 0.5% during approximately 1 hour in order to neutralize pancreatin;
- **Bone staining**: After soft tissue had been sufficiently digested, tadpoles and adults were transferred to a solution containing 10 – 15 mg of Alizarin Red and 100 ml of KOH 0.5%. Usually only 1 or 2 hours were enough for tadpoles and post-metamorphic specimens;
- **Preservation**: After due bone staining, the process of preservation was initiated. Individuals were immersed in crescent proportional solutions of KOH 0.5% and glycerin. The first solution containing 3 parts of KOH 0.5% and 1 part of glycerin (3:1), the second with a ratio of 1:1, the third one with a ratio of 1:3 and the last one of pure glycerin. Each change occurred after 1 or 2 days. In the last solution, tiny crystals of thymol were added to prevent fungal growth.

## RESULTS

### Ontogeny

Body of tadpoles is dorsoventrally flattened during all stages, with fins only at the final inferior portion of the tail. The tail of tadpoles of *Thoropa taophora* reaches its maximum length at GS40, representing up to 70% of total body length at this stage. Table 1 shows the measurements taken from all specimens and fig. 2 illustrates how measurements vary according to Gosner stages. From GS43 and forward the tail begins to be reabsorbed and an abrupt decrease in tail length is evident from GS44 until GS46, when it is totally reabsorbed, thus marking the end of metamorphosis. The anterior limbs emerge from the operculum at GS42, however the larval oral apparatus remains intact at this stage. By GS43 – GS44, the oral apparatus is broken and the mouth has the shape of a small slit, which lengthens posteriorly during subsequent stages. Further at GS46 the specimens look much more like an adult than a tadpole, although they still lack the characteristic dorsal color patterns of the species.

## Chondrocranium description

The description of larval chondrocranium (Figs. 3–4) is based mainly on specimens HCLP – A 141 – 5 (GS35) and HCLP – A 037 – 5 (GS34) because these two specimens had better cartilage staining and none have adult-like or metamorphic structures such as nasal capsules.

### *Neurocranium*

#### *Ethmoidal region*

The suprarostral cartilages support the upper horny beak and articulate with each *cornua trabeculae.* Each suprarostral consists of one central corpus and a lateral ala. The two corpora are fused medially with each other forming a slender cartilaginous bar between both *cornua trabeculae*. Each corpora are fused dorsolaterally to the alae just ventral to the point of articulation of the *cornua trabeculae* with the suprarostrals. Suprarostral alae are flattened, curved posteriorly with a trapezoid shape with a small *processus posterior dorsalis*. The *cornua trabeculae* is a paired horn-like cartilaginous bar that project anteriorly from the *planum trabeculare anticum* and diverge distally, forming a “V” in dorsal view. Distally, they articulate with the suprarostral cartilage at the point of junction between the alae and the corpora. Each *cornua trabeculae* is relatively long, representing 22–28% of the total chondrocranial length and uniform throughout its entire length. In addition, each *cornua* is curved ventromedially, what gives a concave aspect to the cartilage in lateral view. The *processus lateralis trabeculae* is a small protuberance present at the proximal lateral edge of each *cornua* that serves as attachment point to the *ligamentum (l.) quadratoethmoidale,* which in turn, is composed of dense collagen fibers. This ligamentum connects each *cornua trabeculae* to the *processus quadratoethmoidalis*. The *l. cornua-quadratum* is visible in dorsal view as less dense than the *l. quadratoethmoidale* and connects the anterolateral edge of each *cornua trabeculae* to the anterior margin of the *pars articularis quadrati*.

*Orbitotemporal region*

The lateral walls of the braincase are formed by the orbital cartilages (Fig. 4A). Between the posterior margin of the orbital cartilages and the anterior edge of the otic capsule is located the *foramen prooticum*. Two smaller foramens are visible on each side of the posteroventral region of the orbital cartilage: one *foramen opticum* and one *foramen oculomotorium*. The trochlear foramen is the smallest foramen on the orbital cartilage, located dorsally to the previous two and only visible in later-stage specimens which have the orbital cartilages more chondrified. The frontoparietal fontanelle is very large and has the shape of a droplet. In specimens around GS34–36 the *planum intertrabeculare*, a thin sheet of cartilage that grows medially from the *trabeculae cranii*, begins to occlude the *fenestra basicranialis*. Only after this sheet of cartilage starts growing and gets well chondrified it is possible to see the *foramina craniopalatinum* and *caroticum primarium*. Both foramens pierce the cranial floor lateroposteriorly. The *foramen craniopalatinum* is smaller and located anteriorly in relation to the *foramen caroticum primarium*.

*Otoccipital region*

The otic capsules are rhomboid in shape and represent around 21% of the total chondrocranium length. Both capsules are dorsally interconnected by a cartilage bridge, the *tectum synoticum*, which also forms the dorsal edge of the *foramen magnum*. Each otic capsule bear a small triangular *processus anterolateralis* and some few specimens bear an additional *processus posterolateralis* just posterior to the *processus anterolateralis*. Ventrally, on the otic capsule, there is an aperture called *fenestra ovalis*. The two otic capsules are confluent ventrally to the *planum basale* and posteriorly to the *arcus occipitalis*. The *foramen perilymphaticum* is found laterally to the *foramen jugulare*.

*Visceral components*

*Palatoquadrate*

The palatoquadrate of *Thoropa taophora* is narrower anteriorly and expands gradually posteriorly being widest just above the level of the *processus ascendens*. Anteriorly, the palatoquadrate connects to the braincase through the *commisura quadratocranialis anterior*. The *processus quadratoethmoidalis* is the only process of this commissura. It is located on its anterior margin, has a triangular shape and it is much reduced when compared to other species of anurans. This process is the attachment site for the *l. quadratoethmoidale*, which in turn play an important role in the movement of the jaws in anuran larvae. There is no *processus pseudopterygoideus* in the *commisura quadratocranialis anterior* of *T. taophora*. Posteriorly, the palatoquadrate attaches to the braincase through a rod-like cartilage, the *processus ascendens*. This process forms an angle of 80º in relation to the skull and attaches immediately below the posteroventral margin of the *foramen oculomotorium*, thus characterizing the “low” suspensorium (Sokol 1981).

There is no connection to the posterior curvature of the palatoquadrate (otic process of the palatoquadrate) to the larval *crista parotica*, in what would be the larval *processus oticus*. Instead, there is a very dense and short *l. quadratooticum* connecting the anterior margin of the larval *crista parotica* to the otic process of the palatoquadrate. Nonetheless, each palatoquadrate bear two anterior processes: the *processus muscularis quadrati*, located posteriorly and the *pars articularis quadrati*, located anteriorly. The *processus muscularis quadrati* is relatively short, wide and triangular in lateral view, curving dorsally such as it can be seen in dorsal view. The *l. tectum* connects the dorsal edge of the *processus muscularis quadrati* to the neurocranium, but with a much reduced *processus antorbitalis*. On its ventral margin, there is a small depression called *facies articularis hyalis* that serves as attachment point for the ceratohyal articulation of the hyobranchial apparatus. The *pars articularis quadrati* articulates anteriorly with the *processus retroarticularis* of the Meckel’s cartilage.

### *Meckel’s cartilage and infrarostrals*

The Meckel’s cartilage and infrarostral cartilage are paired elements that form the lower jaw in anuran larvae. The Meckel’s cartilage has a sigmoid shape and forms almost a 45º angle to the body axis. This cartilage articulates posterolaterally to the *pars articularis quadrati* through its *processus retroarticularis* and anterolaterally it articulates to the infrarostral cartilage. The *processus retroarticularis* is a rounded protuberance of the Meckel’s cartilage that appears in a darker shade of blue in cleared and stained specimens due to its partial overlap with the *pars articularis quadrati*. The infrarostrals are small paired elements that support the lower horny beak of most anurans. It is found ventromedially in relation to the *cornua trabeculae* and both pieces form the shape of an inverted “V”. In *Thoropa taophora* this cartilage is poorly chondrified and appears in a very light shade of blue in cleared and stained tadpoles. Both infrarostrals are linked medially by connective tissue.

### *Hyobranchial apparatus*

The hyobranchial apparatus of *Thoropa taophora* (Fig. 4B) is composed primarily of the large paired ceratohyalia, the paired hypobranchial plates and the ceratobranchialia. The ceratohyals are paired, wide, flat cartilages attached laterally to the *facies articularis hyalis* of the palatoquadrate. The anterior margin of each ceratohyal bears two processes: the *processus anterior hyalis* and the *processus anterolateralis hyalis*. Both processes are triangular and pointed but the *processus anterior hyalis* is larger and located more medially in relation to the *processus anterolateralis hyalis*. On the posterior medial margin of each ceratohyal there is a large triangular *processus posterior hyalis*. The ceratohyalia are connected medially by a poor chondrifried *copula posterior* which bears a small, but nonetheless well chondrified *processus urobranchialis* on its posterior margin. A diminute elliptical copula anterior is present anterior to the *pars reuniens*.

The hypobranchial plates are narrowly separated medially, and each plate serves as point of attachment for the ceratobranchialia. Posteriorly, the plates leave an ovoid small gap. The branchial basket consists of four ceratobranchialia with numerous long lateral projections. All four ceratobranchialia are united distally via *commisurae terminales*. The ceratobranchial I attaches to the hypobranchial plate by a wide strip of cartilage which bears on its anterior margin a well-developed *processus anterior branchialis* that slightly curves medially and does not contact the ceratohyalia above. The ceratobranchial II is also united to the hypobranchial plate but its connection is much thinner. Ceratobranchialia III and IV are not attached to the hypobranchial plate by cartilage, instead they are attached by connective tissue. Spicules are present dorsally on the proximal margin of all ceratobranchialia. These structures are less chondrified than the rest of hyobranchial, thus it stains only very faintly with alcian blue.

## **Development of chondrocranial cartilages**

### **Pre-metamorphic stages (until stage 41)**

The *tectum synoticum* possibly begins to be formed while the embryo is still inside the egg, because at GS26 it is already well formed, although incomplete yet. The capsules are only fully bridged around GS29-30. In newly hatched tadpoles (GS26) the *processus muscularis quadrati* is still very small. In mid-stage specimens, around GS34–36, the *planum intertrabeculare* begins to form laterally from the *trabeculae cranii* and anteriorly from the *planum trabeculare anticum*. By GS36, the *taenia tecti transversalis* and *taenia tecti medialis* are completely developed, subdividing the frontoparietal fontanelle in two paired parietal fontanelle (Fig. 5). Chondrocranium remodeling has not started yet in pre-metamorphic specimens, and none of the nasal structures are evident.

## Metamorphic stages (From stages 42 to 45)

### Stages 42 and 44

By GS42 the anterior limbs are fully formed and have emerged from the operculum, but the keratinized larval oral apparatus is still present. The *cornua trabeculae* has shortened and the *taenia tecti medialis* and *transversalis* are still visible. Gosner Stages 42 and 43 are marked by a relative stability in cranium morphology; the most dramatic changes occur from GS44 – 45. By GS44 the *cornua* and the suprarostral cartilages are completely eroded (Fig. 6) and the nasal capsules appear for the first time. Also at this stage, the palatoquadrate is regressing posteriorly and getting smaller; however it still retains the *processus muscularis quadrati*, although erosion of this process has made it smaller than in previous stages. The medial margin of the palatoquadrate now bears a long and slender pterygoid process that extends anteriorly. Meckel’s cartilage has fused with the infrarostrals and is lengthening posteriorly (Fig. 6B). The suspensorium is still composed anteriorly by the *comissura quadratocranialis anterior* and posteriorly by the *processus ascendens*. The otic capsules have grown considerably in size. By this stage, the *planum intertrabeculare* is sufficiently chondrified, so the foramens that pierce the cranium dorsally are visible. The ceratohyalia retain all their processes and overall shape but they have acquired a more oblique posterolaterally orientation (Fig. 7). The hypobranchial plates have fused medially and the ceratobranchialia degenerated. A pair of anterolateral processes and posteromedial processes is now visible for the first time in the hypobranchial plates.

### Stage 45

The chondrocranium of GS45 specimen (Figs. 8–9) has lost its posterior connection to the braincase through the *processus ascendens*. The Meckel’s cartilages have elongated even more posteriorly, reaching now the medial region of the chondrocranium. The palatoquadrate has regressed even more posteriorly and is now confined to the third posterior half of the chondrocranium. Its *processus muscularis quadrati* is now completely eroded. Posteriorly, there is a sheet of cartilage called *tectum parietale* (sensu Haas 2003) covering the fenestrae parietales. The hyoid apparatus at GS45 (Fig. 9B) resembles more the adult morphology than the larval. The ceratohyalia have lost all their processes and is now curved posterolaterally, resembling the adult hyalia. The posteromedial process remains cartilaginous. The laryngeal cartilages have appeared for the first time with a distinct cricoid cartilage and bronchial and esophageal processes.

### Development of nasal cartilages

The nasal cartilages, or nasal capsules, support the olfactory organs in post-metamorphic individuals and usually develop during metamorphosis. Its development commences at GS44, when most of larval structures are being remodeled. In adults the nasal capsules remain entirely cartilaginous, except for the septomaxilla that ossifies during metamorphosis.

### Stage 42

The *processus antorbitalis* begins extending laterally, the *cornua trabeculae* has shortened and the *tectum nasi* is being formed. The anterior maxillary process is present as a small blunt process. Oblique cartilage is developing and its posterior edge almost contacts with the anterior maxillary process.

### Stage 43

The *processus antorbitalis* has extended more laterally and bears a long anterior maxillary process, which is connected with posterior edge of oblique cartilage. The *cornua trabeculae* has shortened even more and *tectum nasi* is arising posteriorly to the *cornua*.

### Stage 44

The *crista subnasalis* and the alary cartilage appear for the first time. Inferior prenasal cartilages are present but are still poorly chondrified, indicating recent formation. Septomaxilla is chondrified but not mineralized yet. Anterior maxillary process is well developed but the posterior process is not seen yet (Fig. 7). The *planum terminale* of the oblique cartilage and the triangular process of the post-nasal wall are almost in contact, forming the paranasal commissure. Also, at this stage, the *septum nasi* extends anteriorly from the *planum trabeculare anticum* as a pointy strip of cartilage dividing both sides of the nasal capsules. The *planum antorbitale* is formed. This element is a robust wall of cartilage that is located between the braincase and the maxillary and delimits the anterior portion of ocular orbit.

### Stage 45

At this stage the nasal cartilages resemble a lot the adult ones in morphology. The distal ventral end of the *planum antorbitale* is composed by the *planum triangulare* which now bears the anterior and posterior maxillary processes. The anterior process is longer than the posterior and in adult specimens it invests the posterior margin of the *pars facialis* of the maxillary. The posterior process is synchondrotically fused to the pterygoid process of the palatoquadrate which is now restricted posteriorly. The inferior prenasal cartilage is curved posteriorly and has a fang-like morphology, located anteriorly to the *crista subnasalis*. Superior prenasal cartilage is smaller than the inferior, and in older specimens it invests the pre-maxillaries. The *crista subnasalis* is located anteriorly to the *planum triangulare* and also invests the *pars facialis* of the maxillary in post-metamorphic specimens. The *crista subnasalis* is not bifurcate and it is slightly curved posteriorly, almost in contact with the anterior maxillary process of the *planum triangulare*. The oblique cartilage has a semilunar shape and delimits the anterior wall of the fenestra nasolateralis, which is one of the openings of the nasal capsules. Ventrally to the oblique cartilage is located the alary cartilage. The paranasal commissure is fully formed at this stage, connecting the *planum terminale* of the oblique cartilage with the *planum antorbitale*.

### Stage 46

In post-metamorphic individuals the nasal capsules remain cartilaginous; the only bony element being the septomaxilla. The *tectum nasi* covers the medial anterior wall of the braincase dorsally, whereas the *septum nasi* covers it ventrally. Both are not mineralized in any specimens surveyed. The *planum antorbitale*, which bears a long triangular post-nasal wall process that extends anteriorly, separates the ocular orbit from the nasal capsules. The *planum triangulare* is the distal end of the *planum antorbitale* and bears two processes: the anterior and posterior maxillary process. The first is longer and invests the anterior end of *pars facialis* of the maxillary whereas the second process is shorter and is fused to pterygoid process of the palatoquadrate cartilage. The alary cartilage is dorsal to the oblique cartilage both extending postero-laterally, however the distal end of the oblique cartilage (*planum terminale*) extends further than the alary cartilage, towards the triangular post-nasal wall process. The crista subnasalis is relatively long and overlaps with the *pars facialis* of the maxillary almost at the level between the maxillary and pre-maxillary. The inferior and superior prenasal cartilages are ventrally arcuate thin rod-like cartilages that abut the alary process of the pre-maxillaries.

## Ossification sequence

Although cranial and postcranial ossification initiate roughly at the same developmental stage (GS40), ossification of postcranial elements proceed faster. For instance, by GS43 more than 80% of postcranium ossification has initiated, while not even 20% of the cranium is ossified at the same stage (Fig. 10). By GS44, the postcranium skeleton reaches an index of 1, which indicates it is fully ossified. However, at the same stage, only approximately 50% of the cranium has ossified and by GS45 only 60% of the cranium begins to ossify. Thus, nearly half of the cranium of *T. taophora* ossifies post-metamorphically.

The first ossification center in the cranium appears at GS40, around the jugular foramen, which represents the exoccipital (Table 3). In the postcranial skeleton, the first ossification centers in the neural archs are also seen by GS40. These centers grow rostro-caudally during the subsequent stages until all neural arches are ossified by GS43. The forelimbs remain cartilaginous at GS40, however the diaphyses of all bones that compose the hindlimbs are well ossified, including the elongated tibiale and fibiale. These centers progress rapidly towards the epiphyses during subsequent stages. The transverse processes II, III and IV appear ossified at GS41, together with the cervical vertebra. The remaining transverse processes (V–VIII) appear fully ossified for the first time in GS44 specimens (Fig. 11). The GS42 is marked by ossification of forelimb bones. At this stage, the humerus and radioulna show ossification centers for the first time in the series studied herein. Metatarsals of digits IV and V show one diminute ossification center each by GS42 and by GS44 all of them are ossified (Fig. 12). The metacarpal of digit I also show one tiny ossification center. In the pectoral girdle, the first elements that appear ossified are the coracoids and the scapulas at GS42. In the pelvic girdle, the medial region of the ilium appears ossified and by GS44 it is ossified throughout all of its length. The GS43 is marked by a relative stability in ossification of new elements; the only elements that show new ossification at this stage are the sacral diapophyses and the ischium on the pelvic girdle.

On the other hand and contrary to GS43, GS44 is the stage that exhibits the most dramatic changes in cranial ossification (Fig. 13). The pre-maxillaries and the maxillaries first appear in GS44 specimens as completely and partially ossified elements, respectively. Inside the newly formed nasal capsules, the septomaxilla appears completely ossified by GS44. The angulosplenials show a small ossification center at the posteromedial margin of Meckel's cartilage. The nasals appear as an oblique-oriented thin sliver of bone. Frontoparietals show small signs of ossification, especially along its lateral margins. The parasphenoid has its alae and cultriform process entirely ossified. All the hand phalanges appear mineralized by GS44, as well as the transverse processes IV–VIII in the axial skeleton (Fig. 11). The clavicles and cleithrum appear for the first time in the pectoral girdle. The ossified coccyx and hypochord are present and begin to extend posteriorly to fuse into the adult urostyle. By GS45 most of the postcranial skeleton is completely mineralized and new ossification are seen only at the dentaries and on the mentomeckelians in the lower jaws. The remaining cranial elements that appear ossified in adult specimens (prevomers, sphenethmoid, palatines, squamosals, pterygoids, quadratojugals and prootics) ossify post-metamorphically in GS46 specimens. Also in post-metamorphic specimens, the dorsal centrae of vertebrae and the suprascapulas of the pectoral girdle ossify. The clavicles, which appear only as thin elements in GS44 and GS45 specimens, appear much more robust in post-metamorphic specimens and encase completely the dorsal margins of the procoracoid cartilages.

### Description of post-metamorphic skull

The adult skull of *Thoropa taophora* (Figs. 14–15) is not heavily ossified and the maxillary arcade is complete.

#### *Anterior region*

**Pre-maxillary**. The pre-maxillaries are the anteriormost paired dermal elements of each maxillary arch. Each pre-maxillary is composed of three parts: 1) the *pars dentalis*, located ventrally and bearing teeth; 2) the *pars palatina*, located dorsally and 3) an alary process which usually forms a tall dorsal projection (Trueb 1973). Anteriorly, the pre-maxillaries are narrowly medially separated one another by connective tissue that does stain neither by alcian blue nor by alizarin red. Each *pars dentalis* of *Thoropa taophora* bears 8–12 unicuspid teeth that are not curved. The *pars palatina* is quite reduced in *T. taophora*, however its alary process is well developed and projects posterodorsally. Its dorsal margin is bifurcate and is in intimate contact with the nasal capsules.

**Maxillary.** The maxillaries are paired dentated bones, located posterior to the pre-maxillae. As the pre-maxillary, each maxillary is composed of three parts: 1) a dentated element, called *pars dentalis*; 2) a palatal element, called *pars palatina* and 3) a dorsolateral element, called *pars facialis*. The *pars dentalis* is the longest element in the maxillary arcade and bears approximately 30–40 pedicellate teeth. In addition, the *pars dentalis* overlaps the anterior margin of the pterygoid process. The *pars facialis* bears in its dorsal surface a small projection that does not articulate with the alary process of the pre-maxillary.

**Prevomer.** Many authors refer to this bone simply as “vomer”, however, according to Broom (1902), the vomer of mammals is homologous to the parasphenoid of the “lower vertebrates” hence the term “prevomer” is more appropriate for amphibians. The prevomer are paired palatal bones that invest ventrally the olfactory region. In *Thoropa taophora* each prevomer bear three slender rami: an anterior ramus oriented anterolaterally, a pre-choanal ramus oriented laterally and a post-choanal ramus oriented posterolaterally. Moreover, each prevomer has a posteromedial dentigerous process, located slightly anterior to the distal tips of the palatines, that bears very small odontoids. The distal end of the pre-choanal ramus is located posteriorly to the oblique cartilage and anteriorly to the triangular process of the post-nasal wall.

**Palatines**. The palatines are paired bones that invest the ventral surface of the *planum antorbitale*, delimiting the anteroventral portion of the ocular orbit. Located anteriorly to the ocular orbit and posterior to the choanae, in *T. taophora* the palatines are fully ossified and project medially perpendicular to the skull axis. Despite being laminar, its base is slightly expanded.

**Nasals.** The paired nasals partially roof the nasal capsules, leaving the oblique and alary cartilages exposed, as well as the cartilaginous *tectum nasi* medially. The nasals do not articulate medially and do not overlap with the frontoparietals posteriorly. Maxillary process is well developed, investing the maxillaries posterolaterally.

**Septomaxillae.** The septomaxillae are a pair of dermal bones found embedded within the nasal capsules. In lateral view, this element has semilunar shape, and despite its diminutive size, it is easily identified as the only bony element in the cartilaginous structure of the nasal capsules.

### *Sphenethmoid and orbital region*

**Sphenethmoid.** The sphenethmoid is a small, poorly ossified, azygous element located anterior to the frontoparietal and ventral to the nasals. It did not stain with alizarin red, but by the gap it leaves its diamond shape can be inferred.

**Frontoparietals.** The frontoparietals are paired dermal bony elements that roof the skull, protecting the brain dorsally. In *Thoropa taophora* the frontoparietals are relatively small, leaving the frontoparietal fontanelle almost entirely exposed such that the parasphenoid, the bone flooring the brain ventrally, can be seen even in dorsal view. Posteriorly, the frontoparietals are more developed medially but do not fuse to the prootics.

**Parasphenoid**. The parasphenoid is the bone that encases the brain ventrally. It has an inverted “T” shape with a cultriform process that extends anteriorly until the level of the palatines. The cultriform process is not medially keeled in *T. taophora* and its tip is acuminate. Posteriorly, the lateral alae extend laterally until the medial region of the auditory capsules and do not overlap with the pterygoids. The posteromedial process is small and rounded and do not reach the magnum foramen. The optic foramen is large, rounded and located laterally to the parasphenoid.

### *Auditory and occipital regions*

**Pterygoid.** The pterygoids are paired dermal bones that form the adult suspensorium of anurans (Trueb 1973). It has "Y" shape and bears three rami: anterior, medial and posterior. The anterior ramus is the longest, projecting itself antero-laterally and articulating through its pterygoid process with the *pars dentalis* of the maxillary. The medial ramus extends medially, but does not overlap the parasphenoid alae. The posterior ramus is almost parallel to the cranial axis and extends posteriorly.

**Squamosal.** As the pterygoids, the squamosals are paired and triradiate bones. In lateral view this bone resembles a hammer. The zygomatic ramus of squamosal is anteroventrally oriented, it is relatively short, laterally flattened and bears a tiny pointy dorsal projection. The otic ramus of squamosal is laminar forming an otic plate, longer than the zygomatic ramus and posterodorsally oriented. The distal end of this ramus culminates close to the tympanic ring; however they are not in direct contact. The ventral ramus of squamosal is oriented ventrally and articulates with the quadratojugal. This ramus is not in contact with the frontoparietals. Size relationship of squamosal rami: ventral ramus > otic ramus > zygomatic ramus.

**Quadratojugal**. The quadratojugal is a small slender dermal element located far posterolaterally in the skull. In dorsal view, its anterior portion is tapered and bears a maxillary process which articulates with the maxillary through connective tissue. Its posterior portion bears an extension through which the quadratojugal articulates with the ventral ramus of squamosal.

**Exoccipitals**. Although Trueb (1973) considered that the exoccipitals and prootics fuse in most anurans, these elements are separated in *T. taophora* and thus they are herein treated separately. Dorsally, the exoccipitals are small paired bones that form the posteromedial wall of the auditory capsules. They also marginate the foramen magnum and give rise to the occipital condyles. Each exoccipital bears a well-developed occipital crest.

**Prootics**. The prootics are paired small bones that form the anterior margin of the auditory capsules. These elements are small in *T. taophora* and do not overlap with the frontoparietals anteriorly.

*Mandible*

The anuran mandible is generally composed by three paired elements: 1) the mentomeckelian is the anteriormost element and the smallest of the three elements; 2) the dentary is located posterodorsally to the mentomeckelian and 3) the angular is the posteriormost element and also the most elongated.

The mentomeckelian is a small rectangular-shaped dermal bone. Its elements are synchondrotically united medially. The dentary has neither serrations nor teeth throughout all its length. It extends posteriorly until the half of skull length. Each dentary articulates anterodorsally with the mentomeckelians. The angular extends even further posteriorly; it has a sigmoid shape and double the length of the dentary. It is more robust than the dentary and the two are not linked to one another. The coronoid process of angular is located in its distal end, immediately anterior to the suspensorium. This process is relatively elongated, trapezoid-shaped and it serves as attachment point for the adductor muscles of the mandible. The anterior margin of angular is not in contact with the dentary or with the mentomeckelian, instead it bears a cartilaginous Meckel’s cartilage.

*Hyolaryngeal apparatus*

The hyolaryngeal apparatus is composed of the hyoid and its processes plus the laryngeal cartilages. The hyolaryngeal is entirely cartilaginous except the posteromedial process which is mineralized. The hyoid plate composes the central part of the hyoid and bears three processes: the anterolateral process, the posterolateral process and the posteromedial process. The hyoglossal sinus is the space anterior to the hyoid plate and between the hyales. The hyoglossal sinus is as deep as wide and has a “U”-shaped appearance. The hyales are thin, curved and long, extending posteriorly until the mandible articulation. The anterolateral processes are present and are moderate in size. The posterolateral processes are thin strips of cartilage directed posteriorly, reaching approximately one third the length of the posteromedial processes. The posteromedial process supports the laryngeal cartilages. It is directed posterolaterally and mineralized, with its distal cartilaginous tip (Fig. 16). The arytenoids are triangular-shaped valve-like cartilages that compose the laryngeal apparatus in adult anurans. This cartilage is present in both males and females, but it is better developed in males. The cricoid ring is complete with a thin bronchial process.

### *Plectral apparatus*

The tympanum is supported by the tympanic annulus, which has its edges stained in blue indicating a cartilaginous structure. It is round and bears a hyale process oriented posteriorly in its inferior margin. The columella of anurans is responsible for sound transmission, and is often in close contact with the tympanic annulus, with few cases of secondary loss in anurans (Trueb 1973). Usually it has three parts that may be fused or not: *pars externa plectri*, *pars media plectri* and *pars interna plectri*. In *T. taophora*, the collumela is present and its *pars externa plectri* is cartilaginous with a knob-like shape. The *pars media plectri* and *pars interna plectri* are mineralized and fused. In its distal margin, the *pars interna plectri* is expanded forming the basal plate of the stapes.

## DISCUSSION

### Ontogeny

Generally the remodeling of the oral apparatus occurs contemporaneously with the emergence of forelimbs (Gosner 1960). In *Thoropa taophora*, however, the forelimbs emerge from the operculum, but the oral apparatus is retained for at least two more larval stages. This “uncoupling” of developmental events was also reported by Nodzenski & Inger (1990) for *Ansonia longidigita* and *Meristogenys orphnocnemi*. Both species are from Borneo and their larvae live attached upon rocks on the bottom of lotic environments, such as fast-flowing streams. The authors attributed the prolonged maintenance of their larval oral apparatus to their suctorial habit that requires a functional oral apparatus to hold themselves in place against water currents. Similar to these two species, *T. taophora* larvae also utilize their oral apparatus to attach and move on rocks (Verdade et al. 2019). If the oral apparatus of *T. taophora* larvae began to be broken before the forelimbs have emerged or before they were not strong enough, the tadpoles probably would not be able to grasp onto the rocks they live and would get carried away during rainfalls. Reduction of tail length during metamorphic stages, as seen in fig. 2, is typical of anurans that promote reabsorption of the tail as an energy source during metamorphosis. During this period, reorganizations that occur in its chondrocranium prevent the tadpoles from feeding and thus they reabsorb their tails as an energy source (Altig & McDiarmid 1999).

## Chondrocranium morphology

### Comparison with close-related species

There is still some debate about the inter-familial relationships of Cycloramphidae (Grant et al. 2006; Pyron and Wiens 2011; Fouquet et al. 2013; Blotto et al. 2013; Faivovich et al. 2014). Based on the most recent and comprehensive phylogenies currently available concerning Cycloramphidae and its relatives (Pyron & Wiens 2011; Sabbag et al. 2018; Jetz & Pyron 2018; Streicher et al. 2018; Hime et al. 2021), comparisons of larval chondrocranium and adult skull are made with species from the families Alsodidae, Batrachylidae, Cycloramphidae and Hylodidae if data on these structures are available. Unfortunately, at present, no larval chondrocranium has been described for the family Batrachylidae.

#### *Comparison with Cycloramphidae*

The only chondrocranium description of the genus *Cycloramphus* is that of the endotrophic *C. stejnegeri* made by Lavilla (1991). In both *C. stejnegeri* and *T. taophora* the suprarostral cartilages are linked medially. On the other hand, the *processus ascendens* of *T. taophora* attaches immediately below the *foramen oculomotorium*, thus characterizing the “low” suspensorium of Sokol (1981), whereas in *C. stejnegeri* it is attached above this very foramen, thus characterizing the “high” suspensorium. The high suspensorium is rare in anurans and is considered to be the “primitive” condition, found in basal taxa such as discoglossidean and pelobatoidean larvae (Sokol 1981). It is worthy noting that it was also found in some Neobatrachia by Haas (2003). The otic capsules of *C. stejnegeri* bear no processes whatsoever, however in *T. taophora* the otic capsule bears a *processus anterolateralis* and in some specimens a small *processus posterolateralis*. The *cornua trabeculae* of *C. stejnegeri* is significantly shorter than in *T. taophora* larvae. The hypobranchial plates of *C. stejnegeri* are fused medially, whereas in *T. taophora* they are narrowly separated medially. Contrary to *T. taophora*, spicules are absent in *C. stejnegeri*. Some of those differences, such as absence of spicules in *C. stejnegeri,* may be explained by the endotrophic mode of life of this species, in contrast with the exotrophic mode of *T. taophora*. The chondrocranium and hyobranchial apparatus of *T. miliaris* depicted by Dias et al. (*In press*) is very similar, and virtually indistinguishable from the chondrocranium of *T. taophora*. The chondrocraniums of *C. brasiliensis* and *C. lithomimeticus* depicted by the same authors show a dorsal anterior process on the suprarostral cartilages, which is not present in *T. taophora*. In addition, the hyobranchial of *C. lithomimeticus* lack a copula anterior, which is present in *T. taophora*.

### *Comparison with Hylodidae*

The genus *Hylodes*, together with the genera *Megaelosia*, *Crossodactylus* and *Phantasmarana* composes the family Hylodidae (Vittorazzi et al. 2021), which in turn is one of the sister taxa of Cycloramphidae (Jetz & Pyron 2018). Surprisingly, despite several species of *Hylodes* have had their tadpoles described, only three species had their chondrocranium described: *H. nasus* was depicted by Larson & de Sá (1998), *H. ornatus* was described by Bilate et al. (2012) and *H. meridionalis* by Nogueira-Costa & Wachlevski (2015). None of the seven species of *Megaelosia* or *Phantasmarana* have their larval chondrocranium described yet, and from the genus *Crossodactylus*, only *C. gaudichaudii* (Larson & de Sá 1998; Silva-Soares et al. 2015) has its chondrocranium described and depicted.

The adrostral tissue mass is present in *Hylodes meridionalis, H. nasus* and *H. ornatus*, but it is not in *Thoropa taophora*. The *cornua trabeculae* of *T. taophora* is slender than in *H. ornatus* and its *processus muscularis quadrati* does not have any projection, whereas in *H. ornatus* there are two triangular projections. In *H. meridionalis*, the *processus quadratoethmoidalis* is absent; while in *T. taophora* it is present, although reduced. In addition, the pieces composing the suprarostral cartilage of *H. meridionalis* and *H. nasus* are completely separated, whereas in *T. taophora* its corpora are fused medially, and the corpora fuse dorsolaterally to the alae. A copula anterior is present in *T. taophora*, but absent in all three *Hylodes*. Regarding the genus *Crossodactylus*, *T. taophora* is distinguished by its reduced *processus antorbitalis* and by presence of a copula anterior, which is absent in *C. gaudichaudii*. In addition, the suprarostral of *C. gaudichaudii* is tetrapartite, whereas in *T. taophora* the pieces articulate with each other through synchondrosis.

### *Comparison with Alsodidae*

The genera *Alsodes, Eupsophus* and *Limnomedusa* compose the family Alsodidae (Pyron & Wiens 2011; Blotto et al. 2013; Jetz & Pyron 2018). Currently, five species of

*Alsodes* have their chondrocranium depicted: *A. barrioi* (Lavilla 1992), *A. gargola* e *A. neuquensis* (Barrasso et al. 2016), *A.* cf. *norae* (Formas & Cuevas 2017), *A. vanzolinii* and *A. verrucosus* (Formas & Brievas 2004). The chondrocranium of *Alsodes* species share several features in common (Barraso et al. 2016): (i) ovoid or subquadrangular neurocranium, (ii) *cornua trabeculae* well developed, representing more than 20% of chondrocranial total length, (iii) tetrapartite suprarostral cartilage, (iv) presence of adrostral tissue, (v) presence of *comissura quadratorbitalis* connecting the *processus muscularis quadrati* to the neurocranium, (vi) palatoquadrate extending posteriorly to the level of the pila antotica, (vii) *processus anterolateralis* of the crista parotica well developed and (viii) wide *processus pseudopterygoideus* with its tip fused to the *basi cranii.*

Of these character-states, only (i) (ii) and (vii) are also found in tadpoles of *Thoropa taophora*. The suprarostral cartilage of *T. taophora* has two parts, instead of four and adrostral tissue is not present. The *processus muscularis quadrati* is connected to the neurocranium via ligamentum in *T. taophora* instead of a commissura and there is no *processus pseudopterygoideus* in any developmental stage. In addition, some specimens of *A. neuquensis* have their *processus anterolateralis* connected to the posterior portion of palatoquadrate, forming a larval *processus oticus* (Lavilla 1992; Barrasso et al. 2016). This connection is not found in any developmental stage in *T. taophora*. All the four ceratobranchialia of *A. gargola*, *A. barrioi*, *A. vanzolinii* and *A. verrucosus* are fused to the hypobranchial plate, contrary to the character-state found in *T. taophora* in which the third ceratobranchial is the only one not fused. Similarly, the third ceratobranchial of *A. neuquensis* also is the only one not fused to the hypobranchial plate (Barrasso et al. 2016).

The genus *Eupsophus* is composed of species with nidicolous endotrophic tadpoles (Vera Candioti et al. 2011). The only chondrocraniums described for the genus are those of *E. calcaratus* (Vera Candioti et al. 2005), *E. emiliopugini* (Vera Candioti et al. 2011), *E. queulensis* (Cárdenas-Rojas et al. 2007) and *E. nahuelbutensis* (Nuñez & Úbeda 2009). Unlike larvae of *Alsodes*, larvae of *Eupsophus* resemble those of *T. taophora* in not having a *comissura quadratoorbitalis* or a *processus pseudopterygoideus* (Vera Candioti et al. 2011). On the other hand, larvae of *Eupsophus* have shorter *cornua trabeculae* than *T. taophora* (less than 20% of chondrocranial total length in *Eupsophus*; approximately 25% in *T. taophora*). The infrarostral cartilages are fused in *E. nahuelbutensis*. The *tectum synoticum* of *E. emiliopugini* and *E. queulensis* is much more robust (Cárdenas-Rojas et al. 2007; Vera Candioti et al. 2011) than in *T. taophora*. The posterior suspensorium of *E. emiliopugini* and *E. queuelensis* is the intermediate type (sensu Sokol 1981), whereas in *T. taophora* it is a low type suspensorium. The crista parotica is absent in *E. queulensis*, however it is present in *T. taophora*. The ceratobranchialia I and II of *E. queulensis* are not connected by a *comissura terminales* and in *E. emiliopugini* this comissura is poorly developed or even absent as in *E. nahuelbutensis*, whereas in *T. taophora* it is well developed and connects all ceratobranchialia. A copula anterior is absent in all three species, whereas it is present in *T. taophora*.

Regarding the genus *Limnomedusa*, only one species has its larval chondrocranium described: *L. macroglossa* (Alcalde & Blotto 2006). The tadpoles of *Limnomedusa macroglossa* have a *comissura quadratoorbitalis* connecting its *processus muscularis quadrati* to the neucrocranium and a *processus oticus* posteriorly, connecting the posterior bump of palatoquadrate to the otic capsules whereas in *T. taophora* none of these two structures are present. Additionally, adrostral tissue is present and the *cornua trabeculae* of *L. macroglossa* is shorter than in *T. taophora*. Tadpoles of *L. macroglossa* also have a *processus pseudopterygoideus*, unlike tadpoles of *T. taophora* which do not have this process. The *processus ascendens* of *L. macroglossa* attaches to the neurocranium in an intermediate condition, whereas in *T. taophora* tadpoles this attachment is the "low" condition (sensu Sokol 1981). In *L. macroglossa*, only the first ceratobranchial is attached to the hypobranchial plate, but in *T. taophora* all ceratobranchialia are attached either by cartilage or connective tissue. Contrary to other alsodids, a copula anterior is present in *L. macroglossa* as it is present in *T. taophora*.

## Comparison with other hyloidean families

Hyloid frogs encompass almost 53% of all frog species, including many well-known families distributed across the world such as Bufonidae and Hylidae (Streicher et al. 2018). The *foramen prooticum* is a large aperture through which pass the trigeminal and facial nerves in the vast majority of anuran larvae (except Ascaphidae and

Discoglossidae; Sokol 1975). According to Haas (1995), the term “foramen” is more adequate instead of “fissura” since the orbital cartilages do attach on the dorsal margin of the otic capsule in *Thoropa taophora*.

The vast majority of anurans possess a *processus muscularis quadrati* fastened to the neurocranium either by a ligament (*ligamentum tectum*) or by a cartilaginous bridge (*commisura quadratoorbitalis*) that replaces the ligament (Haas 2003). In *T. taophora* the *processus muscularis* attaches to the braincase via *ligamentum tectum* as in dendrobatid larvae (Haas 1995; Krings et al. 2017); however in bufonids (Fabrezi & Vera 1997; Larson et al. 2004; Vera Candioti 2007; Oliveira et al. 2014), odontophrynids (Fabrezi & Vera 1997; Dias et al. 2013; do Nascimento et al. 2013; Dias et al. 2019) and some leptodactylids (Fabrezi & Vera 1997; Larson & de Sá 1998) this attachment is via *commisura quadratoorbitalis*. The *commisura quadratocranialis anterior* of anuran larvae may have only a *processus ethmoidalis* on its anterior margin or an additional *processus pseudopterygoideus* on its posterior margin. In *T. taophora*, although much reduced, only the *processus ethmoidalis* is present, a condition shared with some hylids (de Sá 1988; Fabrezi & Vera 1997; Alcalde et al. 2011; Rodrigues et al. 2017), leptodactylids (Larson & de Sá 1998) and some dendrobatids (Haas 1995), whereas in bufonids (Oliveira et al. 2014), odontophrynids (Fabrezi & Vera 1997; Dias et al. 2013; do Nascimento et al. 2013; Dias et al. 2019) and some phyllomedusids (Dias et al. 2018) the *processus pseudopterygoideus* is present.

Subdivision of frontoparietal fontanelle by growth of *taenia tecti transversalis* and *medialis* occurs in *T. taophora* larvae as early as GS36. It is a common feature among anurans and also occurs in many bufonids, few hylids and some leptodactylids (Larson & de Sá 1998; Alcalde & Rosset 2003; Alcalde et al. 2011; Oliveira et al. 2014). Exceptions such as *Rhinella granulosa*, *R. hoogmoedi*, *R. jimi* and *R. scheinederi* (Oliveira et al. 2014) may be due to analyzing only early stage specimens. The presence/absence of these taeniae is stage dependent, and usually appears in late pre-metamorphic or metamorphic specimens (Haas 2003). Caution must be taken when stating their absence only by analyzing early stage specimens as this may yield confusions in literature. Species of *Odontophrynus* (Odontophrynidae) analyzed to date have a *tectum parietale* roofing the parietal region (Haas 2003; do Nascimento et al. 2013), whereas in *Proceratophrys boiei* (Odontophrynidae), this tectum does not exist and the parietal fontanelles are open (Dias et al. 2013). The tectum parietale seems to be present only in advanced metamorphic specimens of *T. taophora*. The *crista parotica* of *T. taophora* bears one distinct *processus anterolateralis*, which does not reach the posterior curvature of palatoquadrate, thus not forming a *processus oticus*. This condition is also shared with dendrobatids (Haas 1995), some odontophrynids (Fabrezi & Vera 1997; Dias et al. 2013), and most leptodactylids (Larson & de Sá 1998), however it contrasts to *Scinax* (Hylidae) and *Phyllomedusa* (Phyllomedusidae) which have a *processus oticus* (Rodrigues et al. 2017; Dias et al. 2018) and to some other odontophrynids (e.g. *Odontophrynus carvalhoii*; do Nascimento et al. 2013).

The hyobranchial apparatus of all species of these families share the same basic *bauplan*, with wide ceratohyalia connected medially by a *pars reuniens* which has different degrees of chondrification in different species. Posteriorly there are the hypobranchial plates, which are flat sheets of cartilage that support the branchial baskets composed typically of four ceratobranchialia that are united distally by *comissurae terminales* and may be united proximally by *commisurae proximales* or slightly more distally by fusion of *processus branchialis* of ceratobranchialia II and III. In *Thoropa taophora* the ceratohyal bear two anterior processes: the *processus anterior hyalis* medially and the *processus anterolateralis hyalis* laterally and one additional posterior process: the *processus posterior hyalis*. In some dendrobatids (Haas 1995), bufonids (Vera Candioti 2007; Oliveira et al. 2014), hylids (Rodrigues et al. 2017) and odontophrynids (Nascimento et al. 2013) a third process is also present on the anterior margin of ceratohyal: *processus lateralis hyalis*. A small copula anterior is present in *T. taophora*. The presence of this element is highly variable even among close-related taxa and may be even variable among specimens of the same species. For instance, Haas (1995) found the copula anterior in only two specimens of *Epipedobates anthonyi*, in one specimen of *E. tricolor* and in one specimen of *Phyllobates bicolor*. In other taxa its presence seems to be less variable; it is present in the majority of bufonids (Fabrezi & Vera 1997; Larson 2004; Vera Candioti 2007; Oliveira et al. 2014), in centrolenids (Rada et al. 2019) and in some odontophrynids (Nascimento et al. 2013; Dias et al. 2019). However it is absent in the majority of hylids (de Sá 1988; Fabrezi & Vera 1997; Vera Candioti 2007; Alcalde et al. 2011; Rodrigues et al. 2017) and in the majority of leptodactylids (Fabrezi & Vera 1997; Larson & de Sá 1998).

The hypobranchial plates in *T. taophora* do not fuse medially; this condition is shared with some bufonids (Vera Candioti 2007; Oliveira et al. 2014) whereas in leptodactylids the plates are fused only posteriorly (Larson & de Sá 1998). The *processus branchialis* of ceratobranchials II and III of *T. taophora* are not fused and are connected only by connective tissue, thus corresponding to an "open" condition (sensu Haas 2003). This "open" condition is also shared with bufonids such as *Anaxyrus americanus* (Larson 2004) and species of *Melanophryniscus* (Larson et al. 2003). It is also found in the hylid *Scinax skuki* (Rodrigues et al. 2017), in some odontophrynids (Nascimento et al. 2013) and in some leptodactylids (Larson & de Sá 1998). Alternatively the *processus branchialis* of the ceratobranchials II and III may be fused, thus corresponding to the "closed" condition. This condition if found in some dendrobatids (Haas 1995), in some hylids (de Sá 1988; Alcalde et al. 2011) and in some leptodactylids (Fabrezi & Vera 1997; Larson & de Sá 1998). Degree of fusion of the ceratobranchialia to the hypobranchial plates is also subject to variation in taxa. In *T. taophora* all the ceratobranchialia I and II are synchondrostically fused to the hypobranchial plate, whereas the III and IV which are connected only by ligament. In dendrobatids all ceratobranchialia are connected to the plate by ligament (Haas 1995). In bufonid species of *Rhinella* only the ceratobranchial IV is connected to the plate by ligament (Oliveira et al. 2014). In hylids analyzed by Alcalde et al. (2011) the ceratobranchialia II or IV may be attached by ligament to the plate. In odontophrynids ceratobranchialia II and III are connected to the hypobranchial plate in different degrees (Dias et al. 2019).

## Development of nasal cartilages

Descriptions of the development of nasal cartilages in metamorphic tadpoles are very scarce, being available for only a couple of species as noted by Púgener & Maglia (2007). In *T. taophora*, the development of the nasal cartilages commences during metamorphic climax, when larval cartilages are reabsorbed (e.g. *cornua trabeculae* and suprarostrals), however in *Spea bombifrons*, *S. intermontana* and *S. multiplicata*, the first nasal structures arise between GS31–36 (Scaphiopodidae; Púgener & Maglia 2007). The first structures that arise in these species are the *septum nasi* directed anteriorly and the *planum antorbitale* directed laterally. In *Eupsophus calcaratus* (Alsodidae; Vera Candioti et al. 2005) and *E. emiliopugini* (Vera Candioti et al. 2011) the nasal cartilages begin developing between GS37–39. The first structures that arise in these species are the antorbital process and the *septum nasi*. In *T. taophora* the first fully formed structures on the nasal capsules appear at GS44: the processus antorbitalis, the *septum, tectum* and *solum nasi,* the small cartilaginous septomaxilla, the oblique cartilage with its planum terminale directed posteriorly and alary cartilage. Formation of paranasal commissure begins at GS43. Despite the apparent early initial development in species of *Spea*, the major development of nasal capsules occurs in fact during metamorphic stages like all other species mentioned above. Formation of a paranasal commissure occurs in species of *Spea* and is retained in adult specimens as well (Púgener & Maglia 2007). Older works dealing with adult specimens of bufonids also report the presence of a synchondrosis between the *planum terminale* and the *planum antorbitale* (Michael 1961). This commissure is formed by the fusion of the *planum terminale* with the *planum antorbitale*. In adult specimens of *T. taophora*, this commissure is not present anymore, but a lingular process and a triangular process of the post-nasal wall are present instead. These structures are hypothesized as being the remains of the former paranasal commissure (Púgener & Maglia 2007).

## Ecological significance of chondrocranium morphology of *Thoropa taophora*

The morphological characters described by Haas & Richards (1998) in the chondrocranium of suctorial species were not found in *Thoropa taophora* tadpoles analyzed herein. A comparison between the characters described by Haas & Richards (1998) and the ones found in *T. taophora* tadpoles are summarized in table 2. The fact that none of those characters were found in *T. taophora* raises two hypotheses: I) that mouth suction plays little role in adhesion to the substrate or II) that water currents faced by these tadpoles are not so strong that requires chondrocranium specializations.

Considering the first hypothesis, Lutz (1947) and Barth (1956) presumed that these tadpoles attach to the substrate using their oral apparatus. According to Barth (1956), tadpoles grasp on rocks with aid of “hooks” present in two rows anteriorly on labium and three rows posteriorly. These “hooks” however are actually keratinized denticles, which are present in the vast majority of anuran larvae and serve as a feeding structure along with their keratinized jaw sheaths (McDiarmid & Altig 1999). Thus, their role in adhesion is at least questionable. After analyzing internal buccal anatomy of *T. taophora* larvae, Wassersug & Heyer (1983) proposed that these tadpoles are obligate air breathers and, in order to ventilate the lungs, adhesion solely using the mouth would be impossible. Thus, the authors suggested that adhesion by capillarity using its flattened expanded abdomen is more likely. Based on the lack of chondrocranium specializations for continuous suction found in this work, tadpoles of *T. taophora* possibly maintain their position by capillarity, instead of suction with the oral apparatus. Verdade et al. (2019) proposed that the abdominal “flap” found in cycloramphid tadpoles aids in gas exchange through the skin, rather than adhesion as proposed by Wassersug & Heyer (1983). Dias et al. (*In press*) provided evidence that supports skin as playing a major role in gas exchange. Whether cycloramphid tadpoles use their skin, lungs or both to breathe remains obscure, although the skin-lungs hypothesis seems the most plausible one considering our current knowledge.

Considering now the second hypothesis, the tadpoles analyzed by Haas & Richards (1998) face much faster water currents in rivers than *T. taophora* does on wet rocks. Adhesion solely by capillarity in such lotic environment possibly is not enough to avoid being carried away by the water currents. However, tadpoles of *T. taophora* rarely face fast water currents in their habitat, and strong chondrocranium cartilages may not be necessary to maintain their position. In fact, Rocha et al. (2002) verified that smaller tadpoles of *T. miliaris* prefer shallower water currents. Interestingly, a couple of days after a heavy rain, some tadpoles of *T. taophora* were seen being lixiviated from their vertical rock slabs (personal observation), possibly because the water current that was descending the wall was too strong for them to keep attached. Although the habitat tadpoles of *T. taophora* live is rare among anurans, their chondrocranium and hyobranchial apparatus resembles that of typical pond-type larvae (Vera Candioti 2007).

Unfortunately, no other semiterrestrial species has its ossification sequence described, however some terrestrial direct-developers have. Anurans with direct development do not have a free-living larval phase, instead almost all development occurs inside the egg and an adult fully formed hatches (Hanken et al. 1992). The ossification sequence of direct-developing species may deviate from the “typical” sequence of anurans as in *Eleutherodactylus coqui* (Hanken et al. 1992) or may be quite similar to a typical free-living larva as in *Philautus silus* (Kerney et al. 2007). However, none of them have a delayed ossification as *T. taophora* does, and usually the individual that hatches from the eggs already has various bones ossified in its skull (Kerney et al. 2007).

### Ossification sequence and heterochrony

Using the traditional clearing and double-staining technique in tadpoles of *Thoropa taophora* we found initial exoccipital ossification at GS40, however Fidalgo et al. (2018) found exoccipital ossification as early as GS28 using a modern method of synchrotron phase-contrast microtomography (SR-PhC-microCT). Although most studies, including this dissertation, correlate retention of alizarin red stain with ossification, alizarin may fail in detecting early bone formation (Hanken & Hall 1988; Sheil et al. 2014). As such, their results might be due to the greater sensibility of their method in detecting such early bone formation. Another possibility is that they might have mistakenly identified the endolymphatic sacs, which are located near the inner ear and contain calcium crystals in anurans (Pikington & Simkiss 1966), as exoccipital ossification. More recently, Fidalgo et al. (2020) acknowledged the existence of those tiny deposits of calcium near the occipital region in GS28 tadpoles of *T. miliaris*.

In *T. taophora* the the first cranial bones that fully ossify are the exoccipitals, the pre-maxillaries, parasphenoid and frontoparietal. The maxillary, angulosplenial, nasal and septomaxilla appear simultaneously to the parasphenoid and frontoparietal, however the former four bones are only partially ossified whereas the latter two are fully ossified. The suspensorium bones seem to appear after metamorphosis is complete and the bones that compose the upper and lower jaws are only partially ossified at GS45, except the pre-maxillaries which show extensive ossification as early as GS44. Among the first bones to develop in anurans are the frontoparietal and the parasphenoid, which invest the braincase dorsally and ventrally respectively (Trueb 1985; Harrington et al. 2013) and the exoccipital that marginates the neurocranium posteriorly. Bones that compose the mandible and suspensorium usually ossify towards the end of metamorphosis, whereas the sphenethmoid, columella and other neomorphic elements typically appear after metamorphosis is complete (Trueb 1985). Due to the extensive cranial restructuring necessary to attain the adult skull, if the bones that compose the suspensorium (pterygoid, squamosal) or the upper and lower jaws mineralize earlier in ontogeny, cranial restructuration would be compromised (Wassersug 1975; Harrington et al. 2013). Furthermore, mandible ossification becomes important only during the carnivore prey-capturing phase of anurans, which occurs only post-metamorphically, therefore ossification of these elements during the larval phase is not imperative (DeJongh 1968).

Regarding cranial ossification, approximately seven bones appear after metamorphosis is complete in *T. taophora*. A similar pattern is found in specimens of *Eupsophus calcaratus* and *E. emiliopugini* which show little ossification in their skulls during metamorphic stages (Vera Candioti et al. 2005; Vera Candioti et al. 2011). Table 4 summarizes and compares the cranial ossification sequence of *Thoropa taophora* and *Eupsophus calcaratus* and *E. emiliopugini*. In *E. calcaratus*, ten bones appear post-metamorphically (Vera Candioti et al. 2005) and in *E. emiliopugini* virtually all bones ossify after metamorphosis is complete (Vera Candioti et al. 2011). However, unlike those two species, the exoccipital is the first bone to mineralize in the cranium of *T. taophora*, whereas the septomaxilla and pre-maxillary are the first bones ossify in the skulls of *E. calcaratus* and *E. emiliopugini*. In addition, *Eupsophus emiliopugini* also shows delayed loss of its larval mouthparts (Vera Candioti et al. 2011) as occurs in *T. taophora* and in the torrent-dwellers species *Ansonia longidigita* and *Meristogenys orphnocnemis* (Nodzenski & Inger 1990), but ossification sequences for those two species are not available. According to the table of Gosner (1960), the destruction of larval oral apparatus in addition to the emergence of the anterior limbs from the opercular chamber marks the beginning of metamorphosis. The table of Gosner is based mainly on observations of temperate species and does not take into consideration the huge diversity of tadpoles found in Neotropical regions. Thus, deviations from the table are expected in Neotropical anurans, where many developmental modes are unique to that region (Hödl 1990). Unfortunately, postcranial ossification for *E. calcaratus* and *E. emilipougini* are not available for comparison with *T. taophora*.

It has been hypothesized that little ossification in the skull of post-metamorphic specimens of *Eupsophus calcaratus* and *E. emiliopugini* is a paedomorphic character (Vera Candioti et al. 2011), however this statement was not supported by a phylogenetic testing of heterochronies. Heterochrony is by definition a shift in the relative timing of developmental events in relation to the ancestor character-state (Gould 1977; Alberch et al. 1979; Alberch et al. 1985). Many methods were developed in order to reconstruct ancestral character-state and to propose new heterocronic hypotheses (Bininda-Emonds et al. 2002; Jeffery et al. 2005; Harrison & Larsson 2008). Such methods were used to study heterochronic shifts in ossification sequences of anurans (Emerson 1986; Dunlap & Sanchiz 1996; Yeh 2002; Mitgutsch et al. 2009; Harrington et al. 2013), yielding some insights at familial level (Yeh 2002) as well as more general insights about ossification sequence in amphibians as a whole (Harrington et al. 2013). While some authors have suggested the use of temporal landmarks such as age or size to identify heterochronies, these measures are often difficult to obtain and may yield confusions due to different growth rates in different species (Haas 1999; Smith 2001). Thus, the use of the sheer series of events is more reliable (Smith 2001).

The genus *Alsodes* also is phylogenetically close to *Thoropa* (Jetz & Pyron 2018), however ossification sequences of them are scarce. The frontoparietals and parasphenoid are the first bones to mineralize, at GS34 in *A. verrucosus,* whereas in *A. vanzolinii* the chondrocranium is still entirely cartilaginous at this stage (Formas & Brieva 2004). In *T. taophora*, the parasphenoid and frontoparietals mineralize at GS44, after the exoccipitals, which begin mineralization at GS40. In larvae of *A. barrioi*, primordia of ossification are found as early as GS31 on the frontoparietals, parasphenoid and on the occipital condyles (exoccipitals) (Lavilla 1992). Based on these few reports, species of *Alsodes* begin their cranial ossification in pre-metamorphic stages, whereas in *Eupsophus* and *Thoropa* ossification is only found in metamorphic and post-metamorphic stages.

In anurans, cranial ossification tends to occur earlier or at about the same time as postcranial ossification (Gómez et al. 2017). In *T. taophora* cranial and postcranial ossification initiates simultaneously at GS40, however the postcranial ossification progresses much more rapidly than cranial ossification and by GS44 nearly all postcranium is mineralized. Such delayed osteogenesis of the skull in relation to the postcranial skeleton has been correlated to species with rapid-developing tadpoles (Dunlap and Sanchiz 1996), however that hypothesis still lacks support from evidence of more species (Banbury & Maglia 2009). The acceleration of postcranial ossification that occurs in *T. taophora* may represent an adaptation for the semiterrestrial life history of the species, and more specifically as an adaptation for jumping. Metamorphic and even pre-metamorphic tadpoles of *T. taophora* around GS41 are able to perform some small jumps when disturbed, which increase in distance as metamorphosis progresses (personal observation). Since anuran saltation involves the hind limbs, urostyle, sacrum and presacral vertebrae (Emerson and De Jongh 1980), we hypothesize this jumping behavior would not be possible if those elements were not ossified. Other semiterrestrial tadpoles such as the Beddome's Indian frog *Indirana beddomii* (Veeranagoudar et al. 2009) are also able to jump away early in development when disturbed, however an ossification sequence of that species is not available.

### Post-metamorphic cranium morphology

The sphenethmoid of post-metamorphic specimens of *T. taophora* analyzed herein is poorly ossified. Ossification of this element is known to occur post-metamorphically in several anurans (Weisbecker & Mitgutsch 2010). Trueb (1973) stated that the prootics and exoccipitals are “indistinguishably fused in modern anurans”, forming the otoccipital. However, in *T. taophora*, these elements are not fused and both bones are well-ossified and clearly distinguishable in young post-metamorphic specimens. Older specimens were not analyzed to check if these two bones really fuse to form the otoccipital. Many of the characteristics found in the craniums of arboreal species described by Trueb (1973) are present in *T. taophora*, as the frontoparietals covering only a small portion of the frontoparietal fontanelle, such that the parasphenoid can be easily seen even in dorsal view. The nasals of *T. taophora* do not articulate medially, the quadratojugals are very thin and both the maxillary and pre-maxillary retain teeth, conditions also found in arboreal species (Trueb 1973). In contrast, terrestrial species such as bufonids tend to have large well-ossified frontoparietals, nasals and sphenethmoid (Pramuk 2006), which strengthen the cranium dorsally.

### Comparison with close-related species

Descriptions of adult craniums of cycloramphids and its relatives are as scarce as larval chondrocranium descriptions. For the genus *Thoropa*, only *T. lutzi* have had its cranium analyzed so far (Lynch 1971). For *Cycloramphus*, only the skull of *C. eleutherodactylus* (Lynch 1971), *C. organensis* (Weber et al. 2011) and *C. stejnegeri* (Lynch 1971) are depicted in the literature. In the same work, Lynch (1971) also described the adult cranium of *Cycloramphus parvulus*. Regarding the family Hylodidae, Lynch (1971) depicted the cranium of *Crossodactylus gaudichaudii*, *Hylodes asper* and *Megaelosia goeldii*. Craniums of alsodids are also poorly documented. The skulls of *Eupsophus roseus* and *Limnomedusa macroglossa* were depicted by Lynch (1971). The cranium of *Eupsophus altor* was described by Nuñez et al. (2012) and of *E. calcaratus* by Lavilla et al. (2010). The cranium of *Alsodes coppingeri* was depicted by Formas et al. (2008).

### *Comparison with Cycloramphidae*

The frontoparietals of *Cycloramphus eleutherodactylus*, *C. organensis*, *C. stejnegeri* and *C. parvulus* contact medially occluding the frontoparietal fontanelle totally, whereas in *Thoropa taophora* the frontoparietals do not contact medially, leaving the fontanelle partially exposed. Posteriorly, the frontoparietals of *T. taophora*, *C. organensis*, *C. stejnegeri* and *C. parvulus* are not fused to the prootics, while in *C. eleutherodactylus* both bones are fused. The nasals of *C. eleutherodactylus*, *C. organensis*, *C. stejnegeri* and *C. parvulus* are wider, contacting medially with one another and posteriorly with the frontoparietals, whereas the nasals of *T. taophora* do not contact medially or posteriorly with the frontoparietals. The prevomers and palatines of *C. organensis* contact with the cultriform process of the parasphenoid, but in *T. taophora* neither contacts this process. In *C. eleutherodactylus*, the palatines are considerably broader than in *T. taophora*. In addition, the palatines of *C. eleutherodactylus* contacts with the prevomer, whereas in *T. taophora* this does not happen. The palatines of *C. eleutherodactylus, C. organensis, C. stejnegeri* and *C. parvulus* are arcuate posteriorly, whereas in *T. taophora* they are almost straight. The anterior ramus of pterygoid is robust in *C. eleutherodactylus*, *C. stejnegeri*, *C. parvulus* but in *T. taophora* it is much thinner. All species have teeth in their prevomers, maxillaries and pre-maxillaries. The hyoid apparatus of species of *Cycloramphus* and alsodids are very similar to *T. taophora.*

### *Comparison with Batrachyla*

Species from the family Batrachyla are peculiar in that they lack a quadratojugal. Lynch (1971) stated that this feature diagnosis the genus *Atelognathus*, however quadratojugals are also absent in *Hylorina sylvatica*, *Batrachyla fitzroya*, *B. leptopus*, *B. nibaldoi* and *C. grandisonae* (Lynch 1971; Basso 1994; Formas 1997; Basso 1998; Meriggio et al. 2004; Basso et al. 2011), all of which compose the family Batrachyla. Thus, absence of quadratojugal might actually be a synapomorphy for the family. The sphenethmoid of *B. leptopus* (Lynch 1971) and *C. grandisonae* (Basso et al. 2011) are larger than in *T. taophora* and the alary process of pre-maxillary of *H. sylvatica* is much more developed than in *T. taophora* (Lynch 1971). The palatines of *Atelognathus nitoi* (as *A. jeinimenensis* in Meriggio et al. 2004 and *A. ceii* in Basso 1998) are much more reduced in comparison to *T. taophora*. The alary process of pre-maxillary in *Hylorina sylvatica* is more developed than in *T. taophora* and the anterior process of prevomer is absent (Lynch 1971), whereas it is present in *T. taophora*. Anterior vomerine process is also absent in *B. fitzroya* (Basso 1994). The frontoparietals of *B. nibaldoi* do not touch medially (Formas 1997), thus exposing the fontanelle underneath; similar to what is found in *T. taophora*.

*Comparison with Hylodidae*

The alary process of pre-maxillary in *Crossodactylus gaudichaudii* and *Hylodes asper* is directed anterodorsally, whereas in *T. taophora* it is directed posterodorsally. The sphenethmoid of *C. gaudichaudii, H. asper* and *Megaelosia goeldii* is large and separates the nasals medially, however in *T. taophora* it is a very small element, that does not stain with alizarin, indicating little mineralization. The frontoparietals of *C. gaudichaudii* and *H. asper* are fused to the prootics and occlude totally the frontoparietal fontanelle, however in *T. taophora* they are not fused to the prootics and do not occlude the fontanelle. The prevomers of *C. gaudichaudii* lack a medial dentigerous ramus. The slender nature of palatines and pterygoid rami of *C. gaudichaudii* and *H. asper* resemble those of *T. taophora*. Posteriorly, the cranium of *C. gaudichaudii* lacks a quadratojugal; however in *H. asper, M. goeldii* and *T. taophora* this element is present articulating anteriorly through connective tissue with the maxillary. Loss of the quadratojugal is not exceptional, since it is frequently absent in smaller frogs or in those with reduced ossification (Trueb 1973). In addition, the quadratojugal usually ossify later in anuran ossification sequences, and thus is more likely to be reduced or completely lost (Weisbecker & Mitgutsch 2010). The otic ramus of squamosal in *C. gaudichaudii* is shorter than its zygomatic ramus; however in *T. taophora* the otic is longer than the zygomatic ramus. In *H. asper* the otic and zygomatic rami have approximately the same length, whereas in *T. taophora* the otic ramus is longer than its zygomatic ramus. Conversely, in *M. goeldii* the zygomatic ramus is much broader relative to the previous species and is in contact with the maxillary. Despite *C. gaudichaudii*, *H. asper* and *T. taophora* all possess teeth in their pre-maxillaries and maxillaries, the teeth of *M. goeldii* are much longer and “fang-like”, i.e. curved posteriorly, as remarked by Lynch (1971).

### *Comparison with Alsodidae*

As noted before by Lynch (1971), adult osteology of Alsodidae and *Thoropa* share some common characters as nasals not contacting with frontoparietals, frontoparietal fontanelle partially exposed and frontoparietal not fused with prootics. In addition, alsodids and *T. taophora* also have slender pterygoid rami and quadratojugal articulating with the maxillary. However, some differences are also noticeable. The sphenethmoid of *Eupsophus altor*, *E. calcaratus* and *E. roseus* is larger and overlies the *tectum nasi* dorsally, whereas in *T. taophora* it is small and does not overlies the nasal cartilages. The sphenethmoid varies greatly in size and presence/absence among anurans (Weisbecker & Mitgutsch 2010). In caecilians, the sphenethmoid protects the olfactory organs underneath from mechanic impacts when burrowing (Duellman & Trueb 1994). Large sphenethmoids may have similar function in anurans; however this has not been tested yet. The zygomatic ramus of squamosal is longer than the otic ramus in *E. altor* and *E. calcaratus*, whereas it is shorter than the otic ramus of *T. taophora*. The occipital condyles of *T. taophora* seem to be more pronounced than the ones of *E. calcaratus*. The tip of the cultrifrom process of parasphenoid of *E. altor* is serrated, whereas in *T. taophora* it is smooth. The palatines of *E. roseus* bear small odontoid ridges, whereas in *T. taophora* they do not. The skull of *Limnomedusa macroglossa* bears various similarities to the skull of *T. taophora* and with alsodids. Both *L. macroglossa* and *T. taophora* have their frontoparietal fontanelle exposed, small sphenethmoid, nasals that do not contact medially one another, frontoparietal not fused with prootics, slender rami of pterygoid, quadratojugal as a sliver of bone articulating with the maxillary. Despite these similarities, the squamosals of both species bear the greater differences. In *L. macroglossa*, the zygomatic and otic rami are short, with no otic plate. However, in *T. taophora* the rami are longer and the otic ramus is flattened, forming an otic plate.

## **Comparison with other hyloidean families**

The pre-maxillaries and maxillaries of *Bufo* and *Rhinella*, both bufonids, bear teeth, as in *T. taophora* (Pramuk 2002; 2006). The skull of most species of these bufonids is heavily ossified with large frontoparietals and nasals that contact medially, obscuring the frontoparietal fontanelle and the nasal capsules (Pramuk 2002; 2006). In *T. taophora* neither of the two bones contact medially. The dorsal dermal bones (frontoparietals and nasals) in bufonids may be exostosed and ornamented (McDiarmid

1971; Pramuk 2002), whilst in *T. taophora* both bones are smooth. The palatines of several bufonids bear a ventral ridge (Pramuk 2006), which is not present in *T. taophora*. In various bufonids (e.g., *Rhinella crucifer* and *R. granulosa*) the zygomatic ramus of squamosal articulates with the maxillary, and the ventral ramus is expanded (Pregill 1981; Pramuk 2002), but in *T. taophora* the squamosal is free from the maxillary and uniform in length. In the genus *Melanophryniscus* the zygomatic ramus of squamosal is absent (McDiarmid 1971; Peloso et al. 2012), whilst in *T. taophora* it is present. The columella is absent in some bufonids as *Melanophryniscus* (Pramuk 2006), but it is present in *T. taophora*. The hyoid plate of *M. setiba* lacks a posterolateral process and its posteromedial process is posterolaterally divergent (Peloso et al. 2012). In addition, its hyoid plate is narrow and its hyales are relatively short.

Despite being the most speciose genus of the family Hylidae with 127 species (Frost 2021), studies on skeletal morphology of species of *Scinax* are scarce and restricted to a few comparative (Baldo et al. 2019) or phylogenetic works (Faivovich 2002). The sphenethmoid of *S. fontanarrosai* is considerably larger than of *T. taophora* and overlaps with the nasals anteriorly. In addition, the frontoparietal fontanelle is much less exposed in *S. fontanarrosai* (Baldo et al. 2019) and *S. icterica* (Faivovich 2002) than in *T. taophora*. The alary process of pre-maxillaries in *S. fontanarrosai* is anteriorly inclined while it is posterodorsally inclined in *T. taophora*~~.~~ The prevomer of *S. fontanarrosai* and *S. pinima* does not bear teeth in its dentigerous process (Baldo et al. 2019), whereas in *T. taophora* it has odontoids. In *S. uruguayus* the posteromedial process of parasphenoid is absent (Baldo et al. 2019), but it is present in *T. taophora*. An otic plate is present on the otic ramus of squamosal in *S. fontanarrosai*, *S. pinima* and *S. uruguyaus* (Baldo et al. 2019), as well as in *T. taophora*. The hyoid of *S. fontanarrosai* bears a long, slender cartilaginous projection on the tip of its posteromedial process, but in *T. taophora* this tip is a small rounded cartilage.

Phyllomedusids are known as leaf or monkey frogs and draw attention from the lay public due to their beauty (Faivovich et al. 2010). Currently, 67 species are allocated in this family (Frost 2021) but osteological data is still extremely scarce and available for no more than a handful species. The sphenethmoid of phyllomedusids is large (Sheil & Alamillo 2005; Ruiz-Monachesi et al. 2016) and the frontoparietals cover the frontoparietal fontanelle entirely in *Callimedusa atelopoides* (Sheil & Alamillo 2005), whereas in *T. taophora* the sphenethmoid is small and the frontoparietals leave the fontanelle partially exposed. The parasphenoid may be short in phyllomedusids, not reaching the palatines and the prevomers lack the dentigerous process (Cannatella 1980; Sheil & Alamillo 2005), however in *T. taophora* the parasphenoid reach the palatines anteriorly and the dentigerous process is present. The zygomatic ramus of squamosal of phyllomedusids is much reduced (Cannatella 1980, Sheil & Alamillo 2005; Ruiz-Monachesi et al. 2016) whereas in *T. taophora* it is normal-sized. The hyoid plate of *Phyllomedusa sauvagii* has a much reduced anterolateral process and a short posterolateral process, however in *T. taophora* both are well developed.

The skull of species of *Leptodactylus* (Leptodactylidae) superficially resembles the skull of *T. taophora*. However, most leptodactylids can be readily distinguished from *T. taophora* by their frontoparietals that occlude the frontoparietal fontanelle (Lynch 1971; Nascimento et al. 2005; Ponssa 2008; Ponssa et al. 2010; 2011; Fratani et al. 2017). In addition, the sphenethmoid of *Leptodactylus* is wide and the tip of cultrifrom process of parasphenoid is furcated (Ponssa et al. 2010; 2011), but in *T. taophora* the sphenethmoid is small and the tip of parasphenoid is acuminate. Moreover, the posteromedial process of parasphenoid of L*eptodactylus nesiotus* reaches the foramen magnum posteriorly (Ponssa et al. 2010), but in *T. taophora* it does not. The hyoids of *Leptodactylus* are very similar to the hyoid of *T. taophora*. The posterolateral process of hyoid of *Physalaemus natteteri* is short (Fratani et al. 2017), whereas in *T. taophora* it is well developed.

## Adult nasal cartilages

The nasal cartilages of anurans have been overlooked for a long time; even in recent phylogenies dealing with large datasets utilizing adult morphological characters (e.g. Faivovich 2002; Grant et al. 2006) not a single structure of the nasal capsules was coded as a character. Although these structures can be easily visualized after clearing and staining techniques, the nasal cartilages may be roofed by the nasals or sphenethmoid in species with extensive ossification which renders their observation more difficult. In addition, the lack of a standardized nomenclature may have hindered studies on these cartilages (Púgener & Maglia 2007). The many small cartilages that form the nasal capsules of anurans may provide a rich source of phylogenetic characters and should be more explored in future works. Descriptions of the adult nasal capsules are rare in the literature and are available only for a few species.

In adults, the distal end of the *crista subnasalis* of *Bombina orientalis* (Bombinatoridae; Maglia & Púgener 1998), *Leptodactylus latinasus* (Leptodactylidae; Ponssa & Barrionuevo 2012), *L. podicipinus* (Ponssa et al. 2011), *Phyllomedusa sauvagii* (Phyllomedusidae; Ruiz-Monachesi et al. 2016) is bifurcate, whereas in *T. taophora* it is not. The *solum nasi* of *Phyllomedusa vaillantii* and *Callimedusa atelopoides*, both phyllomedusids, is slightly mineralized, the *crista subnasalis* is bifurcate, and the slender posterior maxillary process projects posteriorly to the posterolateral margin of the orbit, however it is not confluent with the pterygoid process (Sheil & Alamillo 2005). In *T. taophora* however the *solum nasi* is never mineralized, the *crista subnasalis* is not bifurcate and its posterior maxillary process fuses with the pterygoid process In *Pyxicephalus adspersus* (Pyxicephalidae) there is an anterior projection and possibly a posterior projection as well (Sheil 1999). Although it was not mentioned throughout the account on the osteology of *L. nesiotus* (Ponssa et al. 2010), based on their drawing the crista also seems to be bifurcate in this species. Despite the overall similarity between the skull of leptodactylids and cycloramphids, the truncated crista subnasalis of *T. taophora* is much more similar to the crista of *Spea bombifrons* and *S. multiplicata* (Scaphiopodidae; Púgener & Maglia 2007) than the bifurcate condition found in leptodactylids.

The *planum triangulare* of all anuran species described to date bears an anterior and a posterior maxillary process, with the posterior process usually fused to the pterygoid process of the palatoquadrate. The anterior maxillary process of *P. sauvagii* (Ruiz-Monachesi et al. 2016) is much shorter than of *T. taophora*. In some species, the *tectum nasi*, the *solum nasi* and the *septum nasi* may be mineralized: *Leptodactylus latinasus* (Ponssa & Barrionuevo 2012), *L. nesiotus* (Ponssa et al. 2010), *L. podicipinusi* (Ponssa et al. 2011), *Scinax fontanarrosai* (Baldo et al. 2019), *Spea bombifrons*, *S. multiplicata* (Púgener & Maglia 2007). Mineralization of any of these three structures was not found in any specimen of *T. taophora* studied, nor in *P. sauvagii* (Ruiz-Monachesi et al. 2016) and *B. orientalis* (Maglia & Púgener 1998). However, considering that older specimens tend to be more mineralized (Vera & Ponssa 2014); older individuals of these species may present some mineralization in these structures.

The rostral end of *tectum nasi* forms an acuminate process called prenasal process in some leptodactylids (Ponssa & Barrionuevo 2012). Conversely, the rostral end of *septum nasi* also forms an acuminate process in *Spea*, called median prenasal process (Púgener & Maglia 2007). Neither of those are present in *T. taophora*.

## CONCLUSIONS

The chondrocranium of *Thoropa taophora* resembles that of a pond-type larva, even though the habitat it inhabits is unusual among anurans. The anterior limbs and the larval tadpole-like mouth coexist for one of two Gosner stages, which is unusual in anurans. Cranial and postcranial ossification commence simultaneously, even though postcranial ossification progresses faster. The first cranial bones to ossify are the exoccipitals and on the postcranium the first bones that appear are bones of the hindlimbs and the neural arches of vertebrae. By the end of metamorphosis, most postcranial bones are ossified, however most of cranial bones are not. A well-ossified axial skeleton might be useful for the jumping habit of metamorphic *T. taophora*. The adult skull of *T. taophora* is not heavily ossified, however all neomorphic elements, such as collumela are present and the maxillary arcade is complete.

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**FIGURES**

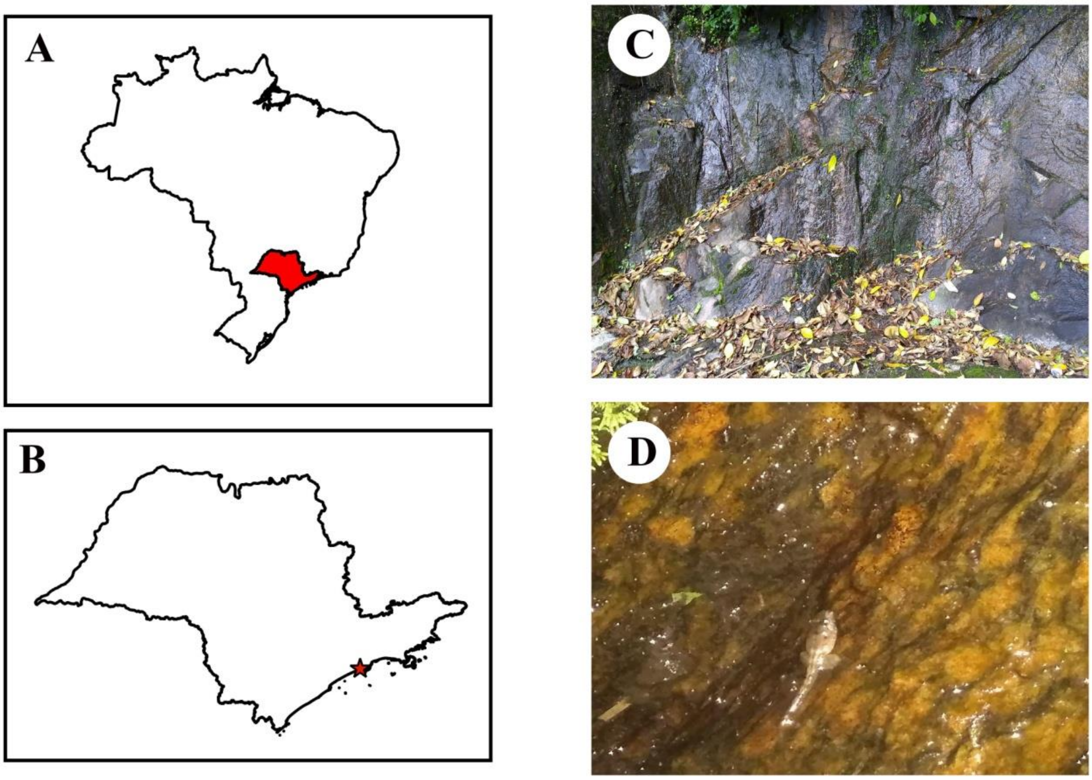

**Figure 1.** Map showing the collection site of the studied specimens. **(A)** Overview of state of São Paulo in Brazil; **(B)** Close-up view on state of São Paulo. Red star indicates municipality of São Vicente, where all specimens were collected; **(C)** Rocky outcrops where specimens live; **(D)** Photo showing tadpole of *Thoropa taophora* attached on the rock. Notice its cryptic coloration against the rocky substrate.

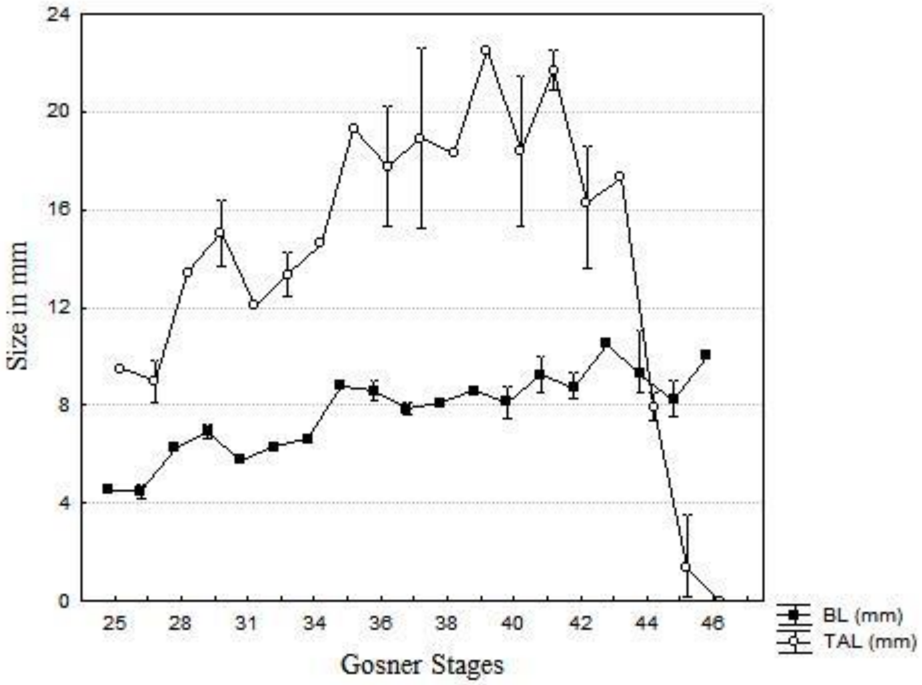

**Figure 2**. Variation of body length (BL), represented by filled squares, and of tail length (TAL), represented by open circles, throughout the ontogeny of tadpoles of *Thoropa taophora*. Tail length decreases dramatically after Gosner Stage 41 as a result of tail reabsorption during metamorphosis. Circles and squares indicate mean values and whiskers indicate minimum and maximum values.

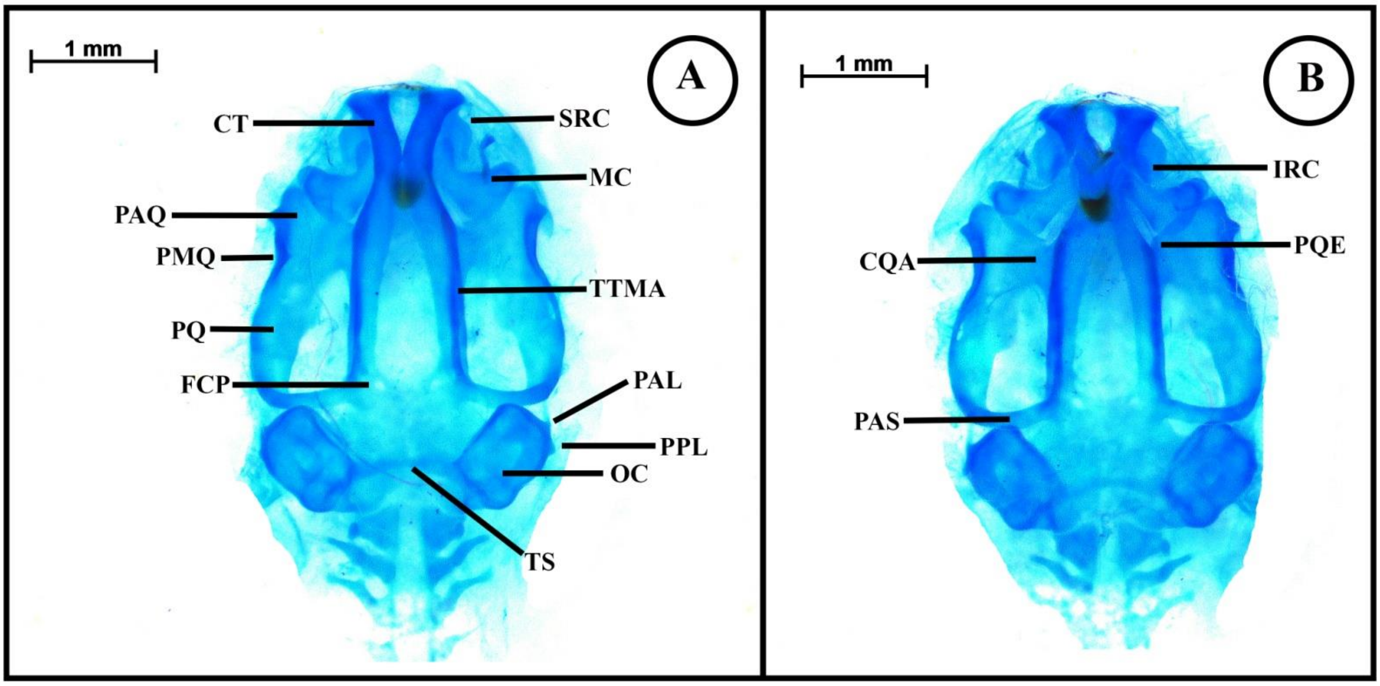

**Figure 3.** Chondrocranium of *Thoropa taophora* at Gosner Stage 34 (specimen HCLP – A 037 – 5). **(A) Dorsal view**. Abbreviations: CT, cornua trabeculae; FCP, foramen caroticum primarium; MC, Meckel's cartilage; OC, otic capsule; PAL, processus anterolateralis; PAQ, pars articularis quadrati; PMQ, processus muscularis quadrati; PQ, palatoquadrate; PPL, processus posterolateralis; SRC, suprarostral cartilage; TS, tectum synoticum; TTMA, taenia tecti marginalis. **(B) Ventral view**. Abbreviations: CQA, commissura quadratocranialis anterior; IRC, infrarostral cartilage; PAS, processus ascendens; PQE, processus quadratoethmoidalis.

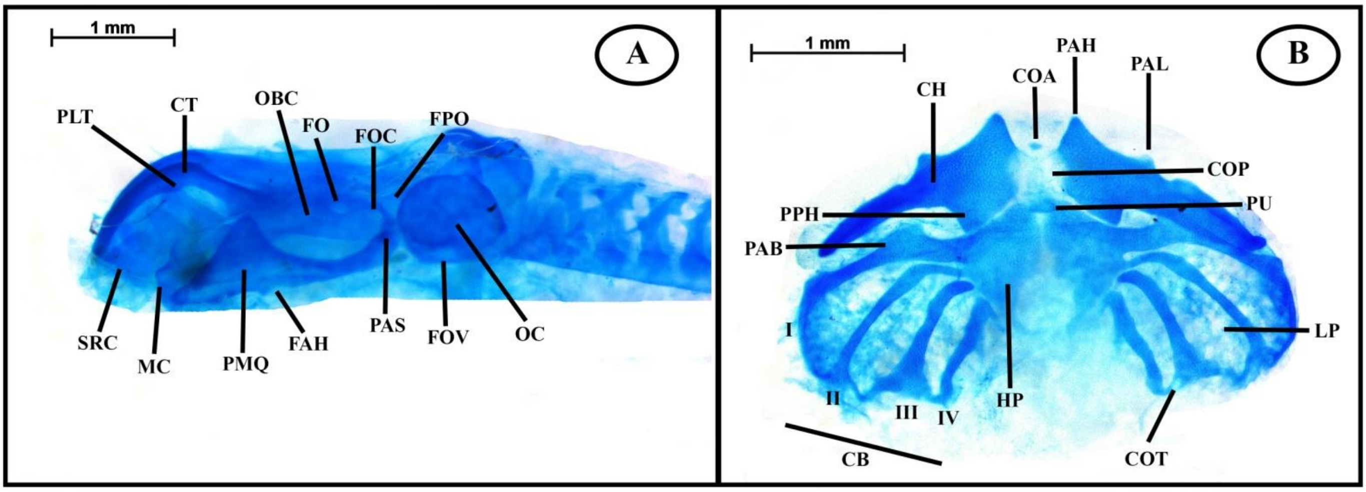

**Figure 4**. Chondrocranium of *Thoropa taophora* at Gosner Stage 34 (specimen HCLP – A 037). **(A) Lateral view.** Abbreviations: CT, cornua trabeculae; FAH, facies

articularis hyalis; FO, foramen opticum; FOC, foramen oculomotorium; FOV, fenestra ovalis; FPO, foramen prooticum; MC, Meckel's cartilage; OBC, orbital cartilage; OC, otic capsule; PAS, processus ascendens; PLT, processus lateralis trabeculae; PMQ, processus muscularis quadrati; SRC, suprarostral cartilage **(B) Hyobranchial apparatus**. Abbreviations: CB, ceratobranchial; CH, ceratohyal; COA, copula anterior; COP, copula posterior; COT, commissura terminales; HP, hypobranchial plate; LP, lateral projections; PAB, processus anterior branchialis; PAH, processus anterior hyalis; PAL, processus anterolateralis hyalis; PPH, processus posterior hyalis; PU, processus urobranchialis.

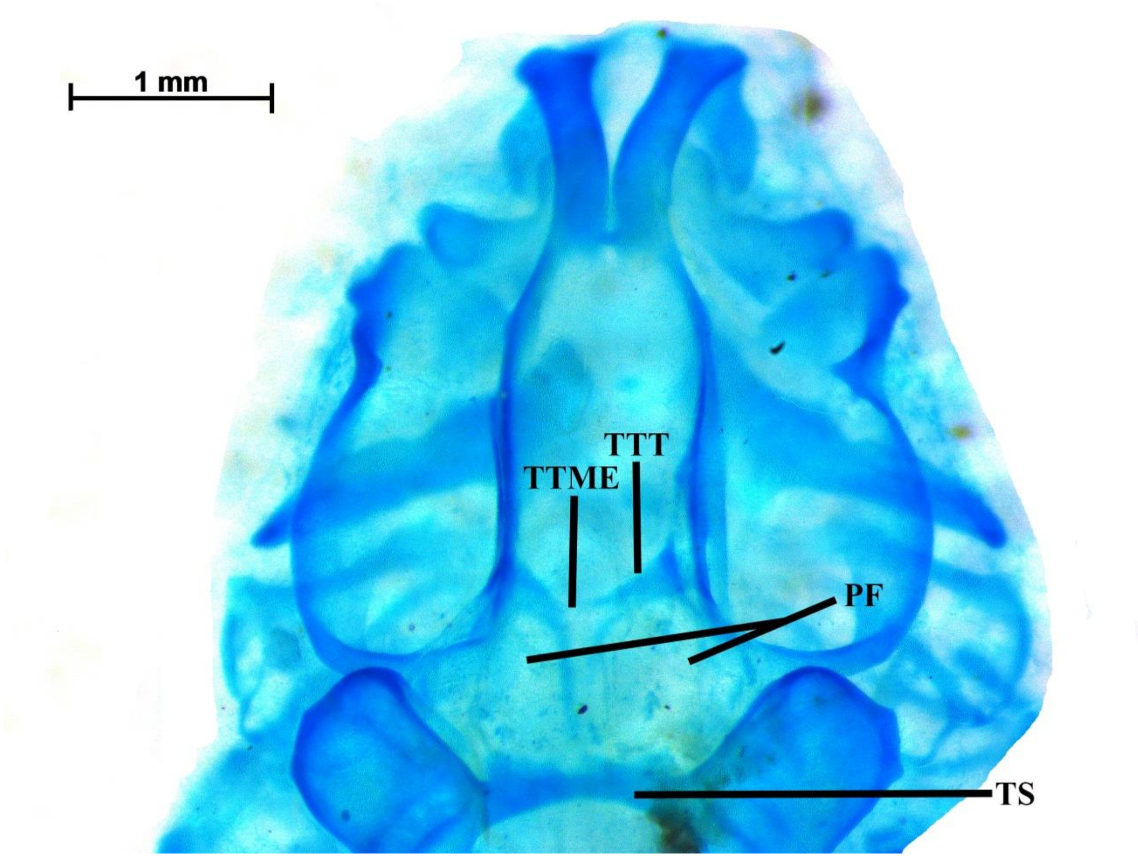

**Figure 5**. Dorsal view of chondrocranium of *Thoropa taophora* at Gosner Stage 36 (specimen HCLP – A 141 – 1) showing the early formation of the parietal fenestrae. Abbreviations: PF, parietal fenetrae; TS, tectum synoticum; TTME, taenia tecti medialis; TTT, taenia tecti transversalis.

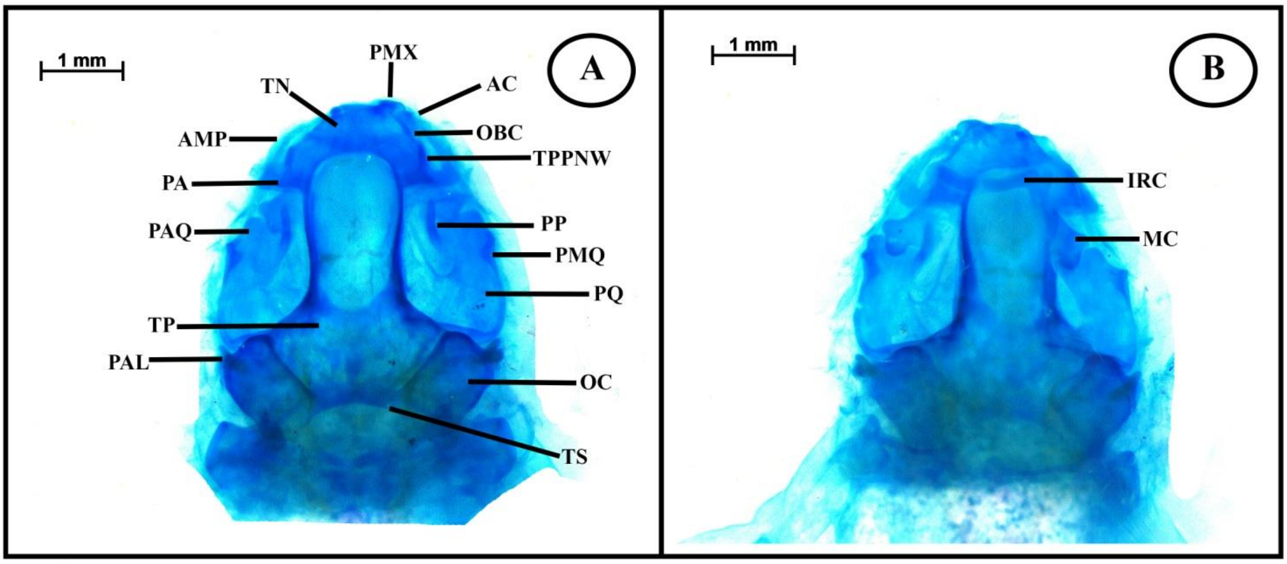

**Figure 6**. Chondrocranium of *Thoropa taophora* at Gosner Stage 44 (specimen HCLP – A 141 – 9/1) stained for cartilage only. **(A) Dorsal view**. Abbreviations: AC, alary cartilage; AMP, anterior maxillary process; OC, otic capsule; PA, processus antorbitalis; PAL, processus anterolateralis; PAQ, pars articularis quadrati; PMQ, processus muscularis quadrati; PMX, pre-maxillary; PQ, palatoquadrate; PT, planum terminale; PP, processus pterygoideus; SMX, septomaxilla; SN, septum nasi; TN, tectum nasi; TP, tectum parietale; TPPNW, triangular process of the post-nasal wall. **(B) Ventral view**. Abbreviations: IRC, infrarostral cartilage; MC, Meckel's cartilage.

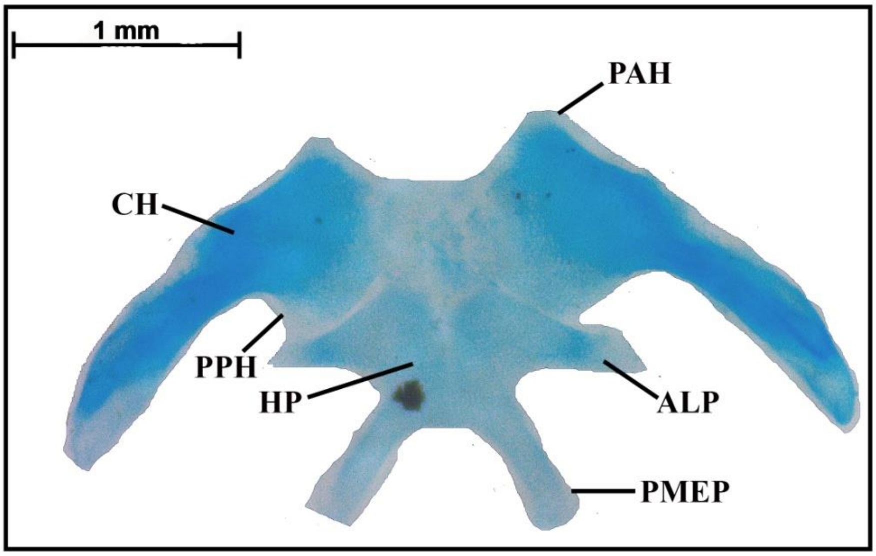

**Figure 7**. Hyobranchial apparatus of *Thoropa taophora* at Gosner Stage 44 (specimen HCLP – A 289 – 1). Abbreviations: ALP, anterolateral process; CH, ceratohyal; HP,

hypobranchial plate; PAH, processus anterior hyalis; PMEP, posteromedial process; PPH, processus posterior hyalis.

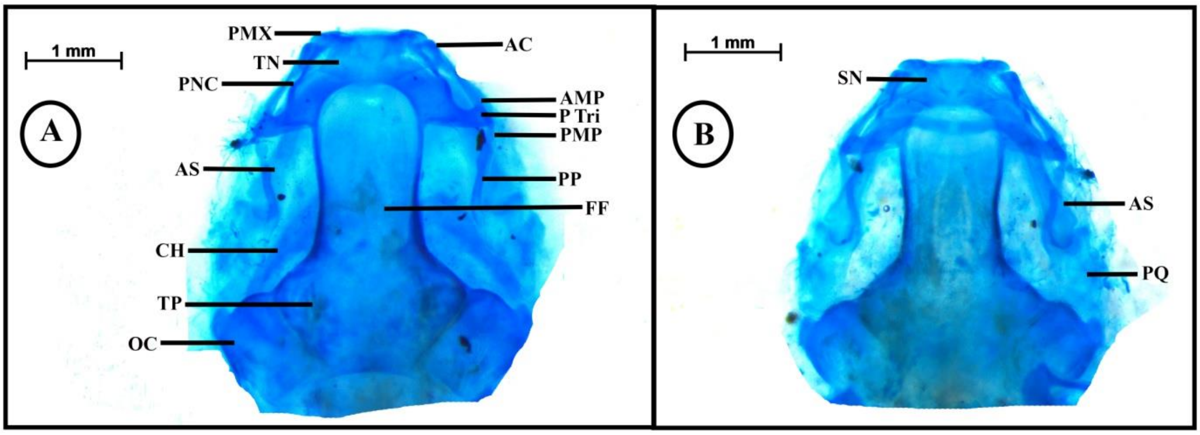

**Figure 8**. Chondrocranium of *Thoropa taophora* at Gosner Stage 45 stained for cartilage only (specimen HCLP – A 261 – 2). **(A) Dorsal view.** Abbreviations: AC, alary cartilage; AMP; anterior maxillary process; AS, angulosplenial; CH, ceratohyal; FF, frontoparietal fenestra; OC, otic capsule; PMP, posterior maxillary process; PMX, pre-maxillary; PNC, paranasal commisure; P Tri, planum triangulare; PP, pterygoid process; TN, tectum nasi; TP, tectum parietale. **(B) Ventral view**. Abbreviations: AS, angulosplenial; PQ, palatoquadrate; SN, solum nasi.

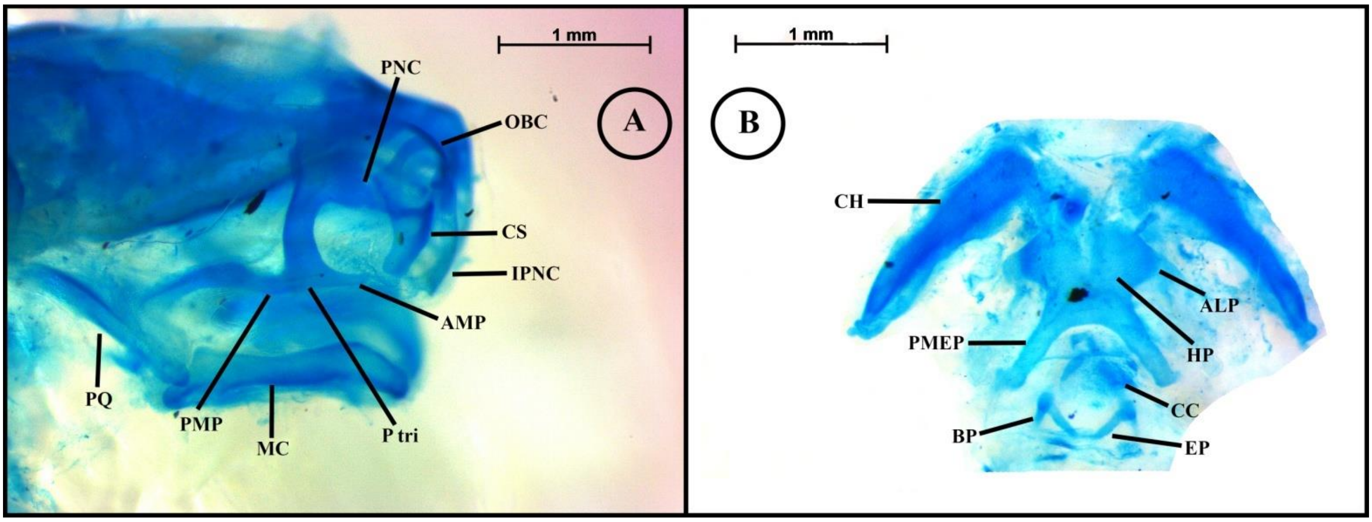

**Figure 9. (A)** Lateral view of chondrocranium and **(B)** Hyobranchial apparatus of *Thoropa taophora* at Gosner Stage 45 (specimen HCLP – A 261 – 2). Abbreviations:

ALP, anterolateral process; AMP, anterior maxillary process; BP, bronchial process; CC, cricoids cartilage; CH, ceratohyal; CS, crista subnasalis; EP, esophageous process; HP, hyoid plate; IPNC, inferior prenasal cartilage; MC, Meckel's cartilage; OBC, oblique cartilage; PMEP, posteromedial process; PMP, posterior maxillary process; PNC, paranasal commisure; PQ, palatoquadrate; P tri, planum triangulare.

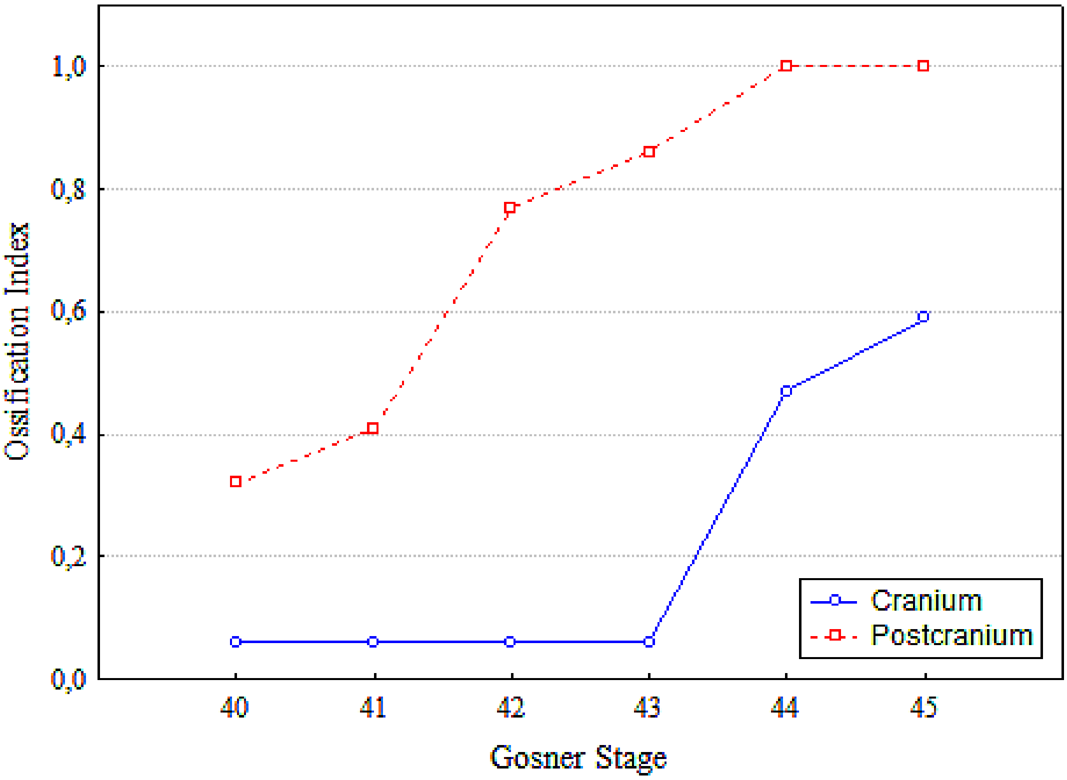

**Figure 10.** Cranium and postcranium ossification indices of *Thoropa taophora*. 0 indicates no bone is ossified; 1 indicates all bones are ossified.

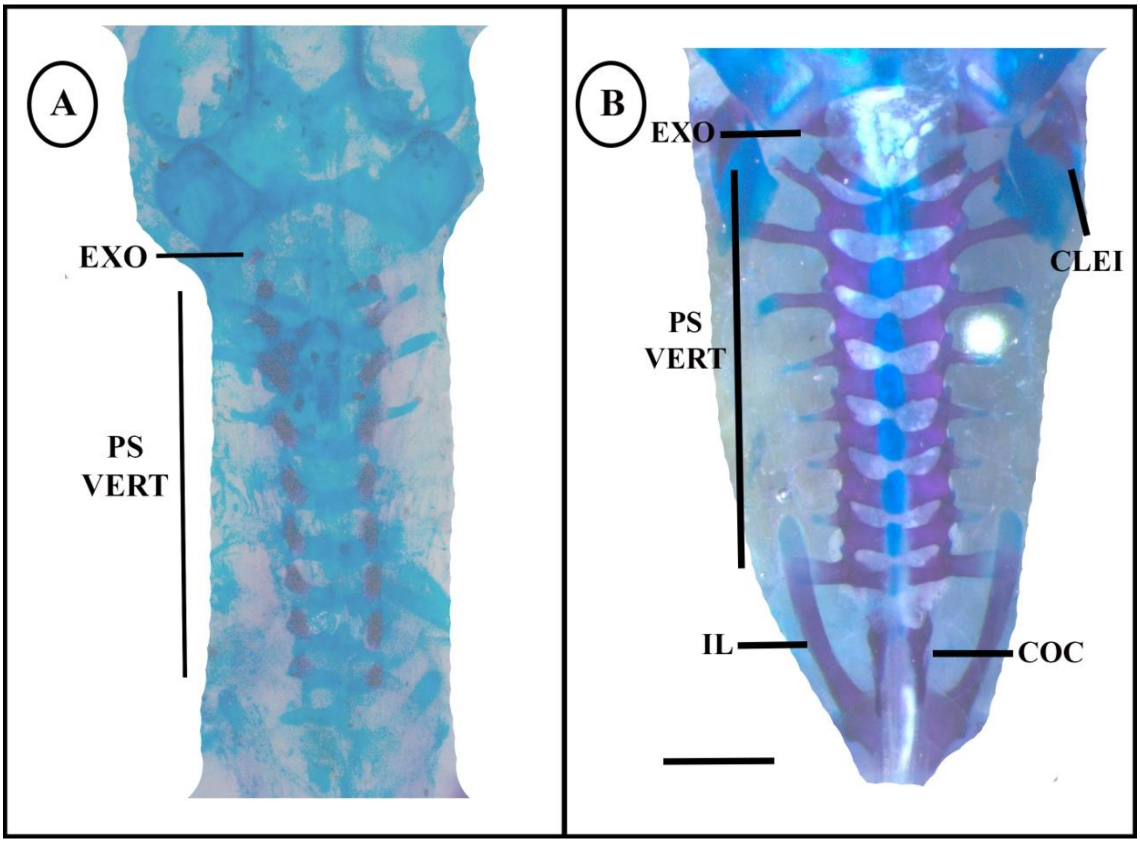

**Figure 11**. Different degrees of ossification in the exoccipital and axial skeleton of *Thoropa taophora* according to developmental stages. (A) specimen HCLP – A 289 – 6 (Gosner Stage 42) (B) specimen HCLP – A 289-1 (Gosner Stage 44). Abbreviations: CLEI, cleithrum; COC, coccyx; EXO, exoccipital; IL, ilium; PS VERT, pre-sacral vertebrae. Scale bar represents 1 mm.

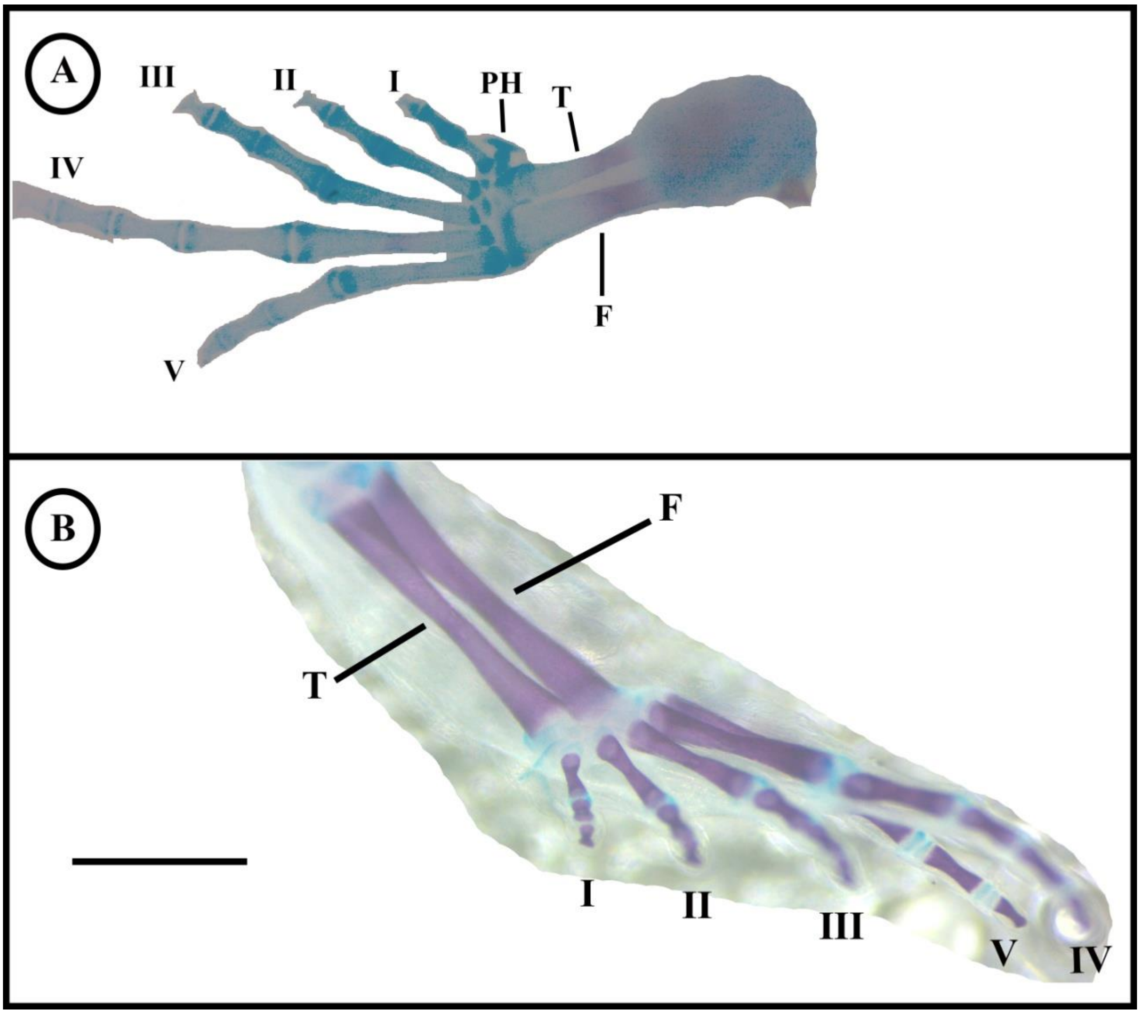

**Figure 12.** Different degrees of ossification in the left feet and tarsal bones of *Thoropa taophora* according to developmental stages. (A) specimen HCLP – A 289 – 6 (Gosner Stage 42) (B) specimen HCLP – A 289-1 (Gosner Stage 44). Abbreviations: F, fibiale; T, tibiale; PH, pre-hallux. Roman numerals indicate order of digits. Scale bar represents 1 mm.

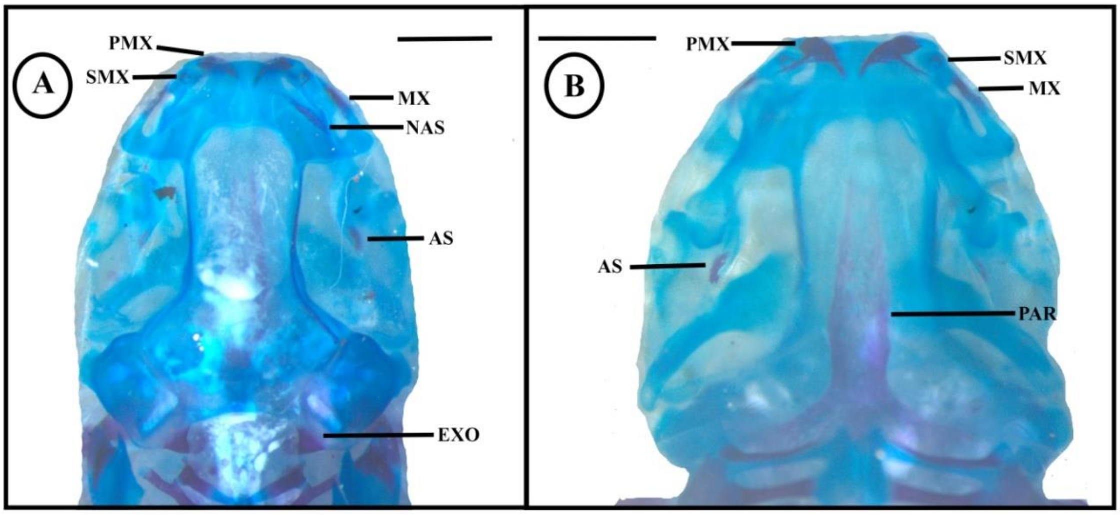

**Figure 13.** Chondrocranium of *Thoropa taophora* at Gosner Stage 44 (specimen HCLP – A 289 – 1) showing ossifications present at this stage. **(A) Dorsal and (B) Ventral views**. Abbreviations: AS, angulosplenial; EXO, exoccipital; MX, maxillary; NAS, nasal; PMX, pre-maxillary; SMX, septomaxilla. Scale bars represent 1 mm.

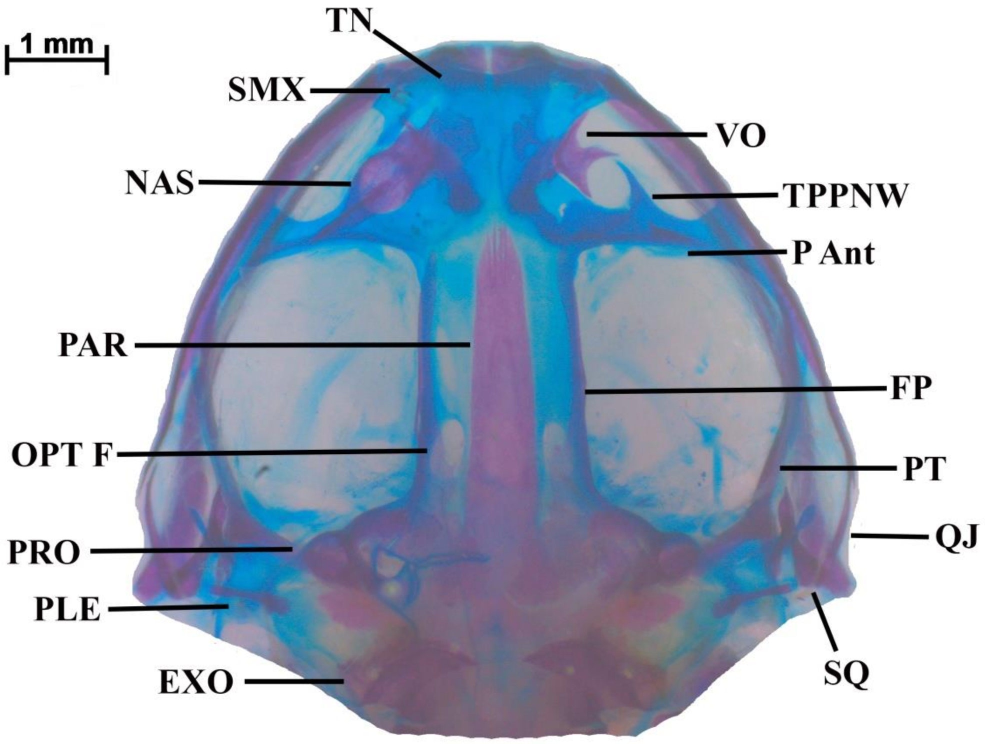

**Figure 14**. Dorsal view of post-metamorphic skull of *Thoropa taophora* (specimen HCLP – A 013; BL = 18 mm). Right nasal bone was removed to allow better visualization of underlying structures. Abbreviations: EXO, exoccipital; FP, frontoparietal; QJ, quadratojugal; NAS, nasal; OPT F, optic foramen; P Ant, planum antorbitale; PAR, parasphenoid; PLE, plectral apparatus; PRO, prootic; PT, pterygoid; SQ, squamosal; SMX, septomaxilla; TN, tectum nasi; TPPNW, triangular process of the post-nasal wall; VO, prevomer.

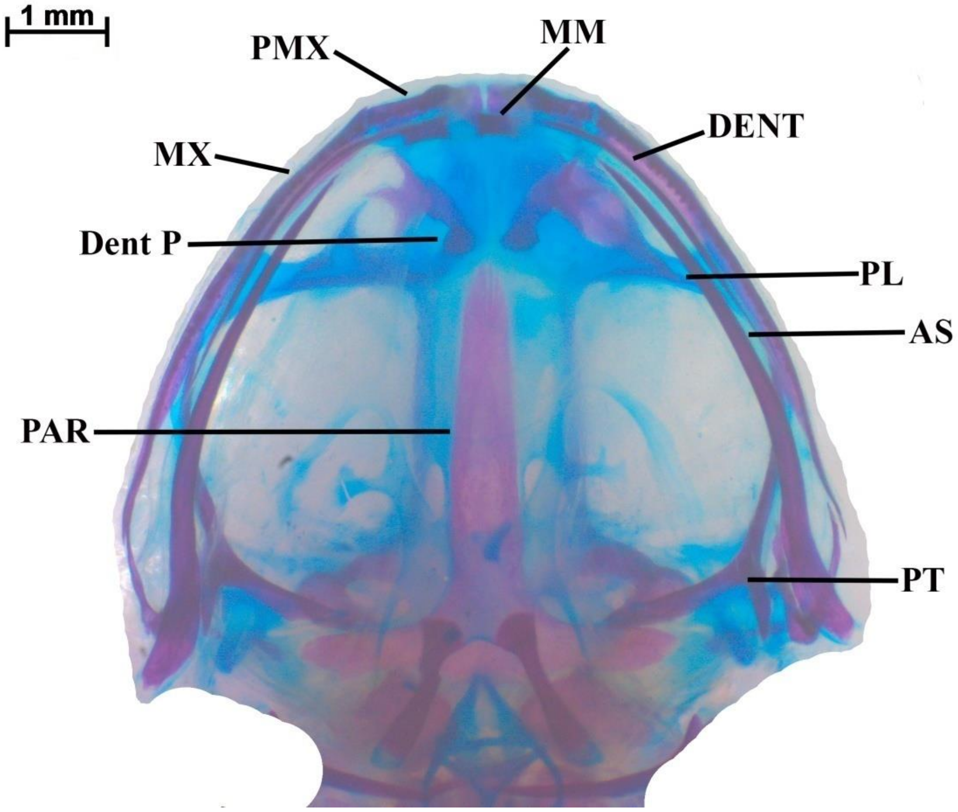

**Figure 15**. Ventral view of post-metamorphic skull of *Thoropa taophora* (specimen HCLP – A 013; BL = 18 mm). Abbreviations: AS, angulosplenial; DENT, dentary; Dent P, dentigerous process of prevomer; MM, mentomeckelian; MX, maxillary; PAR, parasphenoid; PL, palatine; PMX, pre-maxillary; PT, pterygoid.

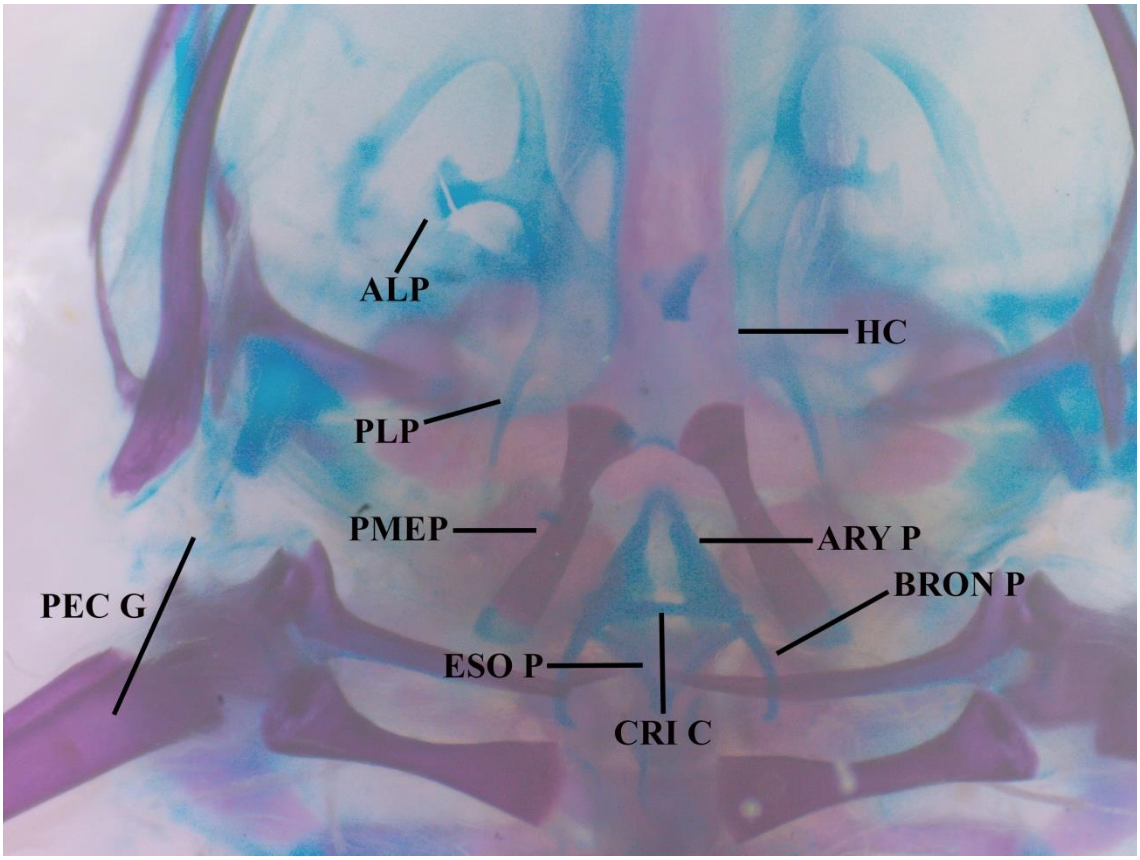

**Figure. 16.** Ventral close-up view of the skull of *Thoropa taophora* showing its hyolaryngeal apparatus (specimen HCLP – A 013; BL = 18 mm). Hyolaryngeal was not removed from the skull to avoid damaging the fragile cartilaginous laryngeal cartilages. Abbreviations: ALP, anterolateral process; ARY P, arytenoid process; BRON P, bronchial process; CRI C, cricoid cartilage; ESO P, esophageal process; HC, hyoid corpus; PEC G, pectoral girdle; PLP, posterolateral process; PMEP, posteromedial process.

## TABLES

**Table 1.** Two measurements (body length = BL; tail length = TAL) of 33 tadpoles and seven post-metamorphic specimens of *Thoropa taophora*. Stages with n ≥ 2 specimens, data are presented as mean ± standard deviation (minimum – maximum).

| Gosner Stage (n) | BL (mm) | TAL (mm) |
|---|---|---|
| 25 (1) | 4.52 | 9.5 |
| 26 (2) | 4.49 ± 0.38 (4.22 – 4.76) | 9 ± 1.22 (8.14 – 9.86) |
| 28 (1) | 6.26 | 13.4 |

| | | |
|---|---|---|
| 30 (3) | 6.92 ± 0.27 (6.66 – 7.20) | 15.07 ± 1.35 (13.68 – 16.38) |
| 31 (1) | 5.78 | 12.06 |
| 32 (2) | 6.31 ± 0.24 (6.14 – 6.48) | 13.35 ± 1.32 (12.42 – 14.28) |
| 34 (1) | 6.6 | 14.62 |
| 35 (1) | 8.8 | 19.3 |
| 36 (2) | 8.59 ± 0.55 (8.2 – 8.98) | 17.78 ± 3.42 (15.36 – 20.2) |
| 37 (2) | 7.86 ± 0.34 (7.62 – 8.1) | 18.9 ± 5.23 (15.2 – 22.6) |
| 38 (1) | 8.1 | 18.28 |
| 39 (1) | 8.6 | 22.5 |
| 40 (2) | 8.12 ± 0.97 (7.43 – 8.8) | 18.40 ± 4.30 (15.36 – 21.44) |
| 41 (2) | 9.25 ± 1.06 (8.5 – 10) | 21.7 ± 1.13 (20.9 – 22.5) |
| 42 (3) | 8.69 ± 0.6 (8.3 – 9.38) | 16.29 ± 2.51 (16.7 – 18.56) |
| 43 (1) | 10.5 | 17.3 |
| 44 (4) | 9.28 ± 1.22 (8.56 – 11.1) | 7.92 ± 0.6 (7.4 – 8.5) |
| 45 (3) | 8.23 ± 0.75 (7.51 – 9) | 1.34 ± 1.89 (0.2 – 3.52) |
| 46 (6) | 16.32 ± 4.57 (10 – 24.3) | 0 |

“n” represents how many individuals of a particular stage were measured.

**Table 2**. Comparison between the chondrocranial characters found by Haas & Richards (1998) in species with suctorial larvae and the ones present in larvae of *Thoropa taophora*.

| **Characters found in suctorial larvae** | **Characters found in larvae of *T. taophora*** |
|---|---|
| Greatest chondrocranium width at the jaw articulation level | Greatest width at the posterior region of palatoquadrate |
| *Cornua trabeculae* expanded and/or fused | *Cornua trabeculae* long and slender |
| Suprarostral cartilages fused | Suprarostral cartilages not fused |
| Robust infrarostral cartilages | Small infrarostral cartilages |
| Large *comissura quadratocranialis anterior* | Regular-sized *comissura quadratocranialis anterior* |

| | |
|---|---|
| Palatoquadrate attached to the skull posteriorly via otic process, basal process and *processus ascendens* | Palatoquadrate attached to the skull posteriorly only via *processus ascendens* |

**Table 3**. Sequence of skeletal ossification of *Thoropa taophora*. The order in which elements ossify after metamorphosis is not necessarily exactly. Brackets indicate the elements first appear ossified together.

| | **Cranium** | **Postcranium** |
|---|---|---|
| | Exoccipital | Ilium<br>Femur<br>Tibiafibula<br>Tibiale<br>Fibiale<br>Neural arches of presacral vertebrae II-VIII |
| | | Transverse processes of presacral vertebrae II-IV<br>Cervical vertebra |
| Metamorphosis onset | | |
| | | Humerus<br>Radioulna<br>Metatarsals<br>Metacarpals<br>Ilium<br>Coracoid<br>Scapula<br>Foot phalanges |
| | | Sacral diapophysis<br>Ischium |
| | Pre-maxillary<br>Maxillary<br>Angulosplenial<br>Parasphenoid<br>Frontoparietal<br>Nasal | Clavicle<br>Cleithrum<br>Transverse processes of presacral vertebrae V-VIII<br>Hand phalanges |
| | Septomaxilla | |
| | Dentary<br>Mentomeckelian | |
| Metamorphosis terminus | | |
| | Prevomer | Suprascapula |
| | Palatine | |
| | Squamosal | |
| | Pterygoid | |

| |
|---|
| Quadratojugal |
| Prootic |
| Sphenethmoid |

**Table 4**. Comparison of cranial ossification sequence of *Thoropa taophora* and *Eupsophus*.

| | *Thoropa taophora* (Present study) | *Eupsophus calcaratus* (Vera Candioti et al. 2005) | *Eupsophus emiliopugini* (Vera Candioti et al. 2011) |
|---|---|---|---|
| | Exoccipital | | |
| Metamorphosis onset | | | |
| | Pre-maxillary<br>Maxillary<br>Angulosplenial<br>Parasphenoid<br>Frontoparietal<br>Nasal<br>Septomaxilla<br>Dentary<br>Mentomeckelian | Septomaxilla<br>Pre-maxillary<br>Maxillary<br>Dentary<br>Angulosplenial<br>Squamosal<br>Pterygoid | |
| Metamorphosis terminus | | | |
| | Prevomer<br>Palatine<br>Squamosal<br>Pterygoid<br>Quadratojugal<br>Prootic<br>Sphenethmoid | Columella<br>Sphenethmoid<br>Exoccipital<br>Frontoparietal<br>Mentomeckelian<br>Neomorphic elements<br>Palatine<br>Parasphenoid<br>Quadratojugal<br>Prevomer | All bones |

## APPENDIX I – MATERIAL EXAMINED

A total of 33 larval and seven post-metamorphic specimens were cleared and double-stained. Specimens' serial numbers are given below; numbers in parenthesis represent their developmental stage according to the table of Gosner (1960). All acronyms are HCLP – A. Larval specimens: 3 specimens belong to lot 141 – 8 (30);

037 – 7 (26); 037 – 1 (28); 037 – 3 (26); 037 – 6 (25); 141 – 3 (26); 141 – 2 (40); 037 – 8 (32); 037 – 10 (32); 037 – 9 (31); 037 – 5 (34); 141 – 5 (35); 037 – 2 (37); 261 – 1 (40); 261 – 3 (41); 264 – 2(38); 288 – 1 (39); 289 – 3 (36); 288 – 2 (41); 289 – 5 (37); 141 – 10 (42); three specimens belong to lot 141 – 9 (44); 261 – 2 (45); 261 – 4 (45); 264 – 1 (42); 289 – 1 (44); 289 – 2 (43); 289 – 4 (45); 289-6 (42). Post-metamorphic specimens: 261 – 5; 263 – 1; 011; 012; 013; 014; 015.