



Old and new hypotheses about the homology of the compound bones from the cheek and otico-occipital regions of the anuran skull

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ABSTRACT

We studied the larval development of compound bones from the otico-occipital and cheek regions in species of the neobatrachian genera *Batrachyla*, *Hylorina*, *Leptodactylus*, *Odontophrynus* and *Pleurodema*. Comparisons were made using a set of *Ambystoma* spp. (Caudata) and *Ceratophrys ornata* (Anura; Ceratophryidae) larvae. As suggested by previous studies, we verified the compound nature of the exoccipital (two centers, anurans only), frontoparietal (one center, most anurans and *Ambystoma*; three centers, some anurans), and squamosal (two centers, all anurans and *Ambystoma*) bones. We discuss old and new homology hypotheses for each of the compound bone centers in the context of the most widely accepted scenario of lissamphibian origins and relationships, i.e., monophyletic Lissamphibia that includes the clade Batrachia (Caudata + Anura) and the most divergent Gymnophiona. Our findings have a direct impact on our understanding of the composition of the skull in Lissamphibia. We recognized the presence of the following bones: (i) opisthotic (fused to the exoccipital) and tabular (fused to the squamosal) in Batrachia (Anura + Caudata) and (ii) supratemporal (fused to the parietal portion of the frontoparietal) in Anura. Separate centers of the parietal were found only in *Pleurodema*.

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1. Introduction

Lissamphibia comprises three groups of extant amphibians: Gymnophiona (also known as apodans or caecilians), Caudata (salamanders), and Anura (toads and frogs). The latter is the most diverse with respect to species number, morphological types and modes of reproduction, among other characteristics (Duellman and Trueb, 1994). Within Anura, cranial bone formation has been addressed mainly by the description of the ossification sequences (see, e.g., Weisbecker and Mitgutsch, 2010 for a recent analysis). But the development and homology of the bones that arise from more than one ossification center (referred to here as compound bones) have largely been ignored. Some names of the anuran bones reflect a compound origin (e.g., angulosplenic and frontoparietal), whereas the names of others that have a compound origin (e.g., squamosal) do not reflect the homology of their ossification centers.

A review of papers dealing with anuran skull development raises a number of questions. Why do commonly used names of some bones (e.g., angulosplenic) reflect their origin from multiple centers of ossification when the presence of two centers has only been demonstrated for a single anuran species, *Xenopus laevis* (Trueb and

Hanken, 1992)? Why do bones for which the presence of two centers has been demonstrated (e.g., squamosal) in at least 16 of 46 genera studied (Table 1) lack a name that reflects an origin from multiple centers of ossification? Table 1 summarizes almost all of the published information on certain bones (exoccipital, frontoparietal, and squamosal) that may originate from more than one center during the larval and postmetamorphic development of the anuran skull. Thus, the main goal of this table is to highlight the prevalence and distribution of this phenomenon across many anuran families.

Historically (de Beer, 1937) and also more recently (Roček, 1981, 1989), authors have considered these questions and discussed the homology of the anuran compound bones in the context of tetrapods. But the most substantial contribution to this topic has been that of Lebedkina (1979). This work, originally published in Russian and recently translated into English (2004), focused on the development of the skull in Caudata. This work is very interesting because Lebedkina also described the development of some anurans and discussed her findings in the context of lissamphibians and tetrapods. From this perspective, the anuran skull pattern was presumed to be the result of losses and fusions of bones that occurred by the paedomorphic processes that operated in the Palaeozoic ancestors of the group (Bolt, 1977). In fact, many bones (e.g., jugal, postfrontal, postparietal, supratemporal, and tabular) are thought to have been eliminated from the anuran skull as a result of these evolutionary processes (Milner, 1988; Roček and Rage, 2000).

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Table 1

Presence (P) or absence (A) of the ventral squamosal center (VSC), dorsal squamosal center (DSC), anterior auditory capsule center (ACC), posterior auditory capsule center (PCC), and lateral frontoparietal center (LFC). Asterisk indicates observations based upon stained and cleared specimens together with histological sectioned material. Absence of asterisk denotes stained and cleared specimens only. 'X' signifies that this aspect of the development was not studied. '?' denotes doubts on the presence/absence of the center under question. The following list of species was studied by Sedra (1949) and Griffiths (1954a) with no evidence for the double origin of the squamosal: *Aglyptodactylus madagascariensis* (Mantellidae), *Colostethus latinasus* (Aromobatidae), *Discoglossus pictus* (Alytidae), *Osteopilus dominicensis* (Hylidae), *Crossodactylus gaudichaudii* (Hylodidae), *Eleutherodactylus martinicensis* (Eleutherodactylidae), *Pleurodema bibroni*, *P. cinereum*, *P. marmoratum* (Leptodactylidae), *Callulops boettgeri* (Microhylidae), *Pelobates cultripes* (Pelobatidae), *Pelodytes punctatus* (Pelodytidae), *Leptobranchium* spp. (Megophryidae), *Silurana tropicalis* (Pipidae), *Nanorana pleskei* (Dicroglossidae), *Pelophylax* spp., *Rana dalmatina* (Ranidae), *Ceratobatrachus guentheri* (Ceratobatrachidae), *Nyctibatrachus major* (Nyctibatrachyidae), and *Boophis madagascariensis* (Mantellidae). The data from the unpublished theses of Altig (1969b) and Brown (1980) were not accessed by the authors and consequently were not included in the table. These studies refer to *Rana aurora*, *R. cascadae*, and *R. pretiosa* (Altig, 1969b) and *Silurana tropicalis* and *Xenopus borealis* (Brown, 1980).

Family	Species	VSC	DSC	ACC	PCC	LFC
Leiopelmatidae	<i>Ascaphus truei</i> (Altig, 1969a)	Single center not clarifying what arm it represents		The "otic" bone formed by at least two centers		A
Alytidae	<i>Alytes obstetricians</i> (Yeh, 2002)	Crude sequence. Detailed osteocranial differentiation events lacking				
	<i>Discoglossus sardus</i> (Pugener and Maglia, 1997)	P	X	P	A	A
Bombinatoridae	<i>Bombina orientalis</i> (Hancken and Hall, 1984; Maglia and Pugener, 1998)	Crude sequence. Detailed osteocranial differentiation events lacking				
	<i>B. bombina</i> (Lebedkina, 2004)	P	A	X		A
	<i>B. variegata</i> (Čihák et al., 2003)					A
Pelobatidae	<i>P. fuscus</i> * (Reinbach, 1939b; Smirnov, 1995; Lebedkina, 2004)		X			P
Scaphiropodidae	<i>Spea bombifrons</i> (Wiens, 1989)	P	P	P	P	?
	<i>S. intermontana</i> (Hall and Larsen, 1998)	P	?	P	?	A
	<i>S. multiplicata</i> (Banbury and Maglia, 2006)	P	A	P	A	A
Bufonidae	<i>Anaxyrus boreas</i> * (Gaudin, 1978)	P	A	P	A	A
	<i>Amietophrynus regularis</i> * (Sedra, 1949; Griffiths, 1954a)	P	P			
	<i>Bufo bufo</i> * (Sedra, 1949; Griffiths, 1954a; Lebedkina, 2004)	P	P: Griffiths, 1954a A: Lebedkina, 2004; Sedra, 1949			
	<i>Atelopus ignescens</i> *; <i>A. varius</i> *; <i>Epidalea calamita</i> *; <i>Phrynomis asper</i> *; <i>Rhinella marina</i> *; <i>R. spinulosa</i> *; <i>R. margaritifera</i> * (Griffiths, 1954a)	P	P	X		
Brachycephalidae	<i>Brachycephalus ephippium</i> (Campos et al., 2010)	P	P			
Ceratophryidae	<i>Ceratophrys cornuta</i> (Wild, 1997a,b)	P	P	P	P	A
	<i>C. calcarata</i> (Wild, 1997b)	P	P	P	P	A
	<i>C. cranwelli</i> (Wild, 1997b)	P	?	P	P	A
	<i>C. ornata</i> (Griffiths, 1954a; Wild, 1997b; material used for comparison in the present work)	P	X: Wild, 1997b A: Griffiths, 1954a	P	P	A: Wild, 1997b; Griffiths, 1954a P: present study, see Fig. 3C
	<i>Chacophrys pierottii</i> (Wild, 1999)	P	P	P	A	A
	<i>Lepidobatrachus laevis</i> ; <i>L. llanensis</i> (Wild, 1997b)	P	P	P	A	A
Leptodactylidae	<i>Leptodactylus chaquensis</i> (Perotti, 2001)	P	P	P	A	A
Microhylidae	<i>Hamptophryne boliviana</i> (de Sá and Trueb, 1991)	P	A	P	A	A
	<i>Dermatonotus muelleri</i> (Fabrezi et al., 2012)	P	A	P	A	A
	<i>Gastrophryne carolinensis</i> (Trueb et al., 2011)	P	?	P	P	A

Table 1 (Continued)

Family	Species	VSC	DSC	ACC	PCC	LFC
Dendrobatidae	<i>Dendrobates auratus</i> ; <i>Epipedobates tricolor</i> (de Sá and Hill, 1998)	Crude sequence. Detailed osteocranial differentiation events lacking				
Eleutherodactylidae	<i>Eleutherodactylus nubicola</i> (Lynn, 1942)	Crude sequence. Detailed osteocranial differentiation events lacking				
	<i>E. coqui</i> * (Hanken et al., 1992)	P	A	P	A	A
Myobatrachidae (Davies, 1989)	<i>Crinia signifera</i> ; <i>Pseudophryne bibroni</i> ; <i>Uperoleia laevigata</i> U. <i>lithomoda</i> ; <i>U. trachyderma</i>	Crude sequence. Detailed osteocranial differentiation events lacking				
Hylidae	<i>Hypsiboas lanciformis</i> (de Sá, 1988)	P	P	P	A	A
	<i>Osteopilus septentrionalis</i> (Trueb, 1966)	P	A	P	A	A
	<i>Phyllomedusa vaillantii</i> (Sheil and Alamillo, 2005)	P	X	P	A	A
	<i>P. boliviana</i> (de Sá and Lavilla, 1996)	P	A	P	A	A
	<i>Pseudacris regilla</i> (Gaudin, 1973)	P	A	P	A	A
	<i>P. triseriata</i> (Stokely and List, 1954)	P	A	P	A	A
	<i>H. japonica</i> *; <i>Hyla arborea</i> (Lebedkina, 2004)	P	A	?	?	?
	<i>Smilisca baudinii</i> ; <i>Tripurion petasatus</i> (Trueb, 1970)	P	?	P	A	A
	<i>Pseudis platensis</i> (Fabrezi and Goldberg, 2009)	P	P	P	A	A
	<i>Acris crepitans</i> (Maglia et al., 2007)	Authors did not provide enough details about center ossification because they studied postmetamorphic ossification				
	<i>Hypsiboas pulchellus</i> (Hoyos et al., 2012)	Crude sequence. Detailed osteocranial differentiation events lacking				
Pyxicephalidae	<i>Pyxicephalus adspersus</i> (Griffiths, 1954a; Haas, 1999; Sheil, 1999)	P	?: Haas, 1999 A: Sheil, 1999; Griffiths, 1954a	P	A	A
Ranidae	<i>R. temporaria</i> (Sedra, 1949; Lebedkina, 2004)	P	P: Lebedkina, 2004 A: Sedra, 1949	?	?	A
	<i>Lithobates pipiens</i> (Kemp and Hoyt, 1969)	P	A	P	P	A
	<i>Pelophilax lessonae</i> *; <i>P. ridibundus</i> * (Lebedkina, 2004)	P	P	?	?	A
Rhinophrynidae	<i>Rhinophrynus dorsalis</i> (Trueb, 1985)	Crude sequence. Detailed osteocranial differentiation events lacking				
Pipidae	<i>Hymenochirus boettgeri</i> ; <i>Pipa myersi</i> (Yeh, 2002)	Crude sequences. Detailed osteocranial differentiation events lacking				
	<i>P. pipa</i> * (Trueb et al., 2000)	P	A	P	A	A
	<i>X. laevis</i> (Griffiths, 1954a; Trueb and Hanken, 1992)	P	A	P	A	A
Racophoridae	<i>Pseudophilautus silus</i> (Kerney et al., 2007)	P	X	P	A	A
Calyptocephalellidae	<i>Calyptocephalella gayi</i> * (Reinbach, 1939a; Griffiths, 1954a)	P	P: Reinbach, 1939a A: Griffiths, 1954a	X		A: Reinbach, 1939a P: Griffiths, 1954a

The present contribution is part of a broader study aimed at determining which of the bones that were formed at advanced stages of development in the Palaeozoic ancestors are now either absent or fused to other bones in the skulls of each lissamphibian order. We address the larval development and homology of each of the centers of the frontoparietal and of the bones from the cheek and otico-occipital regions of the anuran skull. The anuran cheek region (also described as part of the suspensorium fide Trueb, 1973) is composed of a squamosal bone, whereas in temnospondyls there are commonly a supratemporal and tabular bones, and sometimes an intertemporal bone (Romer, 1947). Although some authors have considered the otico-occipital region of the anuran skull to be composed of three bones (the prootic, opisthotic and exoccipital fide Roček, 2003), a more traditional view posits that the prootic and exoccipital fuse to form the oto-occipital bone (Trueb, 1973). The frontoparietal is included in the analysis because it is a compound bone topographically related to most of the cheek and oto-occipital bones.

Thus, the objectives of the present paper were to: (i) review the literature to determine the cheek and otico-occipital bones that are formed from more than one ossification center in anurans; (ii) identify these centers in species of five neobatrachian genera of the families Ceratophryidae, Cycloramphidae, and Leptodactylidae; and (iii) discuss the prevailing hypotheses of homology and, when necessary, reinforce some of them with new evidence in the context of the available knowledge of adults and larvae of extant and fossil tetrapods, mainly of Gymnophiona, Caudata and Dissorophoidea.

2. Materials and methods

We examined a field-collected larval series of *Batrachyla taeniata* (Ceratophryidae), *Hylorina sylvatica* and *Odontophrynus americanus* (Cycloramphidae), *Leptodactylus latinasus*, *Pleurodema borellii* and *Pleurodema bufoninum* (Leptodactylidae). In addition, some *Ceratophrys ornata* (Anura, Ceratophryidae) and *Ambystoma* spp. (Caudata) larvae were used for comparisons. All material is housed in the amphibian collections of Museo de La Plata (MLP) and Instituto Nacional Malbran (CHINM), at MACN, Museo Argentino de Ciencias Naturales Bernardino Rivadavia (Appendix A). Species were identified by checking for diagnostic features and by rearing tadpoles to froglets. Larvae of each species were maintained in separate aquaria with natural light and supplied with aquatic plants and detritus as food. Individuals were removed from the colony at regular intervals, killed, fixed in 5% formalin, staged following Gosner (1960) and cleared and double-stained following the technique of Taylor and Van Dyke (1985). Ossification sequences of all skull bones were recorded to provide a timeframe context for the study (Table 2). To verify features (e.g., otic notch and tympanic ring) of adult skulls and the presence of bones that ossify postmetamorphically, we examined adults of all species. General skull terminology follows de Beer (1937) but the term fenestra frontoparietalis follows Lebedkina (2004). Genera and species level lissamphibian taxonomy follows Frost (2012).

For the discussion of our findings we use the most widely accepted of the three competing phylogenetic hypotheses of amphibian relationships (Laurin and Reisz, 1997; Ruta et al., 2003; Carroll, 2007; Sigurdson and Green, 2011). It establishes a common origin of lissamphibians from dissorophoideans (Temnospondyli), with Anura + Caudata (Batrachia) being the sister group of Gymnophiona (Sigurdson and Green, 2011); alternative phylogenies are discussed by Cannatella et al. (2009).

We think homology assessment is a central problem in evolutionary biology, and agree with Hall (1999), who considered homology to be a hierarchical concept for which different sources of information should be integrated to solve philosophical and

methodological contradictions. Thus we assessed bone homology on the basis of two assumptions: (i) Each ossification center of a compound bone represents a single bone of the ancestors of the group under study (Griffiths, 1954a); (ii) the topology of ossification centers in relation to the underlying cartilaginous endocranial structures is constant across taxa (Lebedkina, 2004).

3. Results

The squamosal, frontoparietal, and the exoccipital bones arise from more than one center of ossification (summarized in Table 1 together with the information available in the literature for the same bones). Because the columella ossifies postmetamorphically in the species we examined, its mode of development is not described. The ossification sequences of all cranial bones of the species studied are listed in Table 2. Centers are described based on the position they have in relation to relevant cranial structures, and the homology of each center is proposed within the respective section (e.g., occipital arch center: exoccipital) and discussed and justified in Section 4. The separate center of the zygomatic squamosal arm has been mentioned for two *Ceratophrys* species (Wild, 1997a,b) and *Leptodactylus chaquensis* (Perotti, 2001), but was not observed in the species of these genera studied by us.

3.1. Occipital arch center: exoccipital

Each exoccipital bone develops from a single perichondral center placed at the occipital arch (Fig. 1A and B). Bone appears at stages 33 (*H. sylvatica*), 35 (*B. taeniata*, *L. latinasus*), 36 (*O. americanus*, *P. borellii*) and 37 (*P. bufoninum*) (Table 2). Once formed, the center of the exoccipital grows toward the posterior wall of the auditory capsule and fuses with the posterior center of the auditory capsule. Such fusion occurs at different stages depending on the species: 35 (*H. sylvatica*), 39 (*L. latinasus*), 40 (*P. bufoninum*), 41 (*O. americanus*, *P. borellii*) and 42 (*B. taeniata*). The later development of the bone is expressed by bone expansion toward reaching the adult condition. The exoccipitals of the species studied invade adjacent areas of the basal plate and the tectum synoticum. In the adults of the species studied, the oto-occipital bone is formed by fusion of the exoccipital and opisthotic bones (see Section 3.2). This oto-occipital bone does not include the prootic except in heavily ossified adults of *O. americanus*. In adults of the other species studied, the area between the oto-occipital and the prootic remains cartilaginous or calcified as indicated by the diffuse Alizarin Red staining, but never truly ossified. The midposterior portion of each exoccipital forms the paired occipital condyles.

3.2. Posterior auditory capsule center: opisthotic

All species studied here present a clearly separate perichondral center placed on the posteromedial wall of the auditory capsule, near the floor of the capsule. We consider this center to be homologous to the opisthotic of other tetrapods, as previous authors have (Roček, 2003). Such a center appears at stages 35 (*H. sylvatica*), 38 (*L. latinasus*), 40 (*O. americanus*, both *Pleurodema*) and 42 (*B. taeniata*) (Table 2) and, once formed, it grows to surround the foramen perilymphaticum inferius (Fig. 1A and B). The center of the opisthotic always appears after the exoccipital and before the prootic, even in species where both otic bones develop at the same stage. Once fused with the exoccipital, the jugular foramen becomes encircled by the oto-occipital bone (Fig. 1C and D). In adults of the species studied the oto-occipital bone becomes fused to the prootic only in *O. americanus*.

Table 2
Ossification sequence of *Hylorina sylvatica*, *Odontophrynus americanus*, *Leptodactylus latinasus*, *Pleurodema borellii*, *Pleurodema bufoninum*, and *Batrachyla taeniata*. Chondral bones are in boldface. Asterisks denote low and diffuse Alizarine staining (calcification).

<i>Hylorina sylvatica</i>	<i>Odontophrynus americanus</i>	<i>Leptodactylus latinasus</i>	<i>Pleurodema borellii</i>	<i>Pleurodema bufoninum</i>	<i>Batrachyla taeniata</i>
			27–29 (39)		
31 (2) 1. Parasphenoid 2. Frontal ² 33 (3) Exoccipital	33 (7) 1. Parasphenoid 2. Frontal ² 34 (9)				
35 (1) 1. Opisthotic 2. Prootic		35 (1) Parasphenoid Exoccipital 36 (2) Frontal ²	36 (1) Exoccipital Parasphenoid	36 (61)	34 (9) Parasphenoid 35 (14) 1. Exoccipital 2. Frontal ² 36 (17)
	37 (1)	37 (10)		37 (32) 1. Exoccipital 2. Parasphenoid 3. Frontal ² 38 (10) Parietal	37 (12)
		38 (4) 1. Opisthotic 2. Prootic 39 (14) Premaxilla	38 (3)		38 (11)
	40 (4) 1. Prootic 2. Opisthotic		40 (19) 1. Frontal ² 2. Opisthotic 3. Parietal 4. Prootic 5. Premaxilla 6. Septomaxilla 7. Nasal 8. Squamosal 41 (4)	40 (5) 1. Opisthotic 2. Prootic 3. Premaxilla 4. Nasal Septomaxilla Squamosal	39 (1) 40 (5)
41 (5) 1. Premaxilla 2. Septomaxilla 3. Nasal Maxilla 4. Angulosplenic 42 (1)	41 (2) Premaxilla Nasal	41 (33) 1. Septomaxilla 2. Nasal 3. Maxilla		41 (8)	
	42 (3) 1. Maxilla Septomaxilla 2. Squamosal Supratemporal	42 (8) 1. Squamosal 2. Vomer			42 (7) 1. Opisthotic 2. Prootic
43 (1) Vomer Squamosal Dentary Pterygoid Supratemporal		43 (8) 1. Angulosplenic 2. Dentary 3. Pterygoid	43 (8) 1. Maxilla Angulosplenic Dentary 2. Pterygoid	43 (1) Maxilla Angulosplenic Pterygoid Supratemporal Septomaxilla	43 (32) 1. Premaxilla 2. Septomaxilla 3. Dentary Maxilla Angulosplenic 4. Nasal Vomer Squamosal Pterygoid 44 (4)
44 (4) 45 (1) Palatine Posteromedial process hyoid Tabular		44 (1)	44 (1) Tabular Supratemporal		
		46 (2) 1. Mentomeckelian Quadratojugal Palatine 2. Posteromedial process hyoid			46 (5) 1. Posteromedial process hyoid Tabular Palatine 2. Mentomeckelian PM Articular* Quadrates* Columella Sphenethmoid

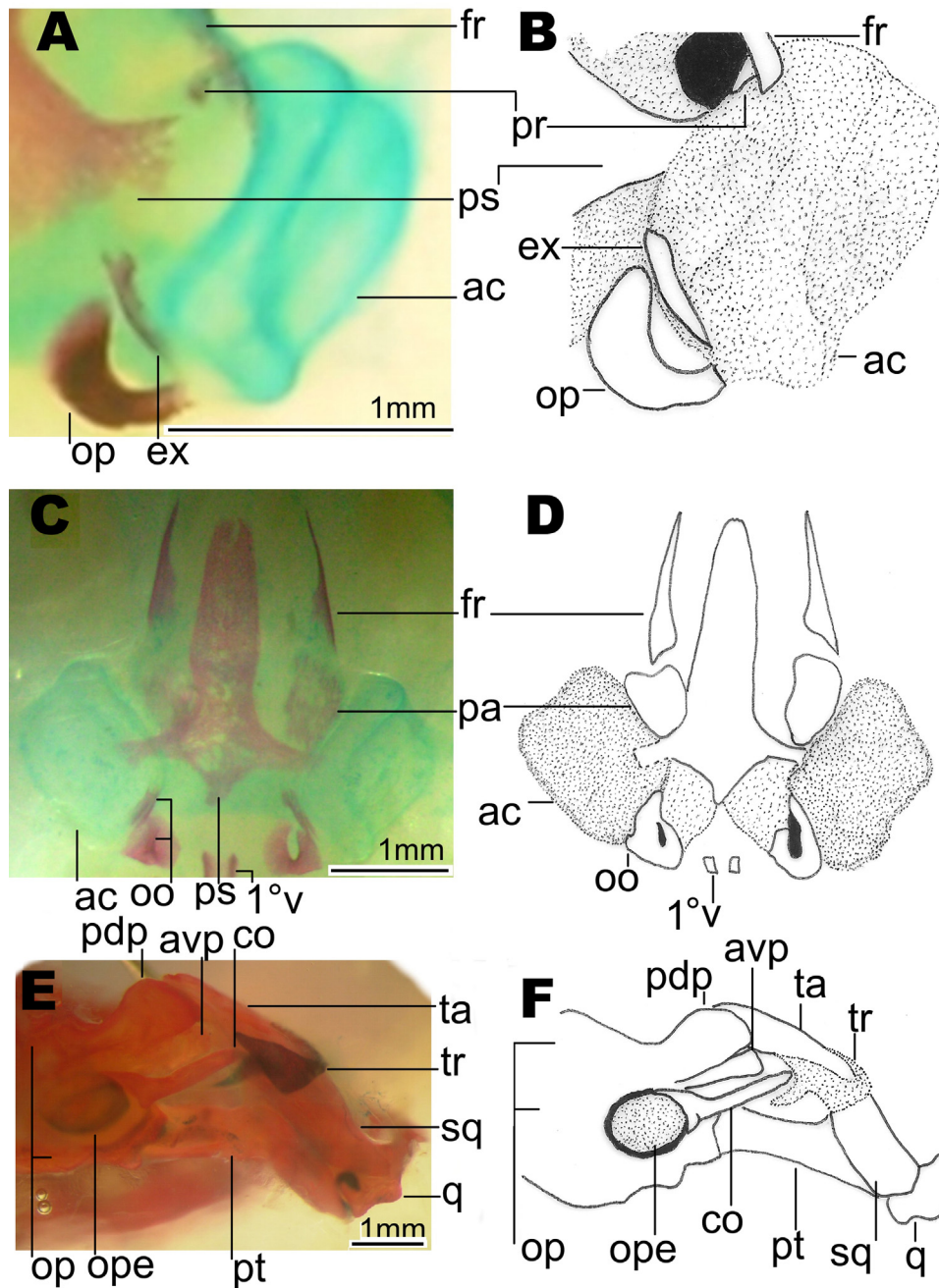


Fig. 1. (A and B) Ventral view of the left auditory capsule in a Stage 38 larva of *Leptodactylus latinasus*. Note the separate perichondral centers of the exoccipital and the opisthotic bones. (C and D) Dorsal view of the orbital and otico-occipital regions of the skull in a Stage 40 larva of *Pleurodema borellii*. Note (i) the separate center of the parietal bone before fusion with the frontal² center and that (ii) the exoccipital and opisthotic centers are fused forming the oto-occipital bone. (E and F) Caudo-ventral view of the right otic capsule in an adult *Odontophrynus americanus*. Note the contact between the posterodorsal parotic process and the tabular (otic squamosal arm) bone, and between the anteroventral parotic process and the squamosal (ventral squamosal arm) bone, as has been described for *Dolesempeton* and *Triadobatrachus* (Sigurdson, 2008). Abbreviations: ac, auditory capsule; avp, anteroventral process; co, columella; ex, exoccipital; fr, frontal (²); oo, oto-occipital; op, opisthotic; ope, operculum; pa, parietal; pdp, posterodorsal process; pr, prootic; ps, parasphenoid; pt, pterygoid; q, quadrate; sq, squamosal; ta, tabular; tr, tympanic ring; 1°v, first vertebra.

3.3. Dermal center of the cranial roof at the level of the optic mesencephalic lobes: parietal

A separate dermal center for the parietal bone was only found in the species of *Pleurodema* (Fig. 1C and D). In this genus, each parietal ossifies later than the frontal² center (frontal² of other authors; Čihák et al., 2003) either during the same developmental stage (40 in *P. borellii*) or one stage later (38 in *P. bufoninum*) (Table 2). The

rounded dermal center of each parietal appears at each side of the fenestra frontoparietalis, behind the level of the tectum transversum, just dorsal to the optic lobes of the mesencephalum, medial to the anterior portion of the anterior semicircular canal and lateral to the taenia tecti medialis. The centers of parietal and frontal² fuse rapidly at the level of the pila antotica at Stage 41 (*P. borellii*) or 40 (*P. bufoninum*). Once fused, the site of the fusion remains identifiable by the presence of a small medial notch.

3.4. Dermal center lateral to quadrate cartilage: ventral squamosal arm: squamosal

The ventral arm of the squamosal bone of the species studied here begins to ossify dermally next to the lateroanterior margin of the processus muscularis quadrati (Fig. 2A and B). Bone appears at Stages 40 (both *Pleurodema*), 42 (*L. latinasus*, *O. americanus*) and 43 (*B. taeniata*, *H. sylvatica*) (Table 2). The center of the ventral arm is first situated at the border between the orbital and ethmoidal regions and then migrates caudally, together with the caudal migration of the articular process of the quadrate cartilage. Finally, it reaches the adult position in the cheek region near the end of metamorphosis, at which time it fuses with the dermal center of the dorsal otic arm of the bone (Fig. 2B–D). Consequently, the adult 'squamosal' is the product of the fusion of two separate and topographically distanced centers: the center of the ventral arm (squamosal *sensu lato*) and the center of the otic arm (tabular; see Section 3.5). The anterior arm of the adult 'squamosal' ossifies during late postmetamorphic development and we could not verify its mode of development.

3.5. Dermal center lateral to the crista parotica: otic squamosal arm: tabular

The oval-shaped dermal center of the otic arm of the squamosal ossifies lateral to the processus anterolateralis of the crista parotica (Figs. 2C, D and 3A, B). Bone appears at Stages 44 (*P. borellii*), 45 (*H. sylvatica*) and 46 (*B. taeniata*) (Table 2). In *L. latinasus*, the center in question could not be observed before fusion with the squamosal, and it was unavailable for *O. americanus* and *P. bufoninum* because of a lack of proper developmental stages. The dorsal and ventral centers of the squamosal in the other species fuse at Stage 46. Once fused, both of them form the otic notch for the attachment of the tympanic ring and the tympanic membrane (Fig. 2E). In adults of the species studied, the tympanic ring is attached to the auditory capsule by a pair of paroccipital processes of the crista parotica (Fig. 1E and F). The dorsal squamosal center represents the otic arm of the adult bone and we consider it homologous with the tabular of Palaeozoic amphibians (see Section 4.4).

3.6. Dermal center dorsal to the anterior semicircular canal: supratemporal

Three of the species studied here present a clearly identifiable dermal center at the dorsum of the anterior semicircular canal and lateral to the parietal portion of the frontoparietal bone (Figs. 2A, B and 3A, B). We consider this center to be homologous to the supratemporal of Palaeozoic amphibians (see Section 4.4). This center appears at Stage 42 (*O. americanus*), 43 (*H. sylvatica*, *P. bufoninum*), and 44 (*P. borellii*) (Table 2). The supratemporal and frontoparietal bones fuse at Stage 44 in *H. sylvatica* and *P. borellii*. Fusion could not be identified in *O. americanus*, *P. borellii* and *P. bufoninum* because it usually occurs after the stages examined in the present study. The supratemporal center was not verified in *B. taeniata* and *L. latinasus*, but was observed in one Stage 43 larva of *C. ornata* used for comparison (see Table 1 and Fig. 3C and D).

4. Discussion

4.1. General comments

All the species studied here revealed center fusion processes during the larval development of certain bones of the otico-occipital and cheek regions. A homology assessment for each compound bone center is explicitly made in the following

paragraphs, together with a complete discussion of the support for each of the homology hypotheses proposed in the past.

4.2. Homology of the perichondral center of the posterior auditory capsule wall

The opisthotic bone is considered to be either absent (Trueb, 1993; Carroll, 2007) or present (Roček, 2003) in anurans, and present in caudates (Goodrich, 1930; Eaton, 1959; Bonebrake and Brandon, 1971; Lebedkina, 2004); although it was considered to be present in Gymnophiona (Marcus et al., 1935), more complete modern studies have failed to verify this bone (Müller, 2006). The anuran opisthotic was misidentified by the authors who worked with larvae of Ceratophryidae, Ranidae and Scaphiopodidae, where the center was interpreted to be either the anterior part of the exoccipital (Kemp and Hoyt, 1969; Hall and Larsen, 1998) or the posterior part of the prootic (Wild, 1997a). But periotic bones (epiotic, opisthotic, prootic) ossify perichondrally at the anterior (prootic), posterior (opisthotic) or dorsal (epiotic) walls of the auditory capsule in all tetrapods that present these bones (de Beer, 1937; Bona and Alcalde, 2009). The center of the opisthotic bone was observed in all the species studied before its quick fusion with the exoccipital to form the oto-occipital bone. Within the species studied, the oto-occipital bone fuses with the prootic only in *O. americanus*. The fusion between exoccipital and opisthotic bones is very common in extant Archosauria and Lepidosauria, but not in Testudines, where exoccipital and opisthotic are not fused (Goodrich, 1930; Lebedkina, 2004; Bona and Alcalde, 2009; present study). The presence of the opisthotic bone is plesiomorphic for anurans because it is present in dissorophoideans such as *Doleserpeton* and in the early Triassic frog *Triadobatrachus* (Rage and Roček, 1989). Interestingly, the anurans studied here and one undescribed species of the trematopid genus *Acheloma* (Temnospondyli) share an identical ossification sequence of the following bones: exoccipital → opisthotic → prootic (Maddin et al., 2010; present study).

Sigurdson (2008) described a remarkable feature of the auditory capsule shared by *Doleserpeton*, *Triadobatrachus*, anurans and primitive caudates but absent in gymnophionans and Lepospondyli. This is the presence of a ventrolateral ledge housing the posterior part of the perilymphatic cistern. The presence of the opisthotic bone in anurans makes the structure of the ventrolateral ledge more consistent when *Doleserpeton*, *Triadobatrachus* and anurans are compared. Finally, it is important to note that, in species with poorly ossified auditory capsules (e.g. *P. borellii*), the ledge is enclosed by cartilage of the capsule only.

4.3. Homology of the ventral squamosal arm

The presence of dorsal and ventral dermal ossification centers of the squamosal bone has been reported for many anuran (Bufonidae, Calyptocephalellidae, Ceratophryidae, Brachycephalidae, Leptodactylidae, Ranidae, Scaphiopodidae) (Reinbach, 1939a; Sedra, 1949; Griffiths, 1954a; Wiens, 1989; Wild, 1997a, 1999; Hall and Larsen, 1998; Perotti, 2001; Lebedkina, 2004; Campos et al., 2010) and caudate (Ambystomatidae, Hynobiidae) (Lebedkina, 2004) families, and they have also been inferred in the fossil stem caudates *Karaurus* and *Kokartus* (Skutschas and Martin, 2011) (Fig. 4). The ventral center ossifies in association with the quadrate cartilage whereas the dorsal center develops lateral to the crista parotica of the auditory capsule in both anurans and caudates (Lebedkina, 2004; present study). The squamosal of amniotes and gymnophionans develops from a dermal center related to the quadrate cartilage (de Beer, 1937; Lebedkina, 2004). Clearly, the single center of amniotes and gymnophionans and the ventral squamosal center of batrachians are homologous because both are related to the quadrate cartilage. But homology assessment

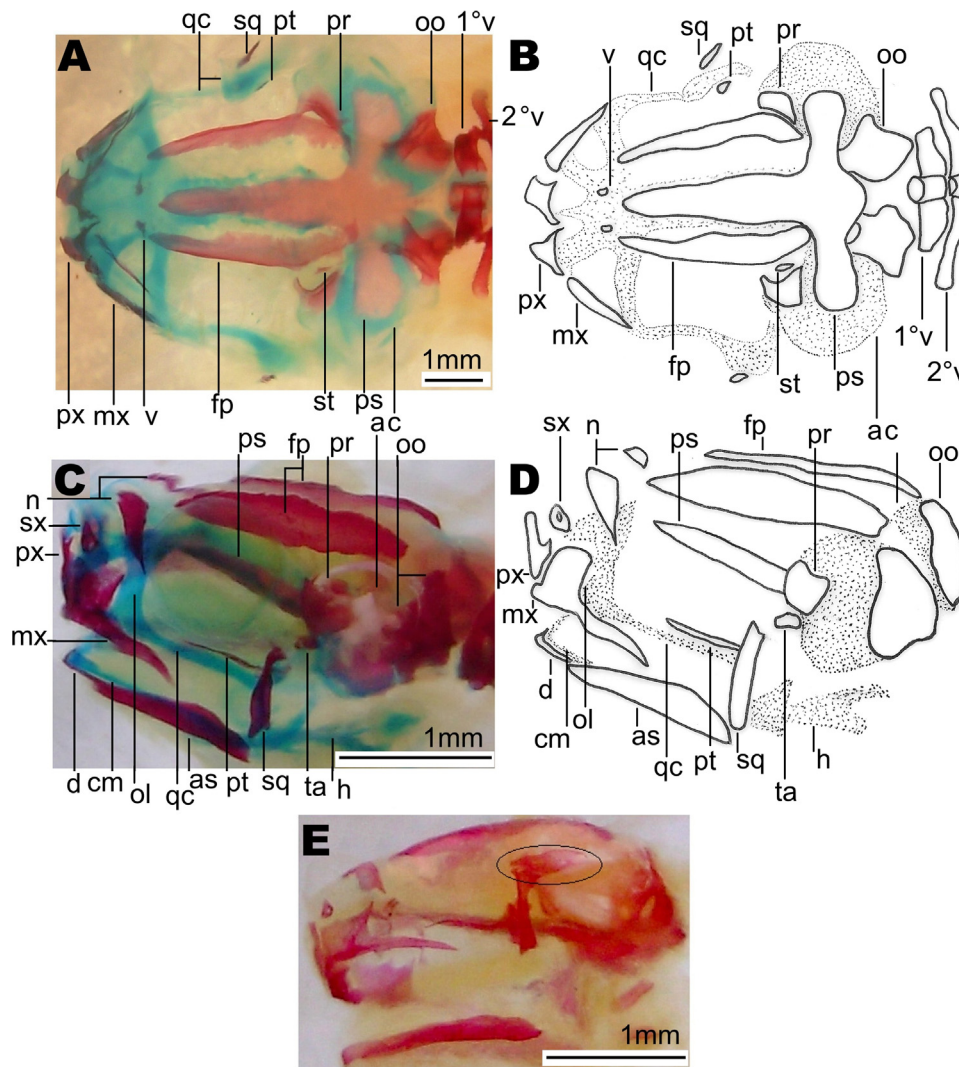


Fig. 2. (A and B) Ventral view of the skull in a stage 43 larva of *Hylorina sylvatica*. Note (i) the separate dermal center of the supratemporal bone before the fusion with the parietal part of the frontoparietal bone and (ii) the dermal center of the ventral squamosal arm (squamosal *sensu lato*). (C and D) Dorsolateral view of a Stage 46 larva of *Batrachyla taeniata*. (E) Dorsolateral view of a more advanced Stage 46 larva of *B. taeniata*. Note the formation of the squamosotabular bone of the adults by the dermal center of the ventral arm of the squamosal (squamosal *sensu lato*) and the dermal center of the otic arm of the squamosal (tabular) before (C and D) and after (E) fusion. The transient suture before complete bone fusion is visible in (E). **Abbreviations:** ac, auditory capsule; as, angulosplenic; cm, cartilago meckeli; d, dentary; fp, frontoparietal; h, hyoid; mx, maxilla; n, nasals; ol, orbitonasal lamina; oo, oto-occipital; pr, prootic; ps, parasphenoid; pt, pterygoid; px, premaxilla; qc, quadrate cartilage; sq, squamosal; st, supratemporal; sx, septomaxilla; ta, tabular; v, vomer; 1°v, first vertebra; 2°v, second vertebra.

between the center related to the quadrate and some of the cheek bones of rhipidistians is rather debatable. Baur (1896) studied the relationship between dermal bones and lateral line sensory canals, concluding that the bone related to the quadrate in stegocephalians corresponds to the preopercular of crossopterygians. Stensiö (1947) re-interpreted the jugal canal as being part of the preopercular canal, suggesting that: (i) the upper preopercular plate of rhipidistians corresponds to the squamosal of stegocephalians, and (ii) the middle and ventral preopercular plates of rhipidistians represent the dorsal and ventral preopercular plates of stegocephalians. But the dorsal and middle preopercular plates of rhipidistians have been interpreted as true squamosals by some authors (Vorobyeva, 2003). Lebedkina (2004) combined data on the cranial development of Batrachia with the ideas of Baur (1896) and Stensiö (1947), concluding that the middle preopercular plate of rhipidistians, the ventral squamosal center of Batrachia and the single squamosal center of Amniota and Gymnophiona are homologous. We think that additional evidence together with a more constant system of cheek bone terminology is needed

before comparisons can be made between bony fish and true tetrapods. Until such evidence and terminology is available, we prefer to use the term squamosal instead of preopercular, as suggested by Lebedkina (2004), for the ventral center of the batrachian squamosal bone.

4.4. Homology of the otic squamosal arm

In the following paragraphs we will discuss the homology hypotheses proposed for the dorsal squamosal center in the context of the dissorophoidean bones. These 'bone hypotheses' are the: (i) postfrontal (Sedra, 1949), (ii) neomorph element (Campos et al., 2010), (iii) supratemporal (Reinbach, 1939a; Griffiths, 1954a; Jarvik, 1967; Lebedkina, 2004) and (iv) tabular (Smirnov, 1995). The intertemporal bone has never been proposed as homologous because it was presumably lost in dissorophoideans (Ruta et al., 2003).

But before developing each hypothesis and their respective implications, we need to remark on some of the aspects of bone

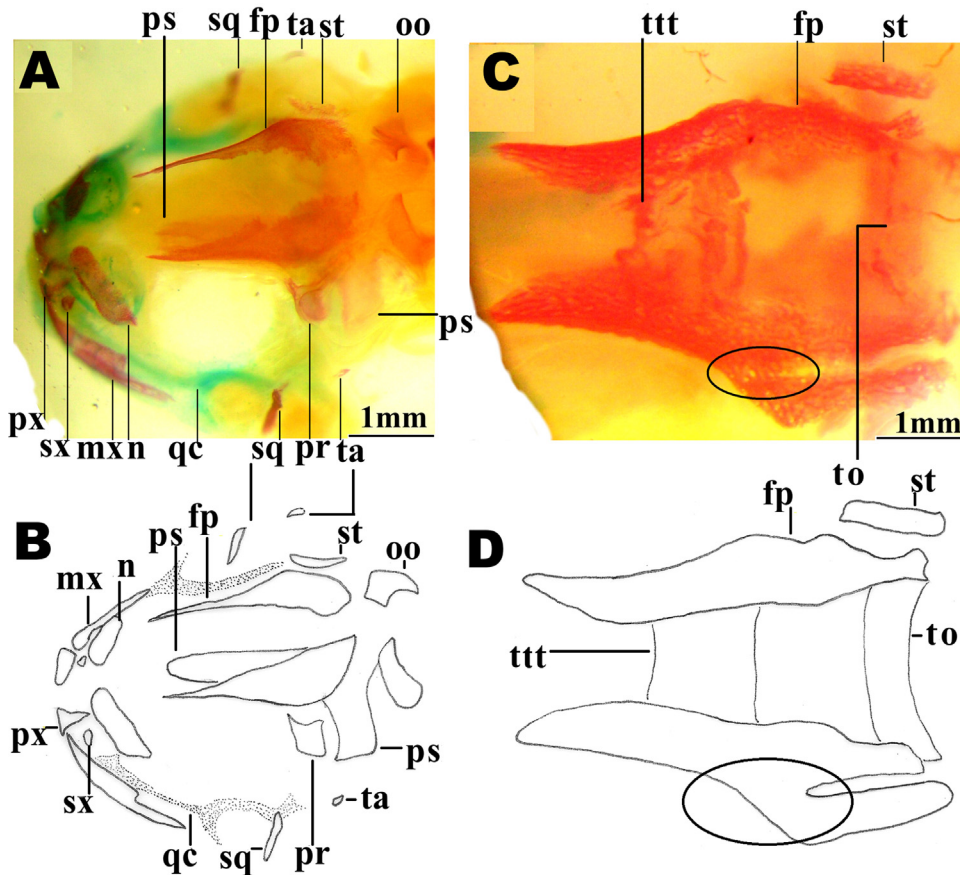


Fig. 3. (A and B) Dorsolateral view of a Stage 44 larva of *Pleurodema borellii*. Note the coexistence of the squamosal, supratemporal, tabular and frontoparietal bone centers before formation of the respective compound bones. (C and D) Dorsal view of the orbital and otico-occipital regions in a Stage 43 larva of *Ceratophrys ornata*. Note the separate center of the supratemporal (right side) and the beginning of the fusion between the supratemporal and the parietal part of the frontoparietal bone (encircled on left side). **Abbreviations:** fp, frontoparietal; mx, maxilla; n, nasals; oo, oto-occipital; pr, prootic; ps, parasphenoid; px, premaxilla; qc, quadrate cartilage; sq, squamosal; st, supratemporal; sx, septomaxilla; ta, tabular; to, tectum sinoticum ossification; ttt, taenia tecti transversalis ossification.

development described in the unique well-studied ossification sequence for larvae of Temnospondyli published by Schoch (1992). In that detailed study, the author described the development of each cranial bone in the larvae of two species of *Apateon* (a fossil more related to the origin of Caudata than Anura). According to Schoch's (1992) descriptions, the most relevant features to our work are that the squamosal bone of *Apateon* presents two centers, one primary and one secondary, whereas the tabular has only one primary center (see Fig. 1 of Schoch's work). The presence of two centers in the squamosal bone of *Apateon* may nullify all the hypotheses in our work. But the centers described by Schoch (1992) are areas of more or less bone deposition instead of separate centers that develop in different regions of the skull and then fuse late in development to form a compound bone, as occurs with the squamosal in anurans. Once such a distinction is made, we are in a position to develop the hypotheses that have been proposed for the dorsal center (otic arm center) of the anuran squamosal.

The 'postfrontal hypothesis' was developed by Sedra (1949) to explain the homology of the 'otic element' (dorsal squamosal center) of *Amietophrynus regularis* and *Calyptocephalella gayi*. Sedra (1949) proposed that the otic element of these frogs represents the postfrontal of early amphibians and certain apodans. The main arguments he considered were: (i) the presence of a postfrontal center placed in the anterolateral side of the auditory capsule in the gymnophionan genus *Ichthyophis* (based on Thyng, 1906), and (ii) the contact between the squamosal and postfrontal bones in the amphibamid *Amphibamus grandiceps* (described as

Miobatrachus romeri by Watson, 1940). Such a contact was also reported for *Eocaecilia micropodia* (Jenkins et al., 2007), a fossil that is not related to Batrachia (Anderson et al., 2008). The main weakness of such a hypothesis is the place where the dorsal squamosal center begins to ossify. The evidence published for lizards, the unique extant tetrapods that have a postfrontal bone, shows that this bone develops in the posterior border of the orbit (Arias and Lobo, 2006) instead of lateral to the auditory capsule (like the dorsal squamosal center) (Lebedkina, 2004; Wild, 1997a; present study).

The 'neomorph hypothesis' of Campos et al. (2010) explains the identity of the 'parotic plates' present in some species of *Brachycephalus*. The intramembranous center of the parotic plate develops in association with the crista parotica (Campos et al., 2010), the place where the dorsal center of the otic squamosal arm ossifies in all anurans (Wiens, 1989; Wild, 1997a; Perotti, 2001; Lebedkina, 2004; present study). Although it is hypertrophied in *Brachycephalus*, the manner in which the parotic plate develops is the same as that of the dorsal squamosal center, suggesting homology, and, consequently, a common ancestry of these structures. As a result, parotic plates of *Brachycephalus* represent the hypertrophied dorsal centers of the otic squamosal arms instead of neomorph structures present only in this genus. Thus we think that the parotic plate (dorsal squamosal center) represents a bone inherited from ancestors rather than a neomorph.

The 'supratemporal hypothesis' was developed by Reinbach (1939a,b) to explain the homology of a dermal center placed lateral to the auditory capsule in *Calyptocephalella gayi* and another center that lies on the roof of the capsule in *Pelobates cultripes*.

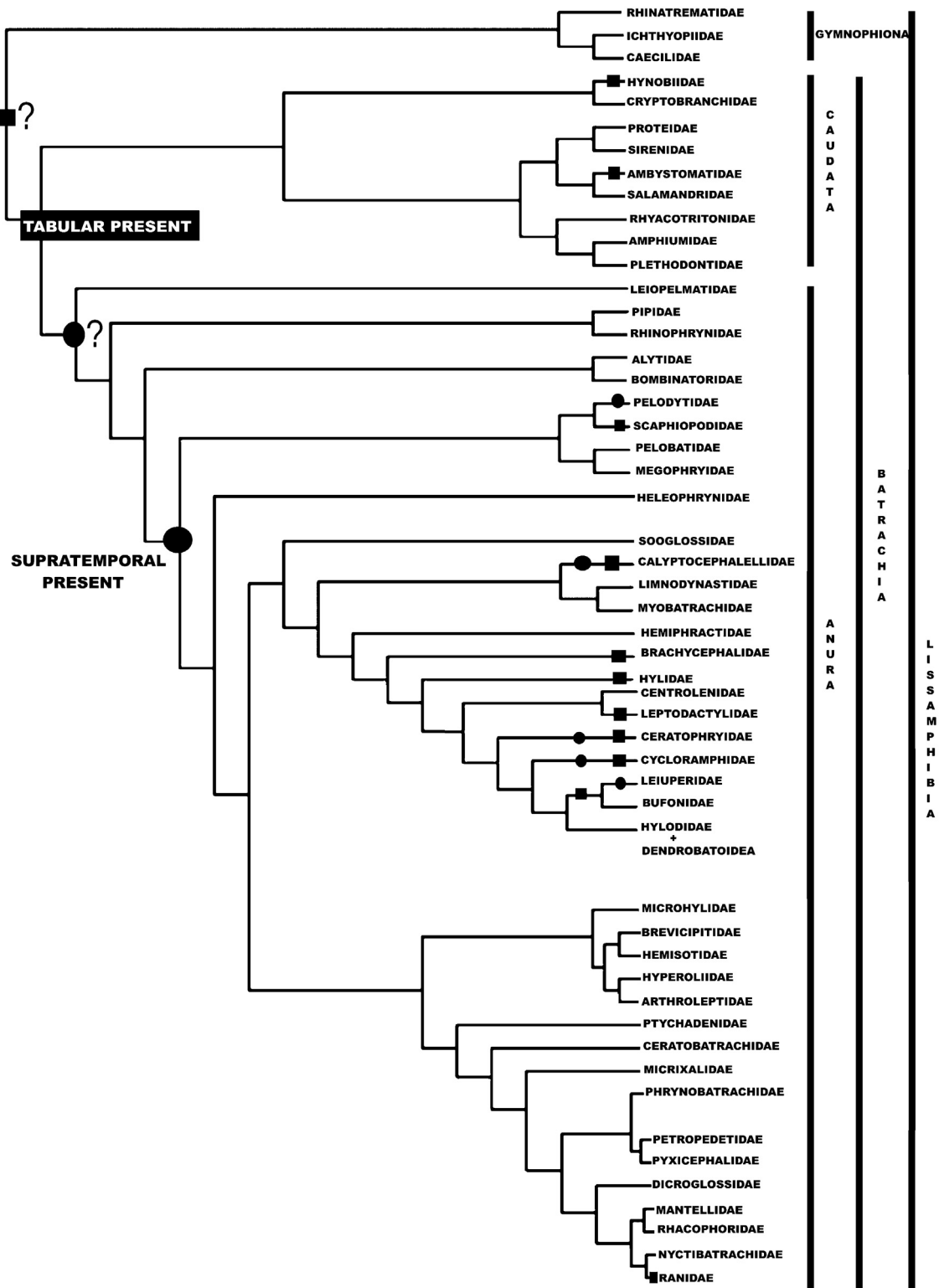


Fig. 4. Phylogenetic tree showing the relationships of the main groups of Lissamphibia as presented by Frost et al. (2006) with modifications on certain terminals according to Grant et al. (2006). Dots mark families for which a separate center for the otic squamosal arm (tabular) has been described. Squares indicate the same but for the supratemporal bone. See Table 1 for literature references. Note that Leiuperidae are now regarded as subfamily Leiuperinae within Leptodactylidae.

Some years later, Griffiths (1954a) erroneously interpreted the dorsal center of the capsule as a supraorbital. Reinbach (1939a,b) justified the homology of both dorsal and ventral auditory capsule centers, introducing the concept of a ‘center movement’. The author explained the position observed in *C. gayi* by a ‘lateral migration’ and applied the concept of ‘upward displacement’ to explain the

position of the center over the auditory capsule (*P. cultripes*). But centers do not move. The center that lies over the auditory capsule in *P. cultripes* was correctly interpreted as supratemporal by Reinbach (1939b), whereas the center that appears lateral to the crista parotica in *C. gayi* is the dorsal squamosal center rather than the supratemporal center. Both centers described by Reinbach are

present in most of the species studied here and in other species described by earlier authors (Smirnov, 1995; Wild, 1997a). As we have demonstrated in the larvae of the species studied here, the coexistence of both centers is a general occurrence rather than a rare phenomenon. In summary, we agree with Reinbach (1939b) that the supratemporal of Dissorophoidea is present in anurans as a separate dermal center that develops over the auditory capsule, but we disagree with the center migration hypothesis that he proposed (Reinbach, 1939a).

The 'tabular hypothesis' was proposed by Smirnov (1995) to assess the 'extra bones' he found in a 9-year-old specimen of *P. cultripes*. The author restricts these bones only to anurans with an accelerated cranial development. However, as demonstrated here, the supratemporal (the dermal center dorsal to the auditory capsule) and tabular (the dermal center lateral to the auditory capsule) bones are usually present in almost all anurans. As these bones are fused to neighboring bones (the supratemporal with the frontoparietal and the tabular with the squamosal) they are consequently omitted in most studies (Fig. 5). Our main argument for supporting the homology between the tabular and dorsal squamosal centers depends on the structure of the otic notch. Bolt and Lombard (1985) considered the skulls of most temnospondyls to be posteriorly emarginated to form the otic notch for tympanum attachment. In dissorophoideans, the otic notch is always formed by the squamosal and tabular, occasionally by the quadrate and rarely by the supratemporal bones (Bolt, 1974). In anurans, the tympanum is attached by a cartilaginous tympanic ring developed from the quadrate together with a bony notch formed by the ventral and otic squamosal arms (Bolt and Lombard, 1985). Milner (1993) pointed out the absence of homology between the otic notch of dissorophoideans (formed mainly by tabular, squamosal and quadrate bones) and anurans (formed by the tympanic ring, originating from the quadrate cartilage, and the squamosal bone only). Other evidence supporting the 'tabular hypothesis' comes from the paroccipital processes for the tympanic ring attachment, which were described by Sigurdson (2008) for *Doleserpeton* and anurans. In *Doleserpeton* the posterodorsal process contacts the tabular, whereas in the species of anurans studied here it contacts the tympanic ring at the level of the otic arm of the squamosal bone (Fig. 1E and F). The main implication of the 'tabular hypothesis' is that the bone composition of the otic (anurans) or temporal (caudate; *sensu* Clack, 2002) notches is almost identical to that of Dissorophoidea.

Finally, squamosal–tabular fusion may also occur as homoplasy in adelospondyls (Lepospondyli), a group in which the presence of a squamosotabular was inferred because of the presence of a single bone occupying the entire cheek region (Andrews and Carroll, 1991). Gymnophionans apparently lack the tabular bone because their squamosal bone develops from a single center (Wake and Hanken, 1982; Müller et al., 2005; Müller, 2006) that, because of its position, should be homologous to the ventral arm of the squamosal bone of Batrachia (squamosal *sensu lato*). Nevertheless, Visser (1963) wrote the following about the squamosal of *Ichthyophis glutinosus* and *Ichthyophis monochromus*: 'Although ontogenetic data are lacking, the size, shape and relationships of the bone in the adult suggest that it is a compound structure. . . ' and 'the ventral portion of this bone undoubtedly represents the squamosal. The dorsal process could be a mere extension of the ventral part, but it seems more likely that it represents a separate element which has at an early stage fused with the ventral anlage' (Visser, 1963). The author also commented on the possibility that the dorsal arm of the bone in gymnophionans may be homologous to the supratemporal bone of other tetrapods. But, at present, Visser's assumptions remain speculative because no ontogenetic evidence has been presented supporting the double origin of the squamosal bone in the Gymnophiona.

4.5. Composition of the anuran frontoparietal

The homology of the cranial roof bones has been the subject of debate for years (Säve-Söderbergh, 1932, 1935; Westoll, 1938, 1943). Both these authors have used the position of the pineal foramen as the main criterion to cast doubt on the traditional view of a direct homology of the frontal and parietal bones between bony fish and tetrapods (Goodrich, 1930; de Beer, 1937). Säve-Söderbergh (1932, 1935) studied the cranium of some crossopterygians and ichthyostegids and inferred the compound nature of the frontal (the fusion of frontal¹ and frontal²) and the parietal (the fusion of parietal¹ and parietal²) bones. The presence of two frontals and two parietals in some crossopterygians and the occasional presence of more than one center in the development of the frontal bone of extant fish were the main evidence used by Säve-Söderbergh. He proposed that tetrapods have a single frontal (frontal¹) and a compound parietal (frontal² + parietal¹ + parietal²) bone that encloses the pineal foramen (Säve-Söderbergh, 1932). The alternative view (Westoll, 1938, 1943) proposes that (i) the frontal of crossopterygians corresponds to the parietal of tetrapods (because of the position of the pineal foramen), and (ii) the parietal of crossopterygians and the postparietal of tetrapods are homologous. To support his view, Westoll introduced the concept of 'posterior shifting' of the roofing bones during the fish–tetrapod transition.

More recently, Lebedkina (1979, 2004) has described three centers for the frontal (f¹, f², f³) and one for the parietal bone in the caudatan and actinopterygian fish she studied, providing strong evidence in support of Säve-Söderbergh's ideas. As a consequence, she modified Säve-Söderbergh's hypothesis as follows (items 1 and 2 have Säve-Söderbergh's names in quotation marks): (1) in ichthyostegids, the 'frontoparietal' corresponds to the fused frontal² and frontal³, and the 'postparietal' represents either the parietal alone or the fusion of both parietal and postparietal; (2) in advanced stegocephalians, the 'frontal' represents the frontal¹ and the 'parietal' the fusion between frontal² + frontal³ with the parietal; (3) the frontoparietal of Caudata represents the fusion of frontals^{1–3} with the parietal; and (4) in Anura, the frontoparietal is formed by the fusion of frontal² and the parietal only. In addition to this clearly open debate about the homology of the dermal roofing bones, Čihák et al. (2003) reported the presence of four ossification centers during the development of the frontal bone in the frog *Bombina variegata*. These centers correspond to frontals^{1,2,3} described for caudates by Lebedkina (2004) plus one previously unknown center without clear homology, the frontal⁴. These centers fuse together before their fusion with the center of the parietal. Finally, Vorobyeva (2003) proposed that the multiple frontal centers found in Batrachia and Actinopterygii may correspond to the numerous postrostral bones of the rhipidistians. In fact, she considered the adult condition of the dermal roofing bone pattern of extant tetrapods to be consolidated in Panderichthyidae. This group of advanced rhipidistians was the first to display the tetrapod ancestral pattern of dermal roofing bones with the paired frontal lying anterior to the paired pineal bone (parietal). In summary, current evidence demonstrates a composition of at least three bones for the anuran frontoparietal: (1) frontals^{1–3} + parietal (*Bombina variegata*; Čihák et al., 2003), (2) frontal² + parietal in bombinatorids, bufonids, dicroglossids, hylids, leptodactylids, lithobatids, ptychadenids and ranids (*Amietia angolensis*, *Epidalea calamita*, *Glandirana tigrina*, *Hoplobatrachus tigrinus*, *Schismaderma carens*, *Pleurodema marmoratum*, *Lithobates catesbeianus*, *Nanorana pleskei*, *Ptychadena mascareniensis*, *Rana iberica*, *Rana temporaria*; Griffiths, 1954b – *Bufo bufo*, *Bombina bombina*, *Hyla arborea*, *Hyla japonica*, *Pelophylax lessonae*, *Pelophylax ridibundus*; Lebedkina, 2004 – *Pleurodema*; present study), and (3) frontal² as a single center which occurs in most anurans (Roček, 1989) including many of those studied in the present work (*Odontophrynus*, *Batrachyla*,

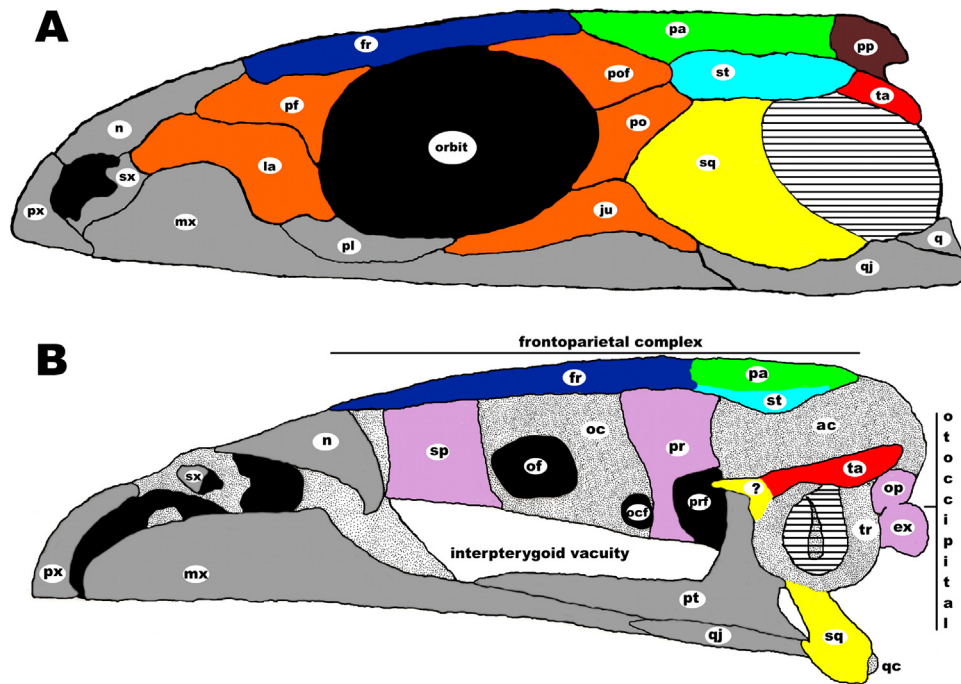


Fig. 5. Lateral view of the skull in (A) the lower Permian amphibamid *Doleserpeton* and (B) a generalized anuran. The bone names from the anuran cheek and otico-occipital regions reflect the hypotheses of bone homology we proposed in the present study. The skull of *Doleserpeton* was modified from Fig. 1 of Sigurdson and Bolt (2010). Graphs are not scaled. Shaded areas represent cartilaginous or calcified areas. Areas with lines represent the tympanum. Black areas are cranial fenestrations and openings. In order to facilitate the comparison between *Doleserpeton* and anurans we used different colors to indicate bone homology (and consequently process of bone fusion). The bones colored violet in the anuran skull (sphenethmoid, prootic, opisthotic, and exoccipital) are present but obscured by the more superficial bones in the skull of *Doleserpeton*. Abbreviations: ac, auditory capsule; ex, exoccipital; fr, frontal²; ju, jugal; la, lacrimal; mx, maxilla; n, nasal; oc, orbital cartilage; ocf, oculomotor foramen; of, optic foramen; op, opisthotic; pa, parietal; pf, prefrontal; pl, palatine; po, postorbital; pof, postfrontal; pp, postparietal; pr, prootic; prf, prootic foramen; pt, pterygoid; px, premaxilla; q, quadrate; qc, quadrate cartilage; qj, quadratojugal; sp, sphenethmoid; sq, squamosal; st, supratemporal; sx, septomaxilla; ta, tabular; tr, tympanic ring; ?, uncertain origin and unknown homology.

Leptodactylus). At present, the addition of the lateral center of the parietal (supratemporal) bone has been reported for species that have frontal² + parietal (*P. bufoninum* and *P. borellii*; present study) as well as those in which the frontoparietal develops from frontal² only (*C. ornata* used for comparison, *Hylorina sylvatica*, *O. americanus*; present study – Pelobates *fuscus*; Reinbach, 1939b; Smirnov, 1995; Lebedkina, 2004–*Calyptocephalella gayi*; Griffiths, 1954a) (Fig. 4).

4.6. Final comments

The presence of more than one ossification center in several bones of the otico-occipital and cheek regions of the anuran skull is demonstrated by our observations in a larval series of five double-stained and cleared species of derived anurans and the evidence available from the literature. Evidence from the literature corresponds to a set of phylogenetically and ecologically diverse anurans (Table 1) and other lissamphibians (mainly salamanders) that have been studied by diverse authors using both histological serial sections and stained and cleared specimens. In the following paragraphs we close this paper by commenting on some of the arguments usually used by the authors that deny the homology of certain ossification centers with some of the bones present in the ancestors of lissamphibians. These arguments are usually contrary to the following three statements: (i) ontogeny is a dynamic phenomenon and certain events are difficult to observe because they occur within a very reduced time frame and, consequently, studies that fail to reveal the presence of certain features do not imply that such features are actually lost; (ii) the absence of a feature in the basal forms of a group does not invalidate homology of the same feature when it is present in derived forms of the group; and (iii) ossification centers evolve and change in many forms across the

history of a group, but this fact does not signify that the centers can move and change their position in the skull, invalidating any comparison regarding its homology.

With respect to point (i), we agree with Lebedkina (2004) and Roček (1989), who clearly stated that the rate of dermal ossification in amphibians is so high that using a limited number of specimens at the relevant stages does not allow proper identification of separate centers of compound bones. This is clear when certain ossification centers have been reported as either absent or present in different works using the same species and techniques (e.g., the center of the otic squamosal arm in *Rana temporaria*, *Bufo bufo* or *Calyptocephalella gayi*; see Table 1 for references). An additional example is the presence (Griffiths, 1954b) and absence (Eaton, 1942) of separate parietal and frontal centers of the anuran frontoparietal bone in the same species (*Rana temporaria* and *Lithobates catesbianus*, among others) studied by different authors that used the same techniques.

With respect to point (ii), the fact that basal anurans may have lost certain centers does not imply that these centers cannot reappear in advanced frogs, such as the neobatrachians studied in the present work. The position and topological relationships of these centers are indicative of their homology, independently of the strict use of the phylogenetic concept of homology.

Finally, regarding the third point, probably the most used in informal debates between colleagues but never demonstrated, is the idea of movement or migration of the ossification centers. Such an idea of bone center evolution implies that ossification centers may change their position in the skull across different taxa. This idea probably arose with the publication of two simultaneous works by Reinbach (1939a,b). In these papers, the author explained the different positions he found for the separate dorsal center of the squamosal bone by using the general concept of ‘center movement’

which implies a 'lateral migration' in the case of *Calyptocephalella gayi* (Reinbach, 1939a) and an 'upward displacement' for *Pelobates fuscus* (Reinbach, 1939b). But such displacement did not actually occur because, as indicated correctly by Roček (2003), Reinbach did observe the lateral center of the parietal bone in *Calyptocephalella* and the dorsal center of the squamosal bone in *Pelobates* but he never had the fortune to observe both centers simultaneously in these species. Thus Reinbach (1939a,b) explained the different position of centers that were actually representing different elements, and consequently were non-homologous. Finally, if the idea of center movement was true, the assumption of homology of any bone would become impossible and neither species comparison nor skull-based phylogenetic analysis would be possible, because the absence of character homology would be highly probable under such circumstances.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.zool.2013.03.002>.

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