

# Development of the skeleton in the dwarf clawed frog *Pseudhymenochirus merlini* (Amphibia: Anura: Pipidae)

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## Abstract

In the present study, we investigate the larval development and metamorphic changes of the skeleton of the small, West African pipid frog *Pseudhymenochirus merlini* Chabanaud, 1920 for the first time. Specimens were cleared and differentially stained for bone and cartilage and the presence or absence of individual bony elements was recorded. *Pseudhymenochirus merlini* is overall similar in larval morphology and development to its sister taxon *Hymenochirus*, but shows differences in ossification sequence. Furthermore, *Pseudhymenochirus* and *Hymenochirus* differ from other pipids by a reduction of the vertebral column to just six presacral vertebrae. This is apparently the result of a modification of the first two vertebrae and a forward shift of the articulation of the pelvic girdle with the vertebral column by at least one vertebra compared to other pipids. The peculiar skeletal characteristics of *Pseudhymenochirus* and *Hymenochirus* do not seem to be a result of miniaturization as often suggested.

## Key words

External gills, metamorphosis, miniaturization, tadpole, vertebral column.

## Introduction

The family Pipidae is a comparatively old clade of anurans (SAN MAURO *et al.*, 2005; ROELANTS *et al.*, 2007; FENG *et al.*, 2017) that contains 41 fully aquatic species in four different genera (AMPHIBIAWEB, 2020). The most prominent members are frogs of the genus *Xenopus*, which includes the established model species *X. laevis* (Daudin, 1802) and *X. (Silurana) tropicalis* (Gray, 1864) (CANNATELLA & DE SÁ, 1993). All pipids are unusual when compared to more generalized anurans and show a number of highly specialized traits in both larvae (ORTON, 1953; HAAS, 2003; ROELANTS *et al.*, 2011) and adults (CANNATELLA & TRUEB, 1988a). The only South American pipids (the genus *Pipa*) are characterized by a highly derived adult morphology (TRUEB & CANNATELLA, 1986; TRUEB *et al.*, 2000) and a specialized form of brood care in which eggs

develop in the modified dorsal skin of the mother and offspring is released either at the tadpole stage or after metamorphosis (TRUEB & CANNATELLA, 1986). Sister taxon to *Pipa* is a clade comprised of the other three pipid genera, *Hymenochirus*, *Pseudhymenochirus*, and *Xenopus*, which are restricted to Sub-Saharan Africa (CANNATELLA & TRUEB, 1988a; IRISARRI *et al.*, 2011). The more recent study of BEWICK *et al.* (2012) found support for a ((*Xenopus*, *Silurana*) (*Pipa*, *Hymenochirus*)) topology. Within *Xenopus*, the *X. tropicalis* clade is consistently recovered as sister taxon to all other *Xenopus* (EVANS *et al.*, 2015), and is recognized as a distinct genus or subgenus, *Silurana*, by some authors.

In contrast to *Pipa*, all species of African pipids lay aquatic eggs and have free-living tadpoles that are char-

acterized by a highly specialized and very divergent tadpole morphology. Unlike most other anurans (but similar to several species of *Pipa*), *Xenopus* has a highly specialized tadpole stage that shows numerous adaptations to microphagous filter feeding (TRUEB & HANKEN, 1992). At the other extreme of the broad spectrum of feeding modes found across anuran tadpoles (ALTIG & JOHNSTON, 1986) are the tadpoles of *Hymenochirus* and *Pseudhymenochirus*, which are comparatively very small, obligate carnivorous tadpoles that ingest their food through a suction feeding mechanism (DEBAN & OLSON, 2002; SOKOL, 1962, 1977). The different feeding modes of pipid tadpoles differ in their functional demands on the larval skeletal morphology. A comparative analysis of pipid morphology and development therefore offers an opportunity to identify and investigate differences in larval morphology and development associated with different feeding modes.

Compared to *Xenopus*, very little information is available on the morphology and development of hymenochirine pipids. For *Hymenochirus*, some information is available on the highly specialized tadpole morphology, which includes traits such as an elongated oral tube supported by the lower jaw, large ceratohyals, and a reduction of the branchial filter, all thought to be associated with macrophagous suction feeding (SOKOL, 1962, 1969, 1977). *Pseudhymenochirus merlini* was first described by CHABANAUD (1920) and occurs predominately in the forested regions of Guinea-Bissau, Guinea and Sierra Leone (IUCN SSC AMPHIBIAN SPECIALIST GROUP, 2013). For *Pseudhymenochirus*, the only data on larval morphology is the external description of the tadpole by LAMOTTE (1963) but no data on tadpole development (skeletal development, ossification sequence etc.) are currently available. For *Hymenochirus*, descriptions of external and internal tadpole morphology exist (SOKOL, 1959, 1962, 1977), but only limited data are available on the development of the skeleton (OLSON, 1998; DE SÁ & SWART, 1999). In this work, we describe the development of the skeleton in *P. merlini* and its ossification sequence and compare it with available information for other pipids.

## Materials and Methods

Eggs were obtained from a breeding colony maintained at the Institute of Zoology, Jena, and raised at 27°C (animal care and sampling protocols followed institutional guidelines). When starting the free-swimming phase after hatching, tadpoles were fed with brine shrimp nauplii (*Artemia salina*). Developing tadpoles were sampled in regular intervals, anaesthetized in a 5% Ethyl-3-aminobenzoate-solution (MS222, Fluka), fixed in 4% neutral buffered formalin and subsequently transferred to 70% ethanol for storage. Specimens were staged using the staging table developed by NIEUWKOOP & FABER (1967; NF) for *Xenopus laevis*.

Specimens were cleared and differentially stained for bone and cartilage using a protocol of TAYLOR & VAN DYKE (1985). For every specimen, the presence of every bony element was recorded. In cases of variation of presence of ossifications within a given stage, we considered ossification to have commenced at a certain stage if an element was present in at least one specimen of a given stage. Images of whole-mount stained larvae were acquired with a Stemi SV 11 stereo microscope (Zeiss) with a ColorViewIII™ camera using the program ANALYSIS® and the images were processed with Adobe Photoshop Elements®. Available for study were 63 larval specimens ranging from NF stage 52 to 66 plus two adults. Specimens have been deposited in the collection of the Department of Zoology, Jena University.

## Results

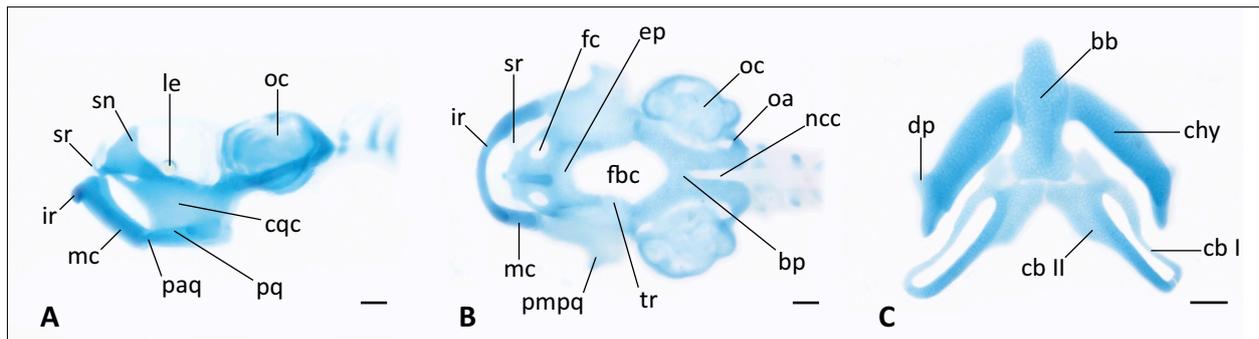
Some elements are initially very small and not readily visible in images of the stage at which they first appear. In these instances, elements are indicated in the figures in a stage in which they are clearly visible to support the description of when an element first appears by showing its shape, position and anatomical context.

### General larval morphology

At hatching, tadpoles were about 2 mm in total length and reached about 12 mm snout-vent length at the end of metamorphosis. Under laboratory conditions the postembryonic development until the end of the metamorphosis took 35 to 44 days. A prominent feature of *P. merlini* tadpoles are the external gills that persist from hatching until stage 59. The external gills consist of three gill rami and protrude outside the spiracle. Overall, our tadpoles of *P. merlini* conformed to the description of LAMOTTE (1963). The tadpoles are very similar in general appearance to tadpoles of *Hymenochirus* (SOKOL, 1962) but show some slight differences in the shape of the cement gland and ventral tail fin (see Discussion).

### Morphology of the chondrocranium

No ossifications are present at stage 52 of development (4 of 4 available tadpoles). The chondrocranium consists of a relatively long but slender basal plate between the otic capsules (Fig. 1). The basal plate contains a deep indentation or canal to accommodate the notochord. The otic capsules are almost completely chondrified, with the exception of the dorsal-most part and the lateral wall, which are rather thin and only incompletely chondrified in parts. Overall, the otic capsules are comparatively large and form the dominant part of the chondrocranium. The trabeculae and ethmoid plate are well chondrified and rather broad and, together with the basal plate, en-



**Fig. 1.** Lateral (A) and dorsal (B) view of the chondrocranium and hyobranchial skeleton (C) of a NF stage 52 tadpole of *Pseudhymenochirus merlini*. bb – basibranchial, bp – basal plate, cb I – ceratobranchial I, cb II – ceratobranchial II, chy – ceratohyal, cqc – commissura quadratocranialis, dp – dorsal process of the ceratohyal, ep – ethmoid plate, fbc – fenestra basicranialis, fc – fenestra choanalis, ir – infra-rostral part of lower jaw (fused with mc), le – ocular lens, mc – Meckel’s cartilage part of lower jaw (fused with ir), ncc – notochordal canal, oa – occipital arch, oc – otic capsule, paq – processus articularis of the palatoquadrate, pmpq – processus muscularis of the palatoquadrate, pq – palatoquadrate, sn – septum nasi, sr – suprarostal, tr – trabecula. Scale bars equal 200  $\mu$ m.

close a prominent fenestra basicranialis. In contrast, the nasal capsule is largely incomplete at this stage and the anterior part of the chondrocranium consists only of the ventral ethmoid plate and a well-chondrified, triangular nasal septum that is pointed upwards. Small, paired, forward projecting suprarostal cartilages are present anterolaterally of the ethmoid plate and project vertically from the anterior margin of the ethmoidal region. Together with the mandibular cartilage, these support the movable oral tube. The palatoquadrate is connected to the chondrocranium via a broad commissura quadratocranialis. The pars articularis quadrati lies on the anterior tip of the palatoquadrate and articulates with the posterior end of Meckel’s cartilage. Both rami of the lower jaw are fused at the symphysis and separate infra-rostral cartilages are absent. However, the anterior part of the lower jaw is more slender and forms a 90 degree angle with the more posterior part of Meckel’s cartilage (see Fig. 1A). This anterior portion resembles infra-rostralia in position and arrangement and presumably represents infra-rostralia fused to Meckel’s cartilage.

*Further development.* By stage 59 the oval ethmoidal plate attained a bluntly rounded shape and the choanae, which are located in the middle of the ethmoidal plate, are enlarged. A cartilaginous tectum nasi has started to form anterolateral of the frontoparietal and dorsolateral to the choanae. From stage 60, the palatoquadrate shifts caudally towards the otic capsule (Fig. 2). The cartilaginous tectum nasi is fully developed and extends, in dorsal view, to the level of the choanae. Scleral cartilages are present as thin rings. All tadpoles have calcium deposits within the endolymphatic sacs of the otic capsule, the braincase and/or within the anterior spinal canal (Fig. 2B–F).

### Morphology and development of the hyobranchial skeleton

In the youngest available specimens (NF52), the larval hyobranchial skeleton is already fully formed. It consists

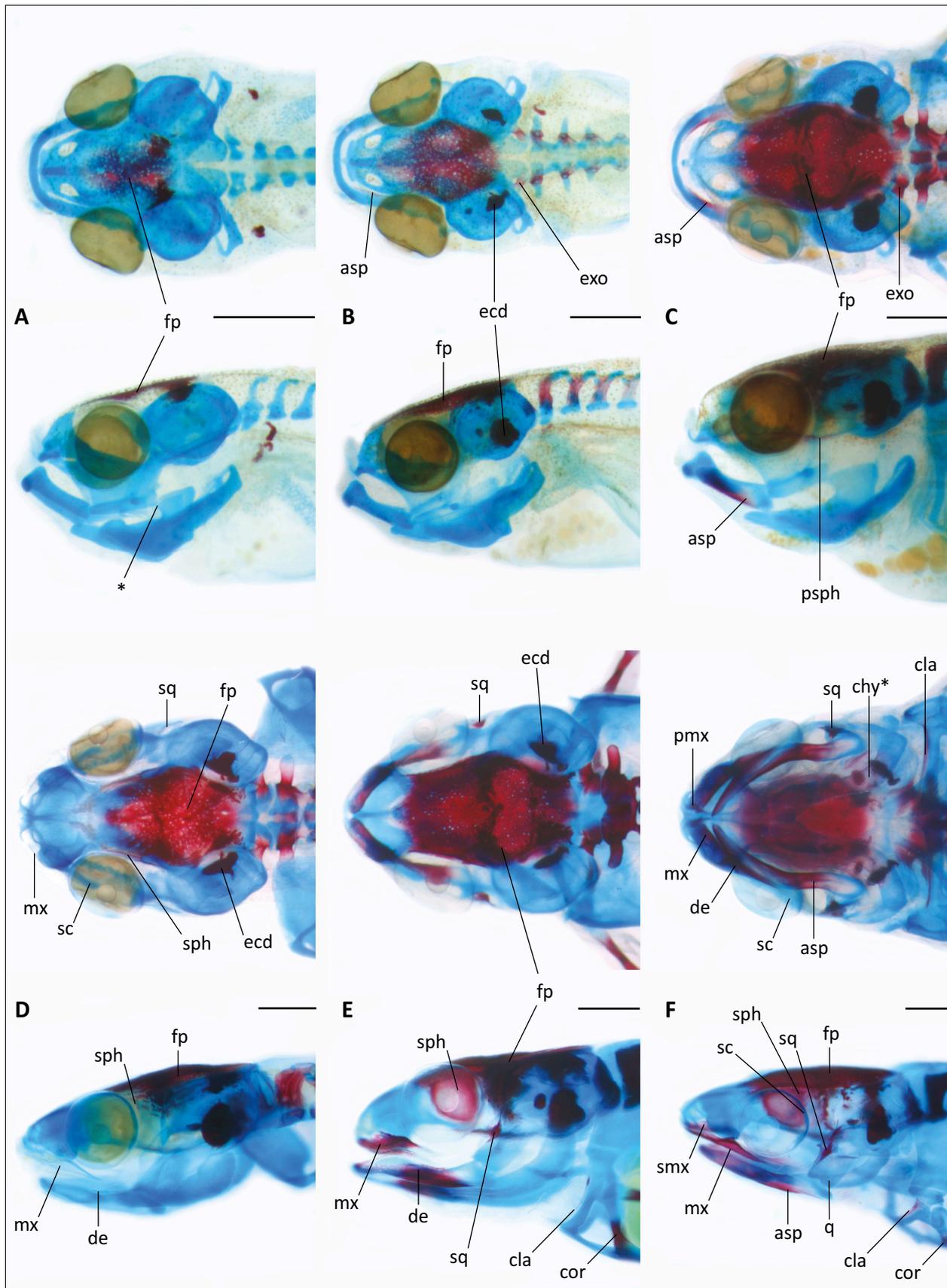
of paired ceratohyals and two pairs of ceratobranchials, all connected by a single, unpaired basibranchial element (Fig. 1C). The ceratohyal is comparatively massive and long-oval in cross section. It has a broad dorsoposterior process (Fig. 1C) that articulates with the palatoquadrate (see Fig. 2A). The ceratobranchials are fused at their proximal and distal ends, forming an elongated, ring-like structure. Ceratobranchial I is rather thin, with its anterior half slightly thicker than its posterior half. Ceratobranchial II is more massive than ceratobranchial I, with about three times the diameter of the latter. Thin but broad flanges are present anteromedially on ceratobranchial II. The basibranchial is a rather stout, T-shaped cartilage, with the cross bar of the T forming the articulation with ceratobranchials I and II on either side. By stage 59, the ceratohyals are extended more caudally, and by stage 60 the transformation of the ceratobranchial and medial basibranchial has started. The anterior cartilaginous process of the hyoid has appeared by stage 61. By stage 64 the ceratohyals begin to ossify (2/6 tadpoles), with ossification starting at the posterior-dorsal margin.

### Ossification of the cranial skeleton

*Stage 53.* Ossification starts with the appearance of the frontoparietal (present in 10 of 10 tadpoles), which at this stage already extends from the posterolateral side of the otic capsule to the larval nasal septum (Fig. 2A).

*Stage 54.* At this stage, the paired exoccipital begins to ossify (2/6 tadpoles) along the margin of the foramen jugulare, posterior to the otic capsule (Fig. 2B).

*Stage 55.* The ossification of the paired angulosphenial (4/4 tadpoles) begins lingual on the ventrolateral margin of the Meckel’s cartilage. The angulosphenial is tapered anteriorly and broadly petering out posteriorly. The unpaired parasphenoid (2/4 tadpoles) ossifies ventral to the braincase, within the centre of the basicranial fenestra (Fig. 2C).



**Fig. 2.** Development of the cranial skeleton in *Pseudhymenochirus merlini*. (A) NF stage 53, (B) NF stage 55, (C) NF stage 59, (D) NF stage 61, (E) NF stage 63, (F) NF stage 65. Top images shows dorsal view in A-E and ventral in F, bottom images shows lateral view. asp – angulosphenial, cla – clavicula, chy\* – ceratohyal ossification, cor – coracoid, de – dentary, ecd – endolymphatic calcium deposit, exo – exoccipital, fp – frontoparietal, mx – maxilla, pmx – premaxilla, psp – parasphenoid, q – quadrate, sc – scleral cartilage, smx – septomaxilla, sph – sphenethmoid, sq – squamosal, \* – articulation between palatoquadrate and ceratohyal. Scale bars equal 1 mm.

*Stage 59.* The ossification of the upper jaw starts with the paired maxilla (3/4 tadpoles) along the posterior margin of the suprarostrals plate. The maxilla is elongated, curved, and its ossification starts from a more medial position to extend rostrally and laterocaudally (Fig. 2D).

*Stage 60.* The paired dentary (4/4 tadpoles) ossifies along the labial side of Meckel's cartilage and, together with the angulosplenial, begins to enclose Meckel's cartilage. The paired squamosal (4/4 tadpoles) is a paired, plate-like dermal bone. It lies anterior of the posterior end of the palatoquadrate (see Fig. 2E). The paired sphenethmoid (2/4 tadpoles) starts developing as a plate-like ossification behind the position of the eye, between the frontoparietal dorsally and parasphenoid ventrally, and forms the lateral wall of the braincase.

*Stage 62.* The paired pterygoid (1/2 tadpoles) ossifies as a small element ventrolateral to the anterior part of the otic capsules.

*Stage 63.* The paired, thin premaxilla (3/4 tadpoles) appears rostral to the cartilaginous nasal capsule (see Fig. 2F). The paired septomaxilla (3/4 tadpoles) appears as a small, narrow, slightly curved rod at the entry of the cavum nasi, dorsal to the maxilla and near the posterior edge of the naris.

*Stage 65.* The paired quadrate (4/6 tadpoles) begins to ossify at this stage. It lies posterolateral to the squamosal and ventrolateral to the pterygoid. The premaxilla and septomaxilla are well developed.

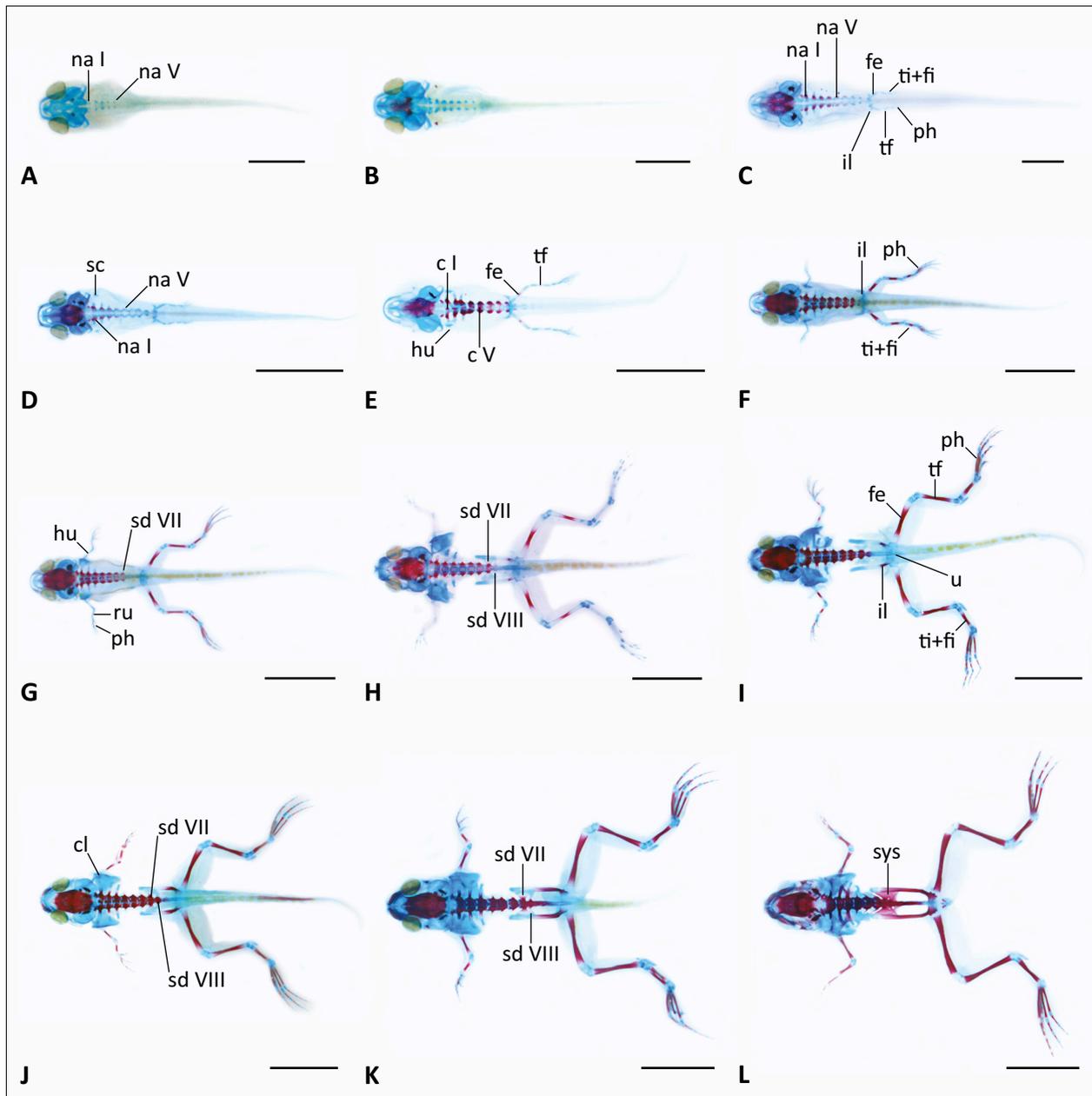
*Stage 66.* The columella (2/4 tadpoles) appears as a sturdy, nearly horizontally oriented, rod-like bone, ventrolateral to the otic capsule, posterior to the squamosal and dorsal to the quadratum. Within the otic capsule, there are ossifications along the semicircular canals.

## Development and ossification of the postcranial skeleton

*Axial skeleton.* At stage 52, the postcranial skeleton consists of five (1/4 tadpoles) to seven (3/4) pairs of cartilaginous neural arches (Fig. 3A). By stage 53, up to 9 pairs of neural arches are present and in the most advanced specimens, the neural arches are more elongated dorsally and possess a posteriorly pointed dorsal process. At the same stage (1/10 tadpoles) ossification of the vertebral column starts with the lateral ossification of neural arches I–III (Fig. 3B). At stage 54 the cartilaginous hypochord appears ventrally of the notochord, at the level of neural arch X. Ossification continues with the lateral ossification of neural arches IV–V (5/6 tadpoles) at the same stage. At stage 55, neural arches VI–VII (2/4 tadpoles) and centrum I (1/4 tadpoles) started to ossify (Fig. 3C). At stage 56 (Fig. 3D) the ossification of the transverse processes had started, with transverse processes II–III ossified in one tadpole and transverse process II–IV os-

sified in the other of the two available tadpoles of this stage. One stage 56 tadpole further showed median ossification of the vertebral centra II–IV. By stage 57 the vertebral centra V–IX (2/2 tadpoles) had started to ossify (Fig. 3E). A cartilaginous sacral diapophysis is present at vertebra VIII at this stage. Ossification of the vertebral centrum VIII (3/3 tadpoles) was first apparent at stage 58 (Fig. 3F). At stage 58, the cartilaginous vertebra X started to degenerate (3/3 tadpoles). By stage 59, the sacral diapophyses of vertebra VII have become broadly triangular (Fig. 3G). A simple, rod-shaped cartilaginous transverse process is present on vertebra VIII. By stage 61 the transverse processes V–VI (1/2 tadpoles) started to ossify and the ossification of the sacral diapophysis (1/2 tadpoles) started at vertebra VII (Fig. 3H). In dorsal view, the transverse processes V–VI are slanted anteriorly, whereas the transverse processes I–IV project laterally at a nearly right angle. At stage 62, the centra of vertebra VII and VIII are fused, as are the transverse processes of these at their lateral margins (Fig. 3I). At the same stage, the hypochord (2/2 tadpoles) started to ossify ventrally, where it is already fused to vertebrae VIII and IX (1/2 tadpoles). At this stage, vertebra IX is only recognizable as a slightly thicker, more ossified area of the urostyle.

*Girdles and limbs.* The first chondrifications of girdle and limb elements are present in a tadpole of stage 53 (1/10 tadpoles), which shows faintly stained ilium, femur, and zygopodial elements, with the femur being the largest and most strongly stained. By stage 54, ilium, femur, tibia and fibula are present in all tadpoles (6/6) and in most (5/6) the first chondrifications of the autopodial elements tibiale and fibulare are present. At stage 55 (Fig. 3 C), metatarsal elements in some (3/4) or all digits are present in all specimens (4/4). The first pectoral elements (scapula, coracoid, procoracoid) and humerus are furthermore faintly chondrified and visible in some tadpoles (2/4) of the same stage. These first anlagen of pectoral elements appear laterally to, and between, the transverse processes of vertebrae I and II. The ossification of the hindlimbs started at stage 57 with the simultaneous onset of ossification of femur (2/2 tadpoles), tibiofibula (2/2 tadpoles), tibiale (2/2 tadpoles), and fibulare (1/2 tadpoles; Fig. 3E). By stage 58, metatarsals (3/3 tadpoles) and the proximal tarsal phalanges II–III (3/3 tadpoles) started to ossify (Fig. 3F). At the same stage, the ilium (3/3 tadpoles) started to ossify as the first pelvic element. Humerus (1/4 tadpoles) and radioulna (1/4 tadpoles) started to ossify at stage 59 (Fig. 3G). At the same stage, ossification of the metacarpals (1/4 tadpoles) and the carpal phalanges (1/4 tadpoles) started. By stage 63 the distal tarsal sesamoid bone (2/4 tadpoles) ossified (Fig. 3J). At the same stage, ossification of the pectoral girdle commenced with the simultaneous ossification of coracoid (4/4 tadpoles), clavícula (3/4 tadpoles), and cleithrum (2/4 tadpoles). At stage 64, the ischium (1/6 tadpoles) started to ossify, and by stage 66 all postcranial ossifications were present (Fig. 3L).



**Fig. 3.** Postcranial skeletal development in *Pseudhymenochirus merlini*. (A) NF stage 52, (B) NF stage 53, (C) NF stage 55, (D) NF stage 56, (E) NF stage 57, (F) NF stage 58, (G) NF stage 59, (H) NF stage 61, (I) NF stage 62, (J) NF stage 63, (K) NF stage 64, (L) NF stage 66, all in dorsal view. c I – centrum of vertebra I, c V – centrum of vertebra V, cl – cleithrum, hu – humerus, il – ilium, fe – femur, na I – neural arch of vertebra I, na V, neural arch of vertebra V, ph – phalanges, ru – radioulna, sc – scapula, sd VII – sacral diapophysis of vertebra VII, sd VIII – sacral diapophysis of vertebra VIII, sys – synsacrum, ti+fi – tibiale and fibulare, tf – tibiofibula, u – urostyle. Scale bars equal 2 mm in A–C and 5 mm in D–L.

## Discussion

*Pseudhymenochirus merlini* shares a number of characters with species of its sister taxon *Hymenochirus* (CANNATELLA & TRUEB, 1988a; IRISARRI *et al.*, 2011). Both taxa are characterized by a unique, derived tadpole that actively hunts prey items and ingests them via suction feeding from a very small size on (LAMOTTE, 1963; CANNATELLA & TRUEB, 1988a, b; KUNZ, 2003). Tadpoles as small as 2 mm in body length are already actively suction-feeding,

and in their early free-swimming stage, hymenochirine tadpoles are among the smallest free-swimming vertebrates and probably the smallest predatory suction feeders (DEBAN & OLSON, 2002). Tadpoles of both taxa are further unusual in retaining external gills throughout most of their larval life until about NF 59 (SOKOL, 1962; LAMOTTE, 1963; this study), which seems to be unique among free-living anuran tadpoles (ALTIG & McDIARMID, 1999). The persistence of the external gills is probably related to the peculiar feeding mode of hymenochirines and the absence of internal gills and opercular folds,

which corresponds with a highly modified hyobranchial skeleton that does not seem to have a respiratory function. One difference in external morphology is the shape of the unpaired cement gland. This gland produces a sticky mucous that hatchlings use to attach themselves to vegetation or other objects. It starts to degenerate once the tadpoles become free-swimming and start to feed actively, and is usually completely reduced by the time hind limb buds appear (SOKOL, 1962). In *P. merlini* tadpoles, the cement gland is more disc-like compared to *H. boettgeri* (Tornier, 1896) and *H. curtipes* (Noble, 1926), where it is more crescent-shaped. A further difference between tadpoles of both genera is the presence of a prominent pre-cloacal part of the tail fin in *P. merlini* (SOKOL, 1962; LAMOTTE, 1963), which is also found in tadpoles of *Pipa* and *Xenopus* (SOKOL, 1977).

At the skeletal level, *P. merlini* shows a number of typical pipoid chondrocranial characters, such as a broad commissura quadratocranialis and a relatively weakly developed processus muscularis of the palatoquadrate, but is otherwise most similar to *Hymenochirus* (SOKOL, 1977; CANNATELLA & TRUEB, 1988a, b; TRUEB & HANKEN, 1992). In tadpoles of both *Pseudhymenochirus* and *Hymenochirus*, suction-feeding is mediated through a tube-like mouth that is mainly supported by Meckel's cartilage, which has an upwards-pointing orientation in rest, and a large but simplified hyobranchial apparatus (SOKOL, 1962; this study). A hyobranchial filter apparatus is missing in both taxa (SOKOL, 1962, 1969; this study). Another similar feature is the presence of small, paired, rod-like suprarostrals cartilages anterolaterally to the ethmoid region. Except hymenochirines, all pipids and its sister taxon, *Rhinophrynus dorsalis* Dumeril & Bibron, 1841, lack separate suprarostrals cartilages (SOKOL, 1977; SWART & DE SÁ, 1999) and DE SÁ & SWART (1999) considered the presence of free suprarostrals a derived character for hymenochirine pipids.

At the level of ossification sequence, however, there are some differences between *Pseudhymenochirus* and *Hymenochirus* (Table 2). OLSON (1998) described the development of the entire skeleton and DE SÁ & SWART (1999) of the cranial skeleton of *H. boettgeri*, and the available data show some differences in the order and timing of the appearance of cranial elements compared to *P. merlini*. As in most anurans (HANKEN & HALL, 1988), the first three cranial bones to ossify in *H. boettgeri* and *P. merlini* are the frontoparietal, exoccipital and parasphenoid, together with the angulosplenial (TRUEB & ALBERCH, 1985; OLSON, 1998). DE SÁ & SWART (1999) reported a more pronounced developmental gap between the appearance of the exoccipital and the parasphenoid than OLSON (1998) or seen in *P. merlini* (Table 2). Frontoparietal, parasphenoid and exoccipital surround and protect the brain (TRUEB & ALBERCH, 1985) and some authors (DE SÁ, 1988; ADRIAENS & VERRAES, 1998; OLSON, 1998) have stressed the role of the parasphenoid in protecting the brain during suction feeding and its resulting early ossification. While this seems plausible, the presence of a parasphenoid does not seem to be a prerequisite

for effective suction feeding as young tadpoles of *P. merlini* and *Hymenochirus* hunt and ingest prey within two to three days after hatching, well before any parasphenoid ossification occurs.

There are further differences in the timing of ossification between the two species, especially of the maxilla, dentary and squamosal, which ossify earlier in *P. merlini* than in *H. boettgeri*. In contrast, the prootic, the ceratohyal and nasal ossify much earlier in *H. boettgeri* than in *P. merlini*. However, we did not observe the formation (and fusion) of a nasal in the material available to us. Specimens at NF stage 66 show a reticulated ossification at the anterior edge of the frontoparietal in a position corresponding roughly to the posterior part of the nasal in *H. boettgeri* (CANNATELLA & TRUEB, 1988a). A nasal might not actually be present as an independent ossification, but investigation of more postmetamorphic material is necessary to confirm this. Most of the postcranial bones – except for the neural arches, tibiofibula, tibial and fibular – also ossify at earlier stages in *H. boettgeri* than in *P. merlini* (OLSON, 1998). However, the two studies of skeletal development in *H. boettgeri* are somewhat difficult to compare because OLSON (1998) only had an incomplete series (her oldest specimens available were at NF stage 63), whereas DE SÁ & SWART (1999) recorded a broad range of variation in the first appearance of elements.

A feature of *P. merlini* as well as *Hymenochirus* (OLSON, 1998) is the ossification of the ceratohyal during metamorphosis, whereas this element remains cartilaginous in other pipids. The ceratohyal plays an important role in suction feeding in the dwarf clawed frogs (SOKOL, 1962, 1969; OLSON, 1998) and its ossification possibly provides more stability and a more rigid attachment site for the associated musculature. All postcranial elements appear during premetamorphosis and metamorphosis in *P. merlini*. In addition, several postcranial sesamoid elements are present mostly in the area of a joint or embedded within a tendon (OLSON, 2000). These elements likely act as spacers for the tendon to increase the lever arm or to prevent pressure damage to the tendon in its course across the joint (NUSSBAUM, 1982; PONSSA *et al.*, 2010). In *P. merlini*, there is a comparatively large number of sesamoid bones, and the high number of sesamoids (though not necessarily homologous in all cases) is thought to be a characteristic of pipids (OLSON, 2000). The power-lever of the sesamoid bones presumably provide an increased power transmission during swimming in *P. merlini*, but are also thought to provide an advantage at digging and jumping, at least in other anurans (NUSSBAUM, 1982). While *P. merlini* make only relatively uncoordinated jumps on land and are not known to dig, individuals are able to live semi-aquatic or even terrestrially in the short term and are known to move around between waterbodies to survive the dry season (KUNZ, 2003).

Among the most remarkable features of *Pseudhymenochirus* and *Hymenochirus* is the reduction of the vertebral column to just six presacral vertebrae (CANNATELLA & TRUEB, 1988a, b; Fig. 4), resulting in one of the

**Table 1.** Ossification sequence of *Pseudhymenochirus merlini* according to the stage at which they first appear; stages are based on NIEUWKOOP & FABER (1967). Light grey boxes and numbers indicate onset of ossification in some of the specimens of that stage. Dark grey boxes indicate the presence of ossification in all specimens.

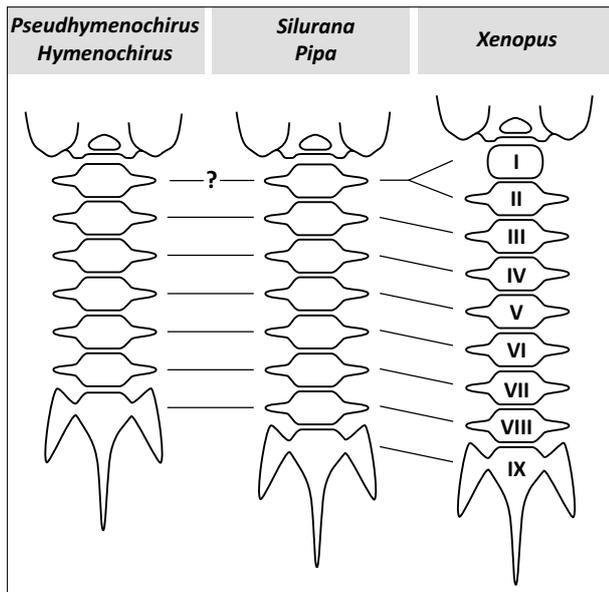
	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
frontoparietal															
exoccipital			2 (6)												
angulosphenial															
parasphenoid				2 (4)											
dentary															
squamosal															
sphenethmoid									2 (4)						
maxilla								3 (4)	3 (4)						
pterygoid											1 (2)				
premaxilla												3 (4)			
septomaxilla												3 (4)			
ceratohyal													2 (6)		
quadrate														4 (6)	
columella															2 (4)
nasal															
prootic															
neural arch I – III		1 (3)													
neural arch IV – V			5 (6)												
neural arch VI – VII				2 (4)											
neural arch VIII															
centrum I – IV				1 (4)											
centrum V – VII															
centrum VIII															
centrum IX															
hypochord/urostyle															
transverse process I – II															
transverse process III – IV				1 (2)											
transverse process V – VI											1 (2)				
transverse process VII – VIII															
femur															
tibiofibula															
tibiale															
fibulare								1 (2)							
metatarsals															
tarsal phalanges															
ilium															
ischium														1 (4)	
humerus									1 (4)						
radioulna									1 (4)						
metacarpals									1 (4)						
carpal phalanges									1 (4)						
coracoid															
clavicula												3 (4)			
cleithrum												2 (4)			
scapula															

shortest known vertebral columns in anurans and vertebrates in general (HANDRIGAN & WASSERSUG, 2007). Most non-pipid anurans have eight presacral vertebrae and this condition is also found in *X. laevis* and presumably other species of *Xenopus*, whereas (adult) *Silurana* and *Pipa*

have seven presacral vertebrae (TRUEB & HANKEN, 1992; DUELLMAN & TRUEB, 1994). Within pipids, the first (atlas) and second presacral vertebrae appear to be fused in all genera except *Xenopus* (CANNATELLA & TRUEB, 1988b). This fusion happens after metamorphosis in *X. (S.) tro-*

**Table 2.** Cranial ossification sequences of Pipidae. Elements are listed according to the stage at which they first appear; stages are based on NIEUWKOOP & FABER (1967). Note that DE SÁ & SWART (1999) and TRUEB & HANKEN (1992) provided ranges for the appearance of ossifications, but elements are listed here at their first appearance.

Stage NF	<i>Pseudhymenochirus merlini</i> this work	<i>Hymenochirus boettgeri</i> OLSON (1998)	<i>Hymenochirus boettgeri</i> DE SÁ & SWART (1999)	<i>Xenopus laevis</i> BERNASCONI (1951)	<i>Xenopus laevis</i> SEDRA & MICHAEL (1957)	<i>Xenopus laevis</i> TRUEB & HANKEN (1992)
49			frontoparietal, angulosplenia			
50			exoccipital			
51		frontoparietal	—			
52		exoccipital	—			
53	frontoparietal	parasphenoid	—	frontoparietal	—	frontoparietal
54	exoccipital, angulosplenia, parasphenoid	prootic	—	frontoparietal	—	frontoparietal
55		—	—	parasphenoid, exoccipital, angulosplenia (medial), maxilla, premaxilla	frontoparietal, parasphenoid	parasphenoid, exoccipital, prootic
56	—	—	parasphenoid	prootic	—	angulosplenia (medial)
57	—	—	—	—	—	maxilla
58	—	angulosplenia	—	nasal	prootic, occipital, maxilla, nasal	premaxilla, nasal
59	maxilla	—	—	dentary	—	—
60	dentary, squamosal, sphenethmoid	—	—	angulosplenia (lateral), septomaxilla, squamosal, pterygoid	septomaxilla, premaxilla, dentary, angulosplenia (lateral and medial)	septomaxilla, dentary, angulosplenia (lateral)
61	—	—	maxilla	columella	—	pterygoid
62	pterygoid	—	—	vomer	—	squamosal
63	septomaxilla, premaxilla	maxilla, nasal, dentary	nasal, sphenethmoid	—	pterygoid	vomer
64	—	—	—	—	squamosal	sphenethmoid
65	quadrate	—	squamosal, prootic, dentary, pterygoid	—	—	pars articularis
66	columella	—	septomaxilla, premaxilla, columella	—	sphenethmoid	—
66 +	nasal(?), prootic	—	—	sphenethmoid, pars articularis, alary cartilage, planum antorbitale	—	operculum



**Fig. 4.** Schematic comparison of the vertebral column in Pipidae. Data based on CANNATELLA & TRUEB (1988a, b), TRUEB *et al.* (2000), TRUEB & HANKEN (1992), and own observations.

*picalis* (HM pers. obs.) and can be observed during ontogeny. In *Pipa*, *Pseudhymenochirus* and *Hymenochirus* evidence for this fusion is the presence of a transverse process and spinal foramen on the first vertebra, which is overall similar in shape to the first vertebra (fused atlas plus second vertebra) in adult *Silurana* (CANNATELLA & TRUEB, 1988a, b; TRUEB *et al.*, 2000; Fig. 4). In their study of *Pipa pipa* (Linnaeus, 1758), TRUEB *et al.* (2000) did not observe this fusion but the smallest specimen available to them already had a single cartilaginous first vertebra with a transverse process and spinal foramen. In our ontogenetic series of *P. merlini* the youngest specimens available (NF 52) already have small cartilaginous neural arches and we also did not observe a subsequent fusion of the first and second vertebra. The first pair of neural arches does not differ markedly in size or shape from the following arches and transverse processes form at the first vertebra shortly afterwards. This seems to suggest that fusion either occurs at an even earlier, likely prechondrogenic stage, or an overall novel morphology of the atlas in *P. merlini*, but further studies at the somitic level would be necessary to confirm this.

*Pseudhymenochirus* and *Hymenochirus* differ from *Pipa* and *Silurana* by having further reduced the number of presacral vertebrae to six (Fig. 4). In some *Pipa*, specimens with just six presacral vertebrae have been documented and this seems to be the result of the incorporation of the seventh (and usually last) presacral vertebra into the synsacrum, which is formed by the eighth and ninth vertebra (TRUEB, 1984; TRUEB *et al.*, 2000; for simplicity, we count the first vertebra as just one and not two). However, in *P. merlini*, the articulation with the ilium is formed by the seventh and eighth vertebrae, with the seventh vertebra making by far the largest contribution to the very broadly triangular transverse process of

the synsacrum. Compared to other pipids, it seems that the articulation of the pelvic girdle with the vertebral column has been shifted forward by at least one vertebra in hymenochirine pipids. The formation of a synsacrum comprising the sacral vertebra, additional postsacral vertebrae, and the urostyle, combined with very broad transverse processes as found in pipids, results in a loss of movement between the sacral complex and the urostyle. This is thought to provide increased stability and better force transmission during swimming (PUGENER & MAGLIA, 2009). The reduced number of free presacral vertebrae in *Pipa*, *Pseudhymenochirus* and *Hymenochirus* would seem to further restrict mobility within the axial skeleton and probably increase stability during swimming.

*Pseudhymenochirus merlini* and *Hymenochirus* share several skeletal characters commonly associated with either miniaturization or paedomorphosis (or both) in anurans and other amphibians (HANKEN, 1985; HANKEN & WAKE, 1993; YEH, 2002; THESKA *et al.*, 2019). Among these are several instances of bone fusion and hypersification (e.g. vertebral fusion, fusion of various carpal elements, fusion of nasals and frontoparietals, fusion of endocranial elements), loss of elements (e.g. ceratobranchials III and IV), morphological novelty (e.g. upward-shifted lower jaw), and appearance of ossifications shifted into the postmetamorphic period (e.g. nasal(?)). Because of the presence of these traits, hymenochirines are commonly considered to be miniaturized (e.g. DE SÁ & SWART, 1999). Intuitively, this would seem to make sense given the extremely small tadpole size and the rather small adult size compared to most other pipids. However, as a result of metamorphosis, tadpole and adult anuran morphology are largely decoupled (SHERRATT *et al.*, 2017; WOLLENBERG *et al.*, 2017), meaning that tadpole morphology might not necessarily constrain adult morphology. Furthermore, *P. merlini* and *Hymenochirus* are not unusually small at metamorphosis and attain adult sizes considerably larger than species commonly considered to be miniaturized (CLARKE, 1996; SCHERZ *et al.*, 2019). Moreover, some ossifications missing in hymenochirines, like vomer, quadratojugal and palatinum are missing in all pipids too, except *Xenopus*, and are apparently not absent as a result of a reduction in body size (CANNATELLA & TRUEB, 1988a, b). On balance, most of the peculiar skeletal characteristics seen in hymenochirines appear to be correlated with either their peculiar larval morphology (e.g. loss of ceratobranchials III and IV) or a result of paedomorphic changes rather than miniaturization as commonly understood.

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