VERTEBRATE ZOOLOGY

65 (1): 81–99
4.5.2015
SENCKENBERG

The "tooth systems" of *Lissotriton vulgaris* (Amphibia: Urodela) with special regard to delayed metamorphosis

Hartmut Greven [1], Thomas van de Kamp [2], Tomy dos Santos Rolo [2], Tilo Baumbach [2] & Günter Clemen [3]

[1] Department of Biology of the Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany (corresponding author); grevenh(at)uni-duesseldorf.de — [2] Karlsruhe Institute of Technology (KIT), ANKA /Institute for Photon Science and Synchroton Radiation (IPS), Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany; thomas.vandekamp(at)kit.edu — [3] Doornbeckeweg 17, 48161 Münster, Germany; gclemen(at)web.de

Accepted 20.iii.2015.
Published online at www.senckenberg.de / vertebrate-zoology on 4.v.2015.

Abstract

A study on the cranial morphology, especially on the tooth bearing (dental) systems of several preserved developmental stages (from early premetamorphic larvae, in which most skull elements were already present and ossified or ossified in part, to transformed adult) of the smooth newt *Lissotriton vulgaris* (Salamandridae) was undertaken. We used µCT (to visualize the ossified elements in general and their relationships to each other) and an overall Alizarinred staining (to at best visualize teeth, replacement teeth, tooth buds and, after removing the oral mucosa, the course of dental laminae). Specimens cleared and stained with Alizarinred and Alcianblue were shown to be less suitable for our questions. In one case we used histological sections to follow the course of dental laminae, and in a second case scanning electron microscopy to show the structure of teeth in detail. The general sequence, growth, and changes of the bony elements including the "dental systems", especially around metamorphosis, known from several other salamandrids are largely confirmed. Concerning the "tooth systems", metamorphic events include the late appearance of the maxillae, resorption of the coronoids and palatines including their tooth-patches, remodelling of the vomer, i.e. resorption of the vomerine larval tooth-patch, formation of the edentate vomerine plate, and outgrowth of the monstichously dentate vomerine bar (typical for salamandrids). We show evidence that the larval vomer is not completey resorbed and that, unlike what has been described for *Salamandra salamandra*, the development of the vomerine bar is probably preceded by a shift of the dental lamina towards the middle of the palate, leaving a broad area between larval vomer and dental lamina. We hypothesize that the connective tissue in this area ossifies later and extends posteriorly forming the vomerine bar. It is noteworthy that in nearly all larvae vomer and intact pterygopalatinum were very close together either on one side or on both sides leading in overwintered larvae to the fusion of the vomer and the palatinal portion of the pterygopalatinum, primarily on one side. The zone of fusion is always characterized by a buccal notch. We think that in *L. vulgaris* the formation of "vomeropterygopalatina" is supported by the close proximity of the two bones and that these bones may fuse due to an imbalance between differentiation- and growth rate (indirectly caused by low temperatures). Approximation and especially fusion of the two bones correspond with the extension of the vomerine dental lamina into the area of the palatine, which temporally provides the latter with teeth. Overwintered larvae show further deviations concerning growth and differentiation of the mouth roof, which can be also interpreted as signs of delayed metamorphosis. They retain, for example, a largely intact dentate palatine, but with some regression of its tooth-patch, while the larval vomer is enlarged anteriorly and posteriorly and its number of teeth has increased; and the largely intact pterygopalatinal bony bridge. Further, maxillae begin to ossify. All larvae obviously have reached a late premetamorphic larval stage before the delay has started.

Kurzfassung

Wir haben die Schädel, insbesondere die „bezahnten Systeme“ einiger konservierter Entwicklungsstadien (frühe Larve, in der bereits die meisten Schädelelemente zumindest zum Teil verknöchert vorhanden waren, bis metamorphosierter Adultus) des Teichmolchs *Lissotriton vulgaris* (Salamandridae) mit Hilfe der µCT (um die Beziehungen der ossifizierten Elemente zueinander zu dokumentieren) und Alizarinrot gefärbter Präparate (die im günstigsten Fall Zähne, Ersatzzähne, Zahnkeime und den Verlauf der Zahnleisten erkennen lassen) untersucht. Aufgehellte und mit Alcianblau/Alizarinrot gefärbte Präparate erwiesen sich für unsere Fragestellung als weniger geeignet. Zudem haben wir eine Serie histologischer Schnitte ausgewertet, um den Verlauf der Zahnleisten zu verfolgen, und in einem Fall die Zähne anhand rasterelektronenmikroskopischer Aufnahmen genauer dargestellt. Hinsichtlich der knöchernen Elemente und der „bezahnten Systeme“ können die für Salamandriden im Wesentlichen bekannten Veränderungen, vor allem im Umfeld der Metamorphose, weitgehend indirekt oder direkt bestätigt werden. Dies sind die späte Bildung der Maxillaria, die Resorption der Coronoide und Palatina einschließlich ihrer Zahnfelder, der Umbau des Vomers, d. h. die Resorption der larvalen Vomerzahnfelder, Bildung der zahnfreien Vomerplatten sowie das Auswachsen einzeilig bezahnter Vomerspangen nach posterior (typisch für alle Salamandriden). Wir haben Hinweise, dass der Larvenvomer nicht vollständig resorbiert wird und dass, anders als z. B. bei *Salamandra salamandra*, der Vomerspangenbildung eine Verlagerung der larvalen Vomerzahnleiste zur Mitte des Gaumens vorausgeht, und so ein breites Areal zwischen verlagerter Zahnleiste und larvalem Vomer entsteht, das sehr wahrscheinlich später ossifiziert und sich posterior zur Vomerspange verlängert. Bemerkenswert ist, dass bei fast alle Larven entweder auf einer Seite oder auf beiden Seiten des Gaumens Vomer und intaktes Pterygopalatinum stark angenähert waren. Dies führte vor allem bei den Laven, die überwintert haben, zu einer Verschmelzung des Vomers mit dem palatinalen Anteil des Pterygopalatinum meist auf nur einer Seite des Gaumens. Die Verwachsungsstelle ist stets durch eine buccal liegende Kerbe gekennzeichnet. Wir gehen davon aus, dass bei *L. vulgaris* die Bildung solcher „Vomeropterygopalatina“ durch die Nähe der beiden Knochen begünstigt wird, diese Knochen aufgrund eines Ungleichgewichtes zwischen Differenzierungs- und Wachstumsgeschwindigkeit fusionieren (u. a. indirekt hervorgerufen durch niedrige Temperaturen), dass damit auch eine Verlängerung der vomeralen Zahnleiste bis in die Palatinalregion einhergeht, die letztere dann vorübergehend auch mit Zähnen bestückt. Larven, die überwintert haben, zeigen noch weitere Abweichungen im Wachstum und Differenzierungsgrad des Munddaches, die ebenfalls als Folgen einer verzögerten Metamorphose gedeutet werden können. Dies sind u. a. die Beibehaltung eines noch weitgehend intakten und bezahnten Palatinum, dessen Zahnfeld jedoch im Abbau begriffen ist, während der larvale Vomer deutlich vorn und hinten vergrößert und die Anzahl seiner Zähne erhöht sind, Vorhandensein einer weitgehend intakten pterygopalatinalen Knochenbrücke sowie Beginn der Ossifizierung der Maxillaria. Alle Larven hatten offenbar ein relativ spätes Praemetamorphose-Stadium erreicht, als die Verzögerung einsetzte.

Key words

Urodela, *Lissotriton*, “tooth systems”, “vomeropterygopalatinum”, delay of metamorphosis; overwintering.

Introduction

In Urodela (= Caudata) the most extensive metamorphic changes are seen in the skull. Many of these processes are mediated by thyroid hormones (TH). Generally, the temporal sequence of these changes is attributed to differential TH-sensitivity of the tissues involved and their responding at different concentrations. Dependence on and sensitivity to these hormones, however, may vary during ontogeny and between taxa, e.g. plethodontids and nonplethodontids (Rose, 1999). Considerable metamorphic changes affect the hyobranchial skeleton and the palate, namely the vomer and palatine, as well as in part the jaws (for review and further literature see Stadtmüller 1936; Reilly, 1986, 1987; Trueb, 1993; Rose & Reiss, 1993; Rose, 1995 a–c, 1999, 2003; Smirnov & Vassilieva, 2003, 2005; Lebedkina, 2004; Smirnov *et al*., 2011).

Jaws, vomers and palatines bear teeth; together with the accompanying dental laminae (tooth producing tissue) they form several “tooth or dental systems”. In a “typical” premetamorphic urodele larva nine systems can be distinguished: (1) the upper jaw arcade (premaxillae fused or not, paired maxillae) with a single continuous dental lamina following the lingual face of the arcade; (2–5) the lower jaw (paired dentaries and paired coronoids), in which each bone is accompanied by its own dental lamina, and (6–9) the palate (paired vomeres and paired palatines fused with the edentate pterygoids), in which also each bone has their own dental lamina clearly separated from each other. In fully transformed specimens the number of these systems is reduced as the coronoid- and palatine-systems are no longer present (see Clemen & Greven, 1994, and literature cited above).

Around metamorphosis the coronoids are reabsorbed, maxillae may develop, and the palate becomes remodelled. Remodelling includes resorption of the palatinal portion of the pterygopalatinum, an anterolateral expansion of the vomer and (in some taxa) a posterior expansion of the vomer (see literature cited above). Further, arrangement of teeth may change from polystichy (tooth patches) to monostichy (single row of teeth) on certain bones, and the typically conical, monocuspid and non-pedicellate teeth of larvae are replaced by pedicellate teeth with apices composed of a lingual and labial cusp (bicuspid teeth) or with otherwise modified crowns (e.g., Greven, 1989; Beneski & Larsen, 1989; Clemen & Greven 1994; Davit-Béal *et al*., 2006, 2007).

Appearance of maxillae, resorption of coronoids, remodelling of the palate (e.g. Rose, 1999; 2003; Smirnov & Vassilieva, 2003; Lebedkina, 2004), and the transformation of teeth (see Gabrion & Chibon, 1973; Greven & Clemen, 1994; Smirnov & Vassilieva, 2003) are TH-inducible. Whether this TH-inducibility also applies to the progressive development of the annular zone that typically divides the transformed tooth in a crown and a pedicel (pedicellate condition) is questionable (Greven & Clemen, 1990; see also the discussion in Smirnov & Vassilieva, 2003).

Naturally, TH-inducible events are highly sensitive to malfunctions of the thyroid. Regarding the skull or some of its elements, this has been repeatedly demonstrated in larvae either treated with thiourea, a goitrogen that inhibits the thyroxine synthesis, to stop or slow down development and metamorphosis, or with thyroxine to accelerate these processes (e.g. Rose 1995 a,b,c; Smirnov & Vassilieva, 2001, 2003; Smirnov *et al*., 2011), in hypophysectomized larvae (Clemen, 1978 a; Greven &

Table 1. Various parameters of the developmental stages of *Lissotriton vulgaris*-specimens used for the present study. Minus and plus signs indicate a slightly less or a slightly further development of the respective stage.

No.	date of collection	TL and SC (cm)	Stage*	skull	preparation	figures	remarks
1	25.07.1975	TL: 1.9 SV: 1.2	I–	complete	µCT, ventral view (Alizarin)	Fig. 1 A–D, 4 A	teeth monocuspid
2	30.07.1975	TL: 2.7 SV: 1.6	I+	complete	µCT	Fig. 1 E–F	teeth monocuspid
3	22.07.1975	TL: 2.3 SV: 1.5	II	complete	µCT, cleared and stained	Fig. 1 G–H, 4 B	teeth monocuspid
4	02.08.1975	TL: 3.9 SV: 2.1	IIIa–IV	complete	µCT, ventral view (Alizarin); partial removal of mucosa	Fig. 2 A–C, 4 C, D	teeth monocuspid; gill remnants, anlage of secondary tongue; lip fringes
5	04.09.1975	TL: 3.8 SC: 2.3	V+	complete	µCT, ventral view (Alizarin)	Fig. 2 D–F, 4 E, F	largely bicuspid teeth on the vomer; monocuspids on the jaws
6	04.04.1978	TL: 4.5 SV: 2.4	IIIa	complete	serial sections	Fig. 5 A–G	teeth monocuspid; short gills, anlage of the secondary tongue, overwinterer
7	19.04.1977	TL: 3.9 SV: 1.9	IIIb		single bones cleared and stained in 2013	Fig. 4 G–I	teeth monocuspid
8	13.04.1975	TL: 3.2 SV: 1.9	IIIb	complete	µCT, ventral view (Alizarin); partial removal of mucosa	Fig.3 A–C, 4 J	teeth monocuspid; overwinterer
9	25.04.1975	TL: 4.0 SV: 1.9	IIIb	without lower jaw	ventral view (Alizarin); partial removal of mucosa	Fig. 4 K	teeth monocuspid; overwinterer
10	25.05.1977	TL: 6.7 SV: 3.7	VII (adult)	complete	µCT	Fig. 3 D–F	teeth bicuspid

* according to Clemen & Greven (2013)

Clemen, 1990), or in larvae simply kept cold (Clemen, 1978 a).

Temperature is an important environmental factor that influences growth rates, timing of metamorphosis, and size at transformation in amphibians (summarized in Wells, 2007). Low temperatures affect the activity of the thyroid either directly or indirectly and are known to retard or inhibit metamorphosis (see Eggert 1934; Moriya, 1983 a, b; Iwasaki & Wakahara, 1999). Larvae of Urodela exposed to cold temperatures slow down or even may stop further development remaining in an arrested stage, but typically continue to grow albeit at a slow rate (summarized in Wells, 2007; see also Bizer, 1978; Petranka 1984; Bechy, 1995; Iwasaki & Wakahara, 1999).

This phenomenon is long-known from overwintering larval urodeles, which often are considerably larger than larvae that metamorphose in the same year the eggs have been spawned (e.g., *Hynobius boulengeri*: Nishikawa & Masafumi, 2008; *Hynobius retardatus*: Iwasaki & Wakahara, 1999; Kanki & Wakahara, 2001; *Ichthyosaura alpestris*: Eggert 1934; several "*Triturus*" spp. summarized in Günther, 1996). To characterize those larvae mostly external morphological changes such as size, degree of gill resorption, and the date of capture were used.

However, external traits are not sufficiently marked to substantiate finer graded differences as "internal" traits may do. Previously we showed that urodele larva captured in the field and identified as overwinterers revealed traits, primarily in the palate, which deviated from normal development resembling those of larvae kept cold. Conversely, larvae of unknown history from the field showing such deviations were classified as larvae with a delayed metamorphosis (Clemen & Greven, 1979, 2013; Jömann *et al*., 2005; Greven *et al*., 2006).

In many populations of the smooth newt *Lissotriton vulgaris* (formerly *Triturus vulgaris*), a common species found throughout most of continental Europe, overwintering of larvae is a well-known phenomenon (see literature cited above). In a previous article focusing on dentition and the course of dental laminae in normal developing larvae, in overwintered larvae and in adults from a natural population of this species, we have already touched the phenomenon of delayed development and metamorphosis (Clemen & Greven, 1979). When checking, however, again this and further material from that time, we decided to use it once more (1) to broaden our previous study, (2) to compare data obtained with the non-invasive µCT and in-toto- preparations stained with Alizarinred, and (3) to add and discuss some unusual traits found in several specimens not considered previously, which appear closely related to delayed development and overwintering.

Material and methods

The samples of *Lissotriton vulgaris* studied herein originated from populations located near Münster, Germany. They were collected in 1975 and 1978 and most have been used in our previous study (see Clemen & Greven, 1979). Specimens were killed by an overdose of MS 222 (Fa. Sandoz), fixed for some days in buffered formalin according to Lillie (see Romeis, 1968) and have since been stored in 70% and 95% ethanol. A few specimens remained for several months in the fixative and were then stored in ethanol. Further, we examined a series of histological sections of a specimen fixed in Bouin at the time of collection (4.4.1978), embedded in Paraplast, sectioned at 7 µm and stained with Azan Heidenhain, Bodian, and hemalum-chromotope (Romeis, 1968).

Finally, the series used for the present study consisted of ten specimens captured at different times and included seven complete skulls, two skulls without lower jaw and one complete skull serially sectioned, ranging from an early premetamorphic larva to an adult. Details regarding the external appearance of the specimens, date of collection and staging (according to Clemen & Greven, 2013; in some cases we used also the developmental status of the tongue; Greven *et al.*, 2013) are summarized in table 1, where specimens are numbered consecutively in Arabic numerals.

Synchrotron microtomography (µ CT)

Seven complete skulls were used for Synchrotron microtomography (see table 1). Scans were performed at the TOPO-TOMO beamline of the ANKA Synchrotron Radiation Facility located at Karlsruhe Institute of Technology (KIT), Germany.

The ethanol-fixed specimens were put into plastic tubes filled with 90% ethanol. The tomographic scans over 180° were taken with a filtered white beam with the spectral peak at about 15 keV. A scintillating screen, which converts X-rays to visible light, was coupled by a white beam macroscope (constructed by Elya Solutions, s. r. o.) to a 12 bit pco.dimax high speed camera. The optical magnification of 3 × led to an effective pixel size of 3.66 µm. 2.500 projections were recorded with an exposure time of 6 ms per frame, resulting in a scan duration of 15 seconds per tomogram.

Before reconstruction, the projections were processed with the phase retrieval ImageJ plugin ANKAphase (Weitkamp *et al.*, 2011). Volume reconstruction was done by the PyHST software developed at the European Synchrotron Radiation Facility in Grenoble, France, and KIT.

For visualization, the tomographic volumes were imported into Amira® (version 5.4.5; Visualization Sciences Group). Volume rendering of the 3D data was done using the *Volren* module of the software.

Resolution of the µCT and microscopic inspection of the Alizarinred preparations allowed distinguishing monocuspid and clearly bicuspid teeth. Incipient bicuspidity could not be recognized. Some details are only seen after rotation and high magnification of the CT images; they were not all pictured.

Staining with Alizarinred

After µCT specimens were stained by adding some crystals of Alizarinred to the ethanol, in which they were stored. Staining with Alizarinred (a few hours) allowed recognizing details such as tooth buds and replacement teeth at least in part. In a few cases we cautiously removed the oral mucosa with a fine forceps after staining with Alizarin to visualize the course of the dental laminae or at least the lamella of connective tissue (*tunica popria*) that borders the non-productive side of the dental laminae facing away from the bone. One larvae was cleared and stained with Alcianblue/Alizarinred (see Dingerkus & Uhler, 1977), and stored in glycerol. Photos were taken with a Keyence VHX 500F digital microscope.

Scanning electron microscopy

To have a closer look on the dentition, the isolated dentigerous bones (vomer, palatopterygoid) of larva 7 (see Tab. 1), previously stained with Alizarinred and stored in glycerine, were dehydrated in an ethanol series, treated with Hexamethyldisilazane (HMDS) (Rumph & Turner, 1998), sputtered with gold and viewed under the SEM Leo 1430 (Fa. Zeiss).

Abbreviations used in the figures

Abbreviations follow largely the Latin terms: ch = *choana* (choana); co = *coronoideum* (coronoid); con = *condylus occipitalis* (occipital condyle); d = *dentale* (dentary); ex = *exoccipitale* (exoccipital); ey = eye; f = *frontale* (frontal); m = *maxillare* (maxilla); Me = Meckel's cartilage; n = *nasale* (nasal); oc = *capsula otica* (otic capsule); os = *orbitosphenoideum* (orbitosphenoid); p = *parietale* (parietal); pa = *praearticulare* (prearticular); pdp = *processus dorsalis praemaxillaris* (*pars praenasalis*, *pars dorsalis*, *pars frontalis*, frontal spine, alary process of premaxilla); pfm = *pars* (if broad) (facial flange of maxilla) or *processus* (if small) *facialis maxillaris*; pf = *prefrontale* (prefrontal); pl = *palatinum* (palatine); pm = *praemaxillare* (premaxilla); po = *prooticum* (prootic); ps = *parasphenoideum* (parasphenoid); pt = *pterygoideum* (pterygoid); ptp = *pterygopalatinum* (palatopterygoid); q = *quadratum* (quadrate); rp = *ramus palatinus*; s = *squamosum* (squamosal); v = *vomer* (vomer); vb = vomerine bar. Anatomical terminology largely follows Trueb (1993) and Rose (2003).

Results

Descriptions of the skull of *Lissotriton vulgaris*, especially development and sequence of ossifications were published by Erdmann (1933) and by Smirnow & Vasiilieva (2003). Apart from some errors that were already commented by Smirnow & Vasiilieva (2003), Erdmann (1933) also takes the posterior extension of the vomer (vomerine bar) in transformed specimens for a "Zahnknochen", i.e. the fusion of the bases of teeth. Further drawings and descriptions of the *L.vulgaris*-skull are found in Marconi & Simonetta (1988), who examined neotenic specimens, and in Haller-Probst & Schleich (1994), who briefly comment on the skull of several transformed "*Triturus*" spp. In these articles the transformed vomer was taken for a vomeropalatinum, i.e. a fusion product of vomer and palatine (see also Stadtmüller, 1936). However, according to the current view the palatine of the majority of Urodela is completely resorbed during metamorphosis as postulated already by Wintrebert (1922) and does not contribute to the vomerine bar, which is formed *de novo* (see discussion). Some sketches of the skull are also depicted in Djorović & Kalezić (2000) and Ivanović *et al*. (2014).

In the present study the organisation of the skull is only used to check the overall progress of development, namely ossifications. Therefore we point under "general remarks" only to some conspicuous events recognized in the respective specimens. Then we focus on the dentigerous bones under the headings "upper jaw", "palate" and "lower jaw". The short "comments" will be deepened in the discussion. Specimens are consecutively numbered largely according to the months of catches.

Larva 1

(stage I-; "young larva") (Fig. 1 A–D, 4 A)

General remarks: Most skull elements are ossified or ossified in part. The paired parietals and frontals are narrow and separated by a large fontanelle. Parts of the exoccipitals are attached to the fully ossified occipital condyles; squamosals are elongated; the otic capsule is present (Fig. 1 A). Orbitosphenoids lie on either side of the parasphenoid and are ossified in part (Fig. 1 B). The parasphenoid articulates with the occipito-otic complex (here exoccipitals); quadrates ares partly ossified (Fig. 1 A, D). Pterygoids do not articulate with the quadrates and the squamosals (Fig. 1 D).

Upper jaw: The premaxilla is unipartite (unpaired), short and dentate; its pars dentalis bears a single row of teeth (Fig. 4 A).This also holds for all following larval stages The *pars palatina* along the lingual side of the *pars dentalis* (not pictured) is small. Maxillae are absent. Interiorly (this location applies for all dentigerous bones) to the row of functional premaxillary teeth replacement teeth (this term includes herein non-attached more or less fully developed teeth and tooth buds with mineralized tooth apices) are present; two replacement teeth each are at the posterior non-ossified ends of the praemaxilla (Fig. 4 A). Two separate dorsal alary processes on the front side of premaxilla (*processus dorsales praemaxillares*) extend beyond the anterior margins of the frontals exposing the fontanelle (Fig. 1 A, C).

Palate: The paired triangular vomeres lie anteromedially to the eye in the same plane with the palatines and ventrolateral to the anterior end of the parasphenoid. Each vomer bears a tooth-patch consisting of approximately 13 teeth and has 11 replacement teeth at the inner side (Fig. 1 B, D, 4 A). Dentate palatine (approximately 19 teeth per palatine and 6 replacement teeth) and edentate pterygoid are connected by a broad bony "bridge" forming together the palatopterygoid. Vomer and palatine have practically no *pars palatina*. The palatopterygoid is clearly separated from the vomer on both sides (Fig. 1 B, D, 4 A). The putative connection on the left side (seen Fig. 1 B) is part of the underlying orbitosphenoid (is seen clearly by image rotation).

Lower jaw: Paired dentaries reveal a frontal symphysis; paired coronoids are present (Fig. 1 B–D). Especially anteriorly dentary teeth are arranged in two rows (not shown); tooth rows slightly overlap the dentition of the coronoids posteriorly. Each coronoid tooth-patch bears approximately 20 teeth; coronoids parallel the prearticulars. Dentaries and coronoids have replacement teeth at their inner side (Fig. 1 B, C).

Comments: We classify this larva as "early" young larva that has the full set of "tooth systems". The palatine bears more functional teeth than the vomer. Presence of replacement teeth along the inner side of these bones indicates highly active dental laminae. The same holds for the coronoids.

Larva 2

(stage I+; "young larva") (Fig. 1 E, F)

General remarks: Parietals are more broadened in the middle. Basal and dorsal parts of exoccipitals have been further grown anteriorly (not shown). The posterior third of squamosals is broadened; the otic capsule is enlarged (Fig. 1 E).

Upper jaw: Premaxilla reveals lingually replacement teeth.

Palate: Vomerine tooth-patches have 18 (left) and 19 (right) teeth as well as replacement teeth at their inner side (Fig. 1 F). Palatopterygoids are complete with a broad bony bridge (Fig. 1 E, F). Palatines bear approximately 15 (right side) and 18 (left side) teeth; replacement teeth are present. Vomer and palatine are very close together. This zone is like a notch widened at the outer (buccal) and narrow at the inner face of the bones. The *partes palatinae* of both are inconspicuous (Fig. 1 E, F).

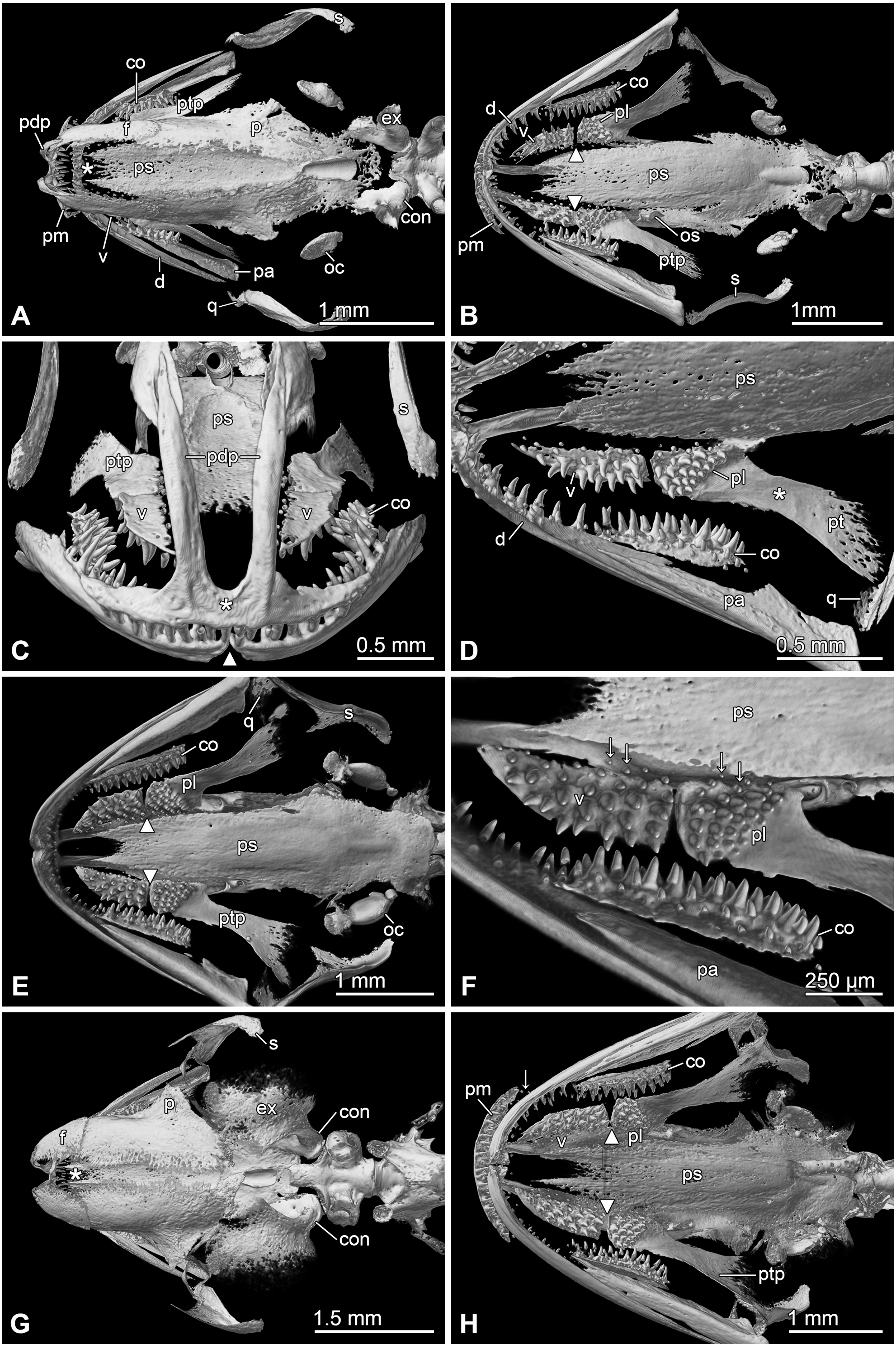
Lower jaw: Dentaries bear a single tooth row; coronoids bear a tooth-patch. Both reveal replacement teeth (Fig. 1 E, F).

Comments: This larva is slightly further developed than larva 1. This is revealed by an increased similar number of established vomerine and palatinal teeth indicating some growth of the vomer. Dental laminae of the coronoids and palatines are still active.

Larva 3

(stage II; "typical larva") (Fig. 1 G, H, 4 B)

General remarks: Exoccipitals are grown further. The fontanelle of the parietal is considerably narrowed (Fig. 1 G). The parasphenoid extends to the occipital region. The anterior processes of the parasphenoid are longer ending at the level of the tip of the vomerine tooth-patches (Fig. 1 H).


s
co
ptp
pdp
f
p
ex
ps
con
pm
v
d
pa
oc
q
1 mm
A
d
co
pl
v
ps
os
pm
ptp
s
1mm
B
ps
s
pdp
ptp
v
v
co
0.5 mm
C
ps
pl
v
pt
d
co
q
pa
0.5 mm
D
q
s
co
pl
ps
ptp
oc
1 mm
E
ps
v
pl
co
pa
250 µm
F
s
p
ex
con
f
con
1.5 mm
G
co
pm
v
pl
ps
ptp
1 mm
H

Upper jaw: As in larva 2; however, a single replacement-tooth is present beyond the ossified ends of the premaxilla (Fig. 1 H).
Palate: Each vomerine tooth-patch has approximately 18 teeth and each tooth-patch of the palatinal portion of the palatopterygoid bears approximately 19 teeth (Fig. 1 H). In the latter, number of replacement teeth is markedly reduced (three teeth on the left and four teeth on the right side) (Fig. 1 H). Rotation of the CT images shows that vomer and palatine are very close together on the right side, and clearly separated on the left side (Fig. 1 H, 4 B).
Lower jaw: As in larva 2.
Comments: Nearly the same as in larva 2. Number of vomerine and palatinal teeth is similar, but number of palatinal replacement teeth is markedly reduced. At this time regression of the palatinal dental lamina begins, which marks the end of the early larval phase (Clemen & Greven, 1979).

Larva 4

(stage IIIa–IV; "late larva" with delay) (Fig. 2 A–C, 4 C, D)

General remarks: The otic capsules are complete. The orbitosphenoids are larger and articulate broadly with the parietal und anteriorly somewhat less broadly with the frontal. Nasals and prefrontals are present (Fig. 2 A, B).
Upper jaw: The premaxilla has a relatively broad pars palatina. The bases of the alary processes of premaxillae are fused. Maxillae have been grown anteriorly meeting the premaxilla (Fig. 2 C, 4 C, D) and bear a single tooth anteriorly (not shown); *partes faciales maxillares* are large and flattened (Fig. 2 A)
Palate: Each vomer has a broad *pars palatina*, one (anteriorly) to four (posteriorly) tooth rows and an edentate anterior expansion that represents the median portion of the future „vomerine plate" (see below) (Fig. 2 B). Vomeres bear approximately 23 functional (tooth patch) and 15 replacement teeth (left side) and 19 functional and 20 replacement teeth (right side). Replacement teeth are also seen in the gap between vomer and palatine and a few in the palatinal area (Fig. 2 B, C). The most anterior vomerine tooth is shifted medially (Fig. 2 B).

Figure 4 C and D suggest a fusion of vomer and palatopterygoid on both sides. However, CT images show a distinct fusion only on the right side (Fig. 2 B), while on the left side a clear gap is seen even interiorly (between the pars palatina of the vomer and the palatine) (Fig. 2 C). Each palatine bears two groups of teeth. Buccaly (exteriorly) there are six to seven small teeth and medially (interiorly) approximately 11 larger teeth (Fig. 2 B, C, 4 C, D). Palatopterygoids are intact; on the right side the palatinal bridge is smaller and somewhat constricted (Fig. 2 B, C, not seen in Fig. 4 C due to the inadequate staining).
Lower jaw: Contrary to the dentaris coronoids have only a few replacement teeth (not shown).
Comments: Previously, a late larval stage in *Salamandra salamandra* was characterized by an increased number of teeth on the vomers, while the number of palatine more or less stagnated and by the presence of already dentate maxillae. Concerning the number of teeth, this holds also for *L. vulgaris*. However in this species and other newt, maxillae develop later. Delay is indicated by broad *partes palatinae* of the vomer (with numerous replacement teeth) and palatines, the edentate front part of the vomer, the reduction of the buccal teeth of the palatines, the larger group of teeth interiorly (premature), and maxillae that contact the praemaxilla (premature). An increase number of vomerine teeth and a distinct decrease of palatinal teeth are traits common for stage IIIa in *S. salamandra* und *L. vulgaris.*

Separation of the palatopterygoid and the vomer has started on one side. Noticeable are the replacement teeth between vomer and palatine.

Larva 5

(stage V+; "midmetamorphic larva") (Fig. 2 D–F, 4 E, F)

General remarks: Quadrates are larger than in larva 4.The prootic articulates with the large orbitsphenoid. Parts of prefrontals contact the frontals und the pterygoid (Fig. 2 D). The parasphenoid articulates laterally with the orbitosphenoids. The parietals have grown together (in part) posteriorly.
Upper jaw: Premaxilla as before. Maxillae bear a single row of teeth anteriorly, whereas the posterior process

← **Fig. 1.** A–H. µCT of the skull of larva 1 stage I– (A–D), larva 2, stage II– (E, F) and larva 3 stage II (G, H). **A** Top view: Note the broad fontanelle (asterisk) between frontals (f) and parietals (p). **B** Ventral view: vomers (v) bear fewer teeth than the palatine (pl); the particles accompanying the dentigerous bones are replacement teeth; note the gap between vomers and palatine (arrowheads). **C** Frontal view: Note fused premaxillae (asterisk) and the strong *processus dorsales praemaxillares* (pdp), symphysis between the dentaries (arrowhead). **D** Ventral view, right side: gap between vomer (v) and platine (pl); note replacement teeth at the inner face of these bones; broad pterygopalatinal bony bridge (asterisk). **E** Ventral view: Small gap (arrowheads) between vomer and palatine (pl). **F** Enlargement of E (right side); note similar numbers of teeth on the vomer (v) and palatine (pl) and the close approximation of the bones; below the replacement teeth (arrows) the *processus dorsalis praemaxillars* is seen. **G** Top view: Note enlargement of exoccipitals (ex) and parietals (p); the fontanelle of the frontals (asterisk) is wider than that of the parietals. **H** Ventral view: the vomer (v) bears fewer teeth than the palatine (pl); both bones are very close together (arrowheads); one tooth bud beyond the end of the premaxilla (arrow). For further abbreviations see page 84.

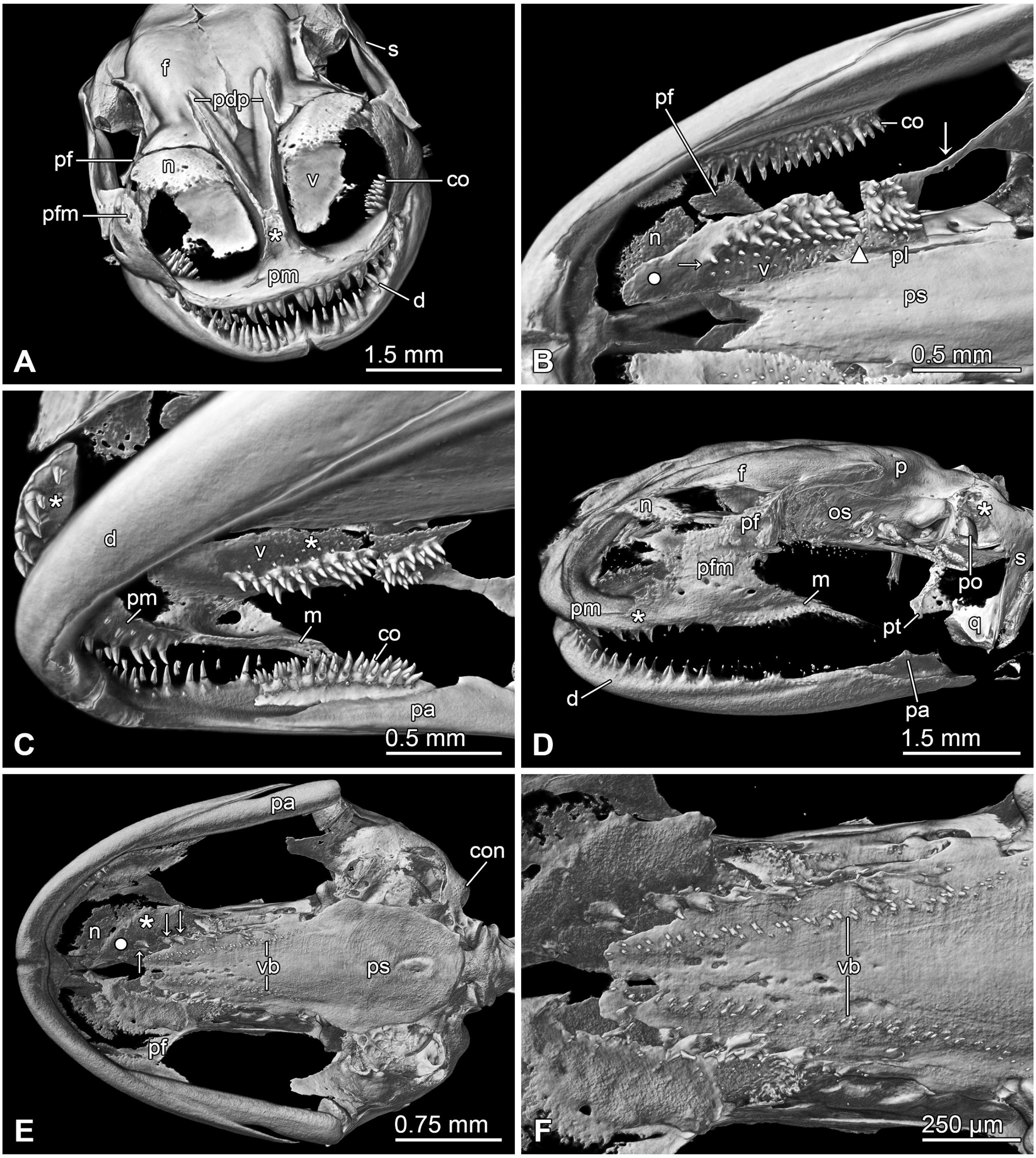


Fig. 2. µCT of the skull of larva 4 (A–C) and larva 5 (D–F). **A** Frontal view: *Processus dorsales praemaxillares* (pdp) are fused at the base (asterisk); *partes faciales maxillares* (pfm) approach the nasals (n). **B** Ventral view, right side: the vomer (v) bears more teeth than the palatine (pl); both bones are fused (arrowhead) showing abroad *pars palatina* (near to the parasphenoid (ps); one vomerine tooth is shifted medially (arrow); note the edentate anterior expansion of the vomer (dot), and the thin pterygopalatinal bridge (large arrow). **C** Ventral view: coronoid (co) without replacement teeth; maxilla (m) is edentate. **D** Lateral view: otic elements (large asterisk); the *pars facialis maxillaris* (pfm) is connected with the prefrontal (pf); orbitosphenoid (os) extends to the prootic (po); maxilla with edentate posterior process (m); overlapping of premaxilla and maxilla (small asterisk); pterygoid (pt) attached to the prootic (po). **E** Ventral view: Note part of the former larval vomer (asterisk), the anterior expansion (point), the large teeth (arrows) and the non-ankylosed teeth along the future dentate vomerine bars (vb). **F** Enlargement of E.: Note bicuspid non-ankylosed teeth of the future vomerine bar. For further abbreviations see page 84.

is edentate. The facial flanges of maxillae articulate with the prefrontals and the maxillae, and overlap the premaxilla in the cheek region (Fig. 2 D). Maxillae bear teeth and show replacement teeth approximately up to the middle of the *pars facialis maxillaris* (Fig. 2 D).

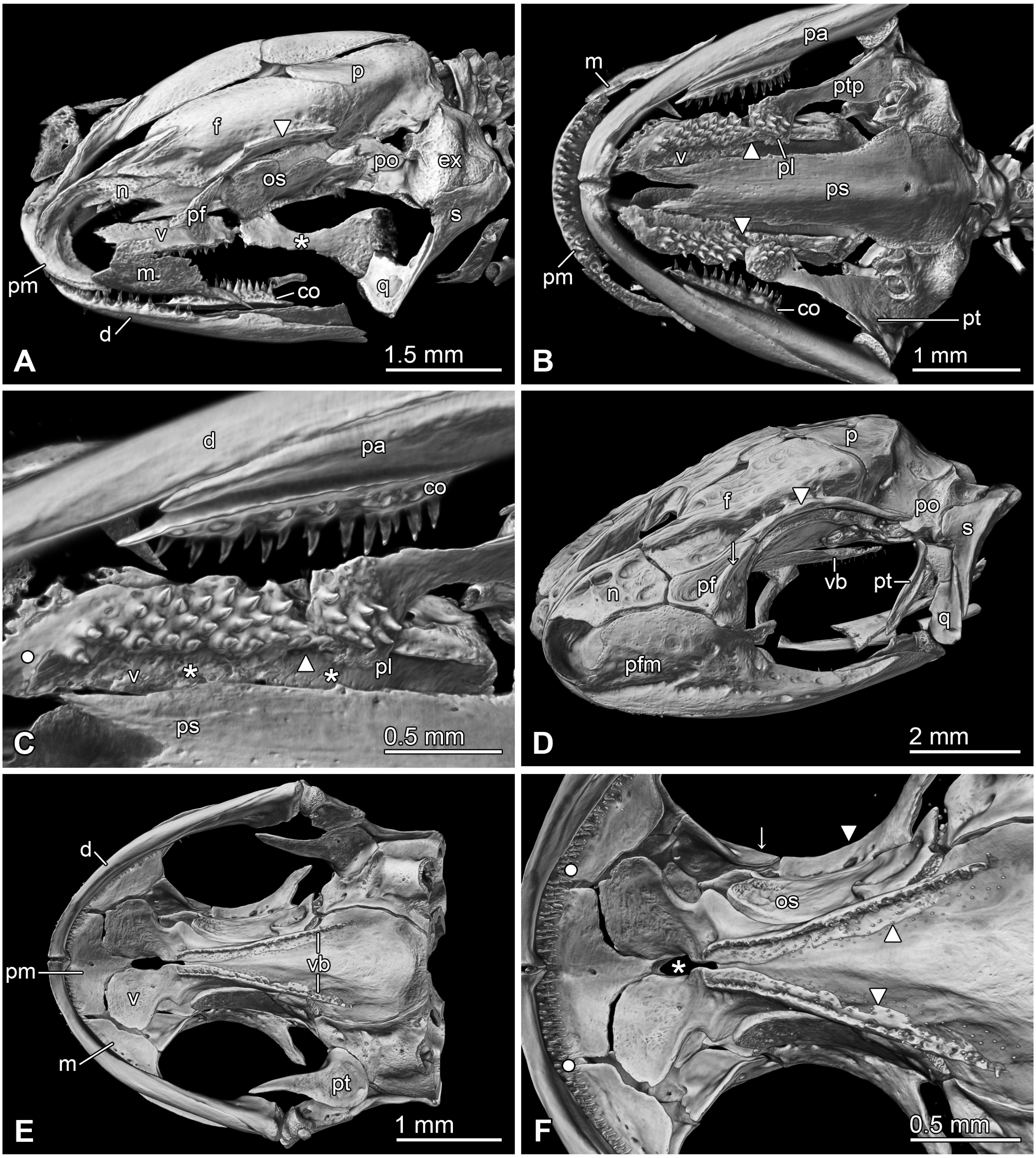
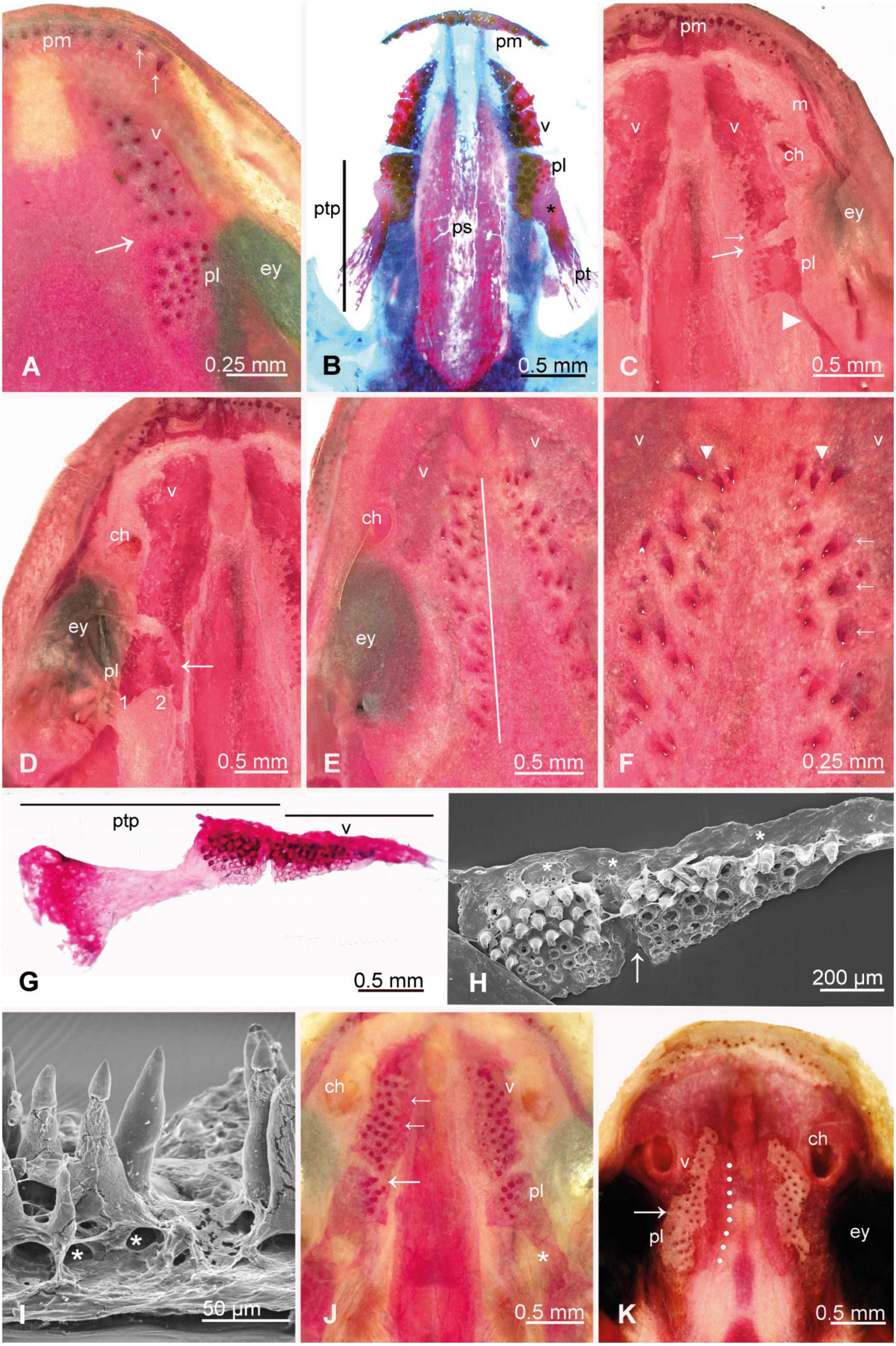


Fig. 3. µCT of the skull of larva 8 (A–C) and adult (D–F). **A** Lateral view; note the broad pterygopalatinal bridge (asterisk); dentition of the dentary (d) parallels a small part of the coronoid (co); frontal crest (arrowhead). **B** Ventral view: Note the close approximation (arrowheads) of vomers (v) and palatines (pl). **C** Enlargement of B: Note fusion (arrowhead) of vomer (v) and palatine (pl) and the continuous broad *pars palatina* (asterisks). **D** Lateral view: Note the prefrontal (arrow) and frontal crest (arrowhead) extending in a free process; vomerine bar (vb). **E** Ventral view: Broad *partes palatinae* of premaxilla (pm), and maxillae (m) articulate with the edentate vomerine plates (v). **F** Enlargement of E: Note the monostichously dentate vomerine bars (arrowheads); hole of the intermaxillary gland (asterisk); crest of the prefrontal (arrow) and the frontal (arrowhead), dental and premaxillary tooth row (white points). For further abbreviations see page 84.

Palate: Vomers are large and elongated anteriorly (Fig. 2 E, F) with remnants of teeth (Fig. 4 E). Their posteromedial margins form the bony surrounding of the choana (Fig. 4 E). On each side a seemingly polystichous row of numerous small bicuspid non-ankylosed teeth (up to 30) spreads over more than the half length of the parasphenoid. These teeth became later attached to the ossifying vomerine bar. Each tooth row begins medially in the


pm
v
pl
ey
0.25 mm
A
pm
v
pl
*
ptp
ps
pt
0.5 mm
B
pm
m
v
v
ch
ey
pl
0.5 mm
C
v
ch
ey
pl
1
2
0.5 mm
D
v
v
ch
ey
0.5 mm
E
v
v
0.25 mm
F
ptp
v
0.5 mm
G
200 µm
H
50 µm
I
ch
v
pl
0.5 mm
J
ch
v
pl
ey
0.5 mm
K

lower third of the largely edentate larval vomer and ends with a slight buccal curvature (Fig. 4 E). Anteriorly there are three transversally arranged monocuspid teeth (Fig. 4 F). The most median tooth of each row is considered as the first tooth of the tooth row of the vomerine bar. Between the former larval vomer and the tooth row of the vomerine bar some large monocuspid teeth are present, most of them without bony support. The palatine is missing; pterygoids approach the quadrates (Fig. 2 D).
Lower jaw: Dentaries show numerous replacement teeth (Fig. 2 D).
Comments: Larvae in the midst of metamorphosis indicated by the absence of the palatine, the presence of large orbitosphenoids and developing teeth that later occupy the vomerine bar. A putative delay is seen by the presence of non-ankylosed teeth between the former vomerine tooth-patch and the tooth rows of the bar, which obviously are from previous dentitions destined for the larval tooth patch.

Larva 6

(stage III a; "late larva" with delay) (Fig. 5 A–G)

Description of the serial section from anterior to posterior focuses mainly on the "tooth systems", namely the course of the dental lamina in the palate.
Upper jaw: Anterior, i.e. before the choana, the dental lamina of the upper jaw (premaxilla) extends into the maxillary area as a small (unproductive) epithelial bulge. Maxillae with their facial flanges are present (Fig. 5 A).
Palate: The vomerine dental lamina, situated ventrally to the vomerine *pars palatina*, is relatively short, but productive (Fig. 5 A). It becomes longer posteriorly; the vomer is strongly connected with the parasphenoid (Fig. 5 B). At the level of the anterior region of the eye an ossifying area on the buccal side of the vomer is seen. A few sections away this zone is very small bearing a single tooth, but the dental lamina is fully developed. This zone represents the transition from the vomer to the palatine (Fig. 5 D). Immediately at the level of the eye lens the dentate palatine bears teeth of different size: The outer (buccal) teeth are small; the inner (medial) teeth are large. In the buccal area osteoclasts are present (Fig. 5 E). The dental lamina ends in the edentate palatinal area (Fig. 5 F). Further sections give evidence that the palatopterygoid is intact (Fig. 5 G). The palatine is strongly connected with the orbitosphenoid (Fig. 5 F).
Lower jaw: Meckel's cartilage is surrounded by the large dentary (buccally and ventrally), the (still) dentate coronoid (dorsally) and the prearticlar (ventrally). Dental laminae of coronoid and dentary are situated lingually (Fig. 5 A, B). Already behind the choana the dental lamina of the coronoid is shortened and non-productive (Fig. 5 B).
Comments: Immediately before fixation, the stage of this specimen (late larva) was defined on the basis of external characters (e.g. short gills) and the date of capture (overwinterer). Histology confirmed this classification among others because of the anlage of the secondary tongue. Delay is indicated by an edentate anterior portion of the vomer (not shown) and reduction of palatinal tooth rows (see Fig. 5 E). We classify this specimen as late larva with delay (stage III a) comparable to larva 4, because the two tooth groups indicate presence of original palatinal teeth, but also palatinal teeth originating from the outgrowing vomerine dental lamina.

Larva 7

(stage III b; "late larva" with delay) (Fig. 4 G–I)

Upper jaw: Maxillae still edentate (not pictured).
Palate: Vomers show a reduced tooth-patch (approximately 15 teeth), with only one (anteriorly) to two (posteriorly) rows of teeth, a broad *pars palatina* medially and a large outer area with numerous former tooth loci. The same applies to the dentate palatinal portion of the intact palatopterygoid, whose *pars palatina* is continuous with that of the vomer. However, here the tooth-patch has

← **Fig. 4 A–K.** Ventral aspect of the mouth roof of larval *Ichthyosaura vulgaris* stained *in toto* with Alizarinred (A, C–F, J, K), cleared and stained *in toto* (B, G), and SEM-pictures (H, I); in C, D and J, K oral mucosa partly removed (see table 1). **A** Larva 1, stage I, left side; teeth are more numerous on the palatine (pl) than on the vomer (v), both with replacement teeth and tooth buds (not clearly seen); vomerine and palatinal tooth patches separated (large arrow); two primary replacement teeth posterior to the premaxilla (small arrows). **B** Larva 3, stage II; vomer (v) and palatine (pl) separated; note the broad palatinal bony bridge (asterisk). **C** Larva 4, stage IIIa-IV; oral mucosa largely removed; note the largely edentate maxillae (m), and the small pterygoplatinal bony bridge (arrowhead); tooth buds and replacement teeth (small arrow); partial fusion of vomer and palatine (large arrow). **D** The same specimen as in C, right side**;** the palatine bears two tooth patches (1,2); note partial fusion of the vomer and palatine (arrow). **E** Larva 5, stage V; non-ankylosed teeth of the future vomerine bar (straight line); edendate remnants of the "larval" vomer (v). **F** Enlargement of E; note the tranverse row of teeth anteriorly (arrowheads) and the large teeth (small arrows) between the "larval" vomer (v) and the median tooth rows of the future vomerine bar. **G** Larva 7, stage IIIb; fusion of the vomer (right side) and the palatopterygoid (ptp). **H** Enlargment of G (SEM): Note the clear zone of fusion (arrow) and the broad *pars palatina* (asterisks). **I** Ditto, buccal view showing the zone of fusion and large pulps (asterisks) of the established teeth. **J** Larva 8, stage IIIb; oral mucosa partly removed; vomer (v) with more teeth than the palatine (pl); note vomerine tooth anlagen (small arrows), the broad pterygopalatinal bony bridge (asterisk), and the fusion of vomer and palatine (arrow). **K** Larva 9, stage IIIb; larval oral mucosa partly removed; note the fusion of vomers and palatines (arrow); the area of the *pars palatina*, vomerine dental lamina and its lingual *tunica propria* (dotted line on the left side) extends to the posterior end of the tooth patch. For further abbreviations see page 84.

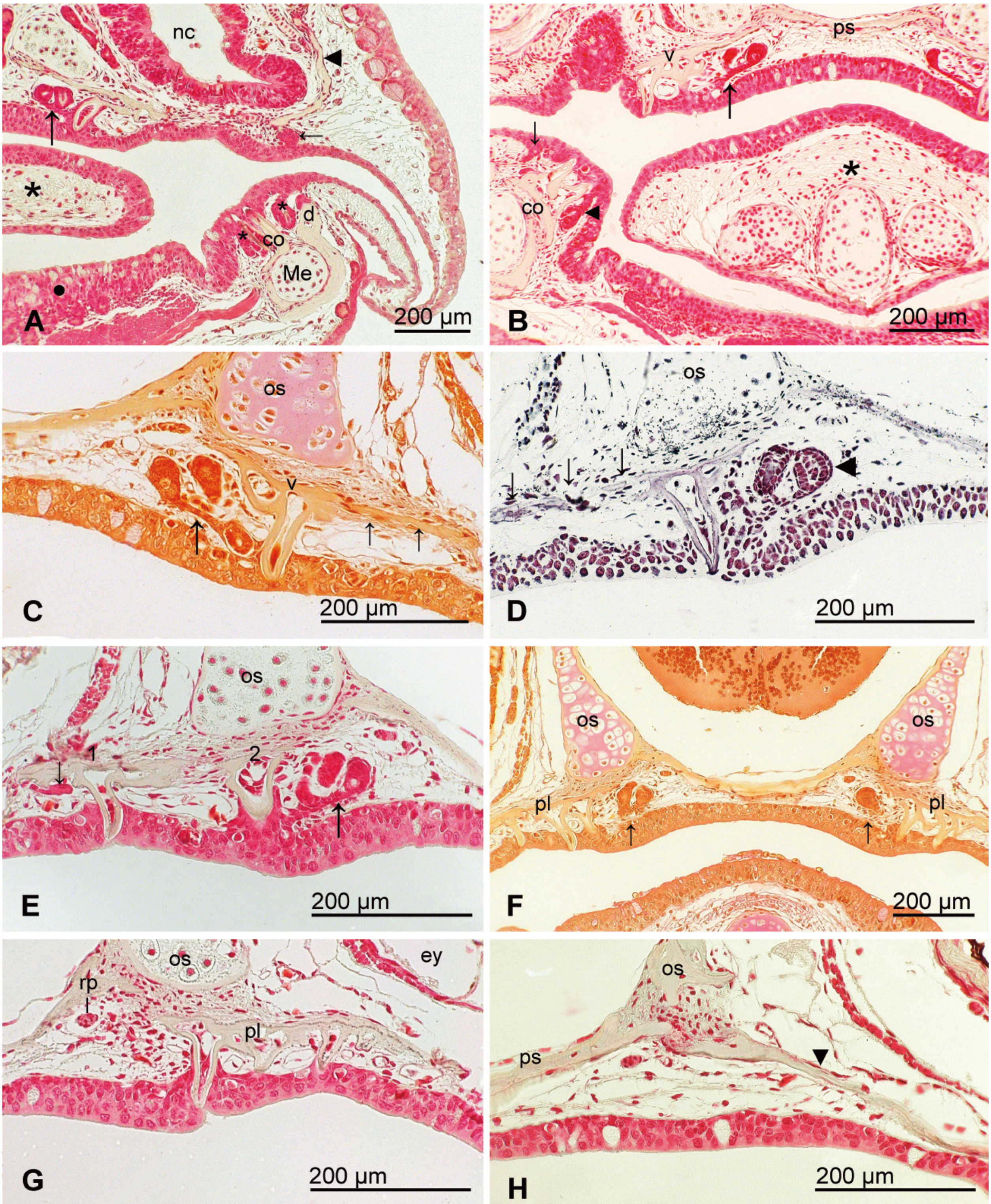


Fig. 5 A–G. Selected examples of serial cross-sections of the skull of larva 6 from anterior (nasal region) to posterior (pterygopalatinal bony bridge). Images switch from the left to the right side depending on quality and information value. **A** Short productive dental lamina of the vomer (large arrow); non-productive dental lamina (small arrow) in the maxillary area; *pars facialis maxillaris* (arrowhead), primary tongue (asterisk), anlage of the secondary tongue (dot); coronoid (co), and dentary (d) with their dental laminae (small asterisks); nc = nasal cavity. **B** Behind the choana; broad vomerine dental lamina (large arrow) along the vomer (v); coronoid (co) with productive dental lamina (arrowhead); unproductive end of the dental lamina of the dentary (small arrow); primary tongue (asterisk). **C** Anterior region of the eye; vomerine dental lamina with tooth buds (large arrow), vomer (v); note the edentate buccal part of the vomer (small arrows). **D** Transition of vomer and palatine (zone of fusion) occupied by a single functional tooth. Note the intact dental lamina with tooth buds (arrowhead) and the incompletely ossified buccal area (arrows). **E** Palatine with small (1) and large teeth (2), the latter near the productive dental lamina (large arrow); osteoclast (small arrow). **F** Detachment of the dental laminae (arrows) from the oral epithelium in the palatinal area (pl). **G** Posterior part of the palatinal tooth patch (pl); the dental lamina (left side) is absent. **H** Part of the edentate pterygoid (arrowhead). For further abbreviations see page 84.

at least three rows of approximately 20 functional teeth. On the right side, the palatine is clearly separated from the vomer; on the left side both bones are fused (Fig. 4 H, I). Buccally the zone of fusion is characterized by a notch (Fig. 4 G, H). Teeth of the buccal side of bones appear somewhat smaller; they are monocuspid, but preservation of their structure is poor and does not allow assessing further details (Fig. 4 H).
Comments: This larva seems to have spent a relative long time in a late phase of development. Delay is indicated by the enlarged palatinal tooth patch accompanied by an obviously active dental lamina and the enlarged vomer, whose number of teeth appears considerably reduced.

Larva 8

(stage III b; "late larva" with delay) (Fig. 3 A–C, 4 J)

General remarks: The occipito-otic complex with the relatively large exoccipitals is further ossified posteriorly. The anterior part of the orbitosphenoid has largely reached its final size. The protruding crests of prefrontal and frontal are clearly seen. The triangular prefrontal, nasals (separated from each other) are enlarged (Fig. 3 A).
Upper jaw: Maxillae have a few established teeth anteriorly.
Palate: Vomers are large; they have an anterior edentate area (Fig. 3 B, C). Vomerine tooth-patches consist of approximately 24 teeth each. Vomer and palatinal portion of the intact palatopterygoid – the bony bridge is quite broad – are very close to each other (left side) or fused (right side) (Fig. 3 B, C). The left palatine bears seven teeth; the right palatine shows an ankylosed tooth in the zone of fusion and a broad pars palatina that is continuos with the *pars palatina* of the vomer (Fig. 3 C). The pterygoidal portion of the palatopterygoid reaches the quadrato-squamosal complex (Fig. 3 A).
Lower jaw: Coronoids possess one or two rows of teeth; replacement teeth appear to be absent.
Comments: Delay of this specimen is characterized by the relatively few teeth of the palatine (<10), the large vomerine tooth-patches, the broad vomerine and and palatinal *partes palatinae* (needs time to develop), the wide pterygoid near the quadrate; presence of nasal and prefrontal, long edentate process of maxillae.

Larva 9

(stage III b; "late larva" with delay) (Fig. 4 K)

Upper jaw: Maxillae with only a few established teeth.
Palate: On both sides of the palate vomer and palatine appear to be fused. The pterygoid is present, but not stained satisfactorily. On the left side the palatine appears largely intact bearing approximately 11 teeth and appears slightly remote from the reduced tooth-patch of the vomer. On the right side, the posterior portion of the palatinal portion of the pterygoid is partly obscured. The more strongly stained area at the inner side of the fused bones represents the area covered by the dental lamina and its tooth anlagen, which is medially bordered by a *tunica propria* that appears as bright line in the preparation.
Comments: In this preparation the oral mucosa could not be removed completely and not all parts were satisfactorily stained, e.g. the pterygoid. Nevertheless this larva is very similar to larva 8 (see above). Noticeable is the (indirect) evidence for a continuous dental lamina.

Transformed, adult

(stage VII) (Fig. 3 D–F)

General remarks: Fully transformed skull with large ossified nasals and prefrontals; prefrontals articulate broadly with the frontals. Both bones form crest-like bulges that border the orbital region dorsally. The crest of the frontal extends posteriorly in a free process (*processus alaris frontalis*). The pterygoid contacts the quadrate and is fused with the prootic (Fig. 3 D). The squamosum fuses with the quadrate; the articular is fully ossified.
Upper jaw: Tooth row of maxillae extends posterior until the posterior edge of facial flange; the *processus posterior maxillaris* remains edentate (Fig. 3 D). The lingual *partes palatinae* of maxillae and premaxilla are broad abutting the anteriorlateral edentate parts of the vomer ("vomerine plates"); the *partes faciales maxillares* are very large articulating with the nasals and prefrontals (cheek region).
Palate: Vomers form part of the bony margin of the choanae; the edentate vomerine plates show broad articulations with premaxillae and maxilla (see above) and leave a hole in the center (intermaxillary gland). The posterior part of each vomer plate becomes narrower extending in the straight vomerine bars with bears a single row of teeth (Fig. 3 E, F).
Lower jaw: Dentaries bear a single tooth row that extends beyond the upper jaw dentition posteriorly.

Discussion

Life cycle of *Lissotriton vulgaris* and the specimens examined

Transformed individuals of *Lissotriton vulgaris* usually have a biphasic life cycle: an aquatic breeding period of about 120 day with a peak approximately in the midst of this period, and a terrestrial period (e.g. Bell & Lawton, 1975; Günther, 1996). Bell & Lawson (1975) distinguished an early, a main and a late period of oviposition that generated an early, a main and a late cohort of larvae. Accordingly, larvae from the different cohorts may metamorphose at different times and, thus, the time with metamorphosing individuals covers a period up to October. Despite the fact that earlier in the year eggs will take longer to develop, at a given time different developmental stages originated from the different oviposition periods have been found in a pond. These developmental stages were calculated by means of length frequency. Larvae from the late cohort, however, overwinter, may even actively feed during this time, but generally slow down or arrest development and finish metamorphosis in the following year (Bell & Lawton, 1975). Surely, our series, although incomplete in some respects, contained overwintered larvae. This is indicated by the date of capture and the length of the specimens. These larvae can be most likely expected to show signs of delayed metamorphosis.

On the basis of the organisation of the mouth roof, three larvae (1–3) captured in July were classified as stage I and II being largely consistent with "normal" development. The same holds for larva 5 classified as stage V. One larva (4) from August and a further larva (6) from April (overwinterer) were classified as stage III a showing a slight delay. Date of capture (all have been caught in April) and total (remarkably variable) length (3.2–4.5 cm) of the remaining three larvae reveal them as individuals, which had spent the winter in the pond. All were classified as stage IIIb, i.e. larvae with clear signs of delay (see Clemen & Greven, 2013).

Our starting point was a premetamorphic larva, in which the majority of skull elements and all "tooth systems" (except for the maxillae in the upper jaw) were already present and ossified at least in part, whereas the main metamorphic events of the hyobranchium and palate are still to come. The endpoint of our series was a fully transformed adult specimen. Details concerning development of cranial elements in several Salamandridae are found in the literature (e.g. *Salamandra salamandra*: Stadtmüller, 1924; *Lissotriton vulgaris*: Erdmann, 1933; Smirnov & Vassilieva, 2003; *Notophthalmus viridescens*: Reilly, 1986, 1987; *Pleurodeles waltl*: Corsin, 1966; Smirnov & Vassilieva, 2001; Lebedkina, 2004; *Triturus karelinii*: Lebedkina, 2004). Sequence of ossification of the single elements differs slightly among species and authors (see also table 1 in Rose, 2003). This is often due to the fact (at least in part) that complete series of developmental stages were not available and/or that stages can not be defined with the desirable precision.

Concerning *L. vulgaris*, Smirnov & Vassilieva (2003) distinguished early, mid-larval and late appearing bones. The early bones are not TH-inducible and develop in the order (1) coronoid, palatine, dentary, vomer; (2) premaxilla, squamosal, angular, pterygoid, parasphenoid; and (3) parietal and frontal. The mid-larval bones, also not TH-inducible are (4) exoccipital, orbitosphenoid, quadrate, and prootic; Finally the TH-inducible late bones follow, which are (5) maxilla, prefrontal, and nasal. Also TH-inducible is the remodelling of the palate, which includes disintegration of the palatine, anterior expansion of the vomer to meet the maxilla and premaxilla and formation of the vomerine bar. In addition, the coronoid is lost and the articular ossifies (see also Reilly, 1986, 1987).

The "tooth systems"

The general organisation and metamorphic changes of the tooth bearing systems of *Lissotriton vulgaris* have been described previously (e.g. Clemen & Greven, 1979, 1994; see also Smirnov & Vassilieva, 2003) and correspond to that of other salamandrids (see literature cited above). However, with regard to the fusion of vomer and palatinal portion of the palatopterygoid (see 4.3.) and delayed metamorphosis (see 4.4.) we briefly summarize here some events of the normal development in this species using data from literature, in which, however, dental laminae are largely ignored, and own partly unpublished findings. Comments will be made on (1) tooth generations and dental laminae, (2) splitting of the pterygopalatine and resorption of the palatine and its tooth patch; (3) the anterolateral growth of the vomers, and (4) the posterior outgrowths of the vomers (= vomerine bars).

Ad 1. Most of the dentigerous bones of *L. vulgaris* appear early in development (see above), whereas maxillae appear around metamorphosis. Generally, ossification of dentigerous elements and ankylosis of functional teeth is preceded by the formation of the dental lamina that already produces tooth buds without the supporting bone. This does not hold for the maxillae that begin to ossify more posteriorly. Ossification then spreads anteriorly and posteriorly. Anteriorly maxillae contact the already existing dentate premaxilla (Erdmann 1933; Clemen & Greven, 1979; Smirnov & Vasssilieva, 2003). The dental laminae of maxillae, however, arise from the dental lamina of the premaxilla that grows out in posterior direction (see Greven & Clemen, 1994; Smirnov & Vassilieva, 2003).

As usual in transforming Urodela (e.g. Greven, 1989; Davit-Béal *et al.*, 2006, 2007), all dentigerous bones bear teeth that first are monocuspid and undivided. They are replaced by subpedicellate teeth, which are monocuspid revealing a trace of a dividing zone, and these teeth are replaced gradually by bicuspid teeth during metamorphosis. This holds also for the late appearing maxillae, but obviously does not apply to the growing vomerine bar that bears predominatly bicuspid teeth already from the start (see also Accordi & Mazzarini, 1992). Palatine and coronoids disintegrate so early (coronoids with a small delay) that they very probably never receive bicuspid teeth (Clemen & Greven, 1979).

Ad 2. In all metamorphosing (and some paedomorphic) urodeles the bony pterygopalatinal "bridge" undergoes resorption, thus separating the palatine from the pterygoid. This event has been considered as a key feature indicating onset of metamorphosis or that metamorphosis is underway (Wilder, 1925; Reilly, 1986, 1987). The separation process starts before the palatinal tooth patch is resorbed and this resorption is preceded by a posterior-anterior regression of the accompanying dental lamina, which already takes place long before the disintegration of the bony "bridge" (Clemen & Greven, 1979).

Ad 3. A new formation in metamorphic salamandrids is the anterolateral edentate expansion of the vomer ("vomerine plate") that joins the upper jaw arcade and surrounds in part the choana (e.g. Trueb, 1993; Rose, 2003). According to the prevailing opinion the larval vomer is completely replaced by the adult ("definite") vomer (Wintrebert, 1922; Rose, 2003; Lebedkina, 2004). However, the larval vomer appears to contribute to the "definite" vomer, especially to the edentate median part that lies between the vomer plate and the vomerine bar (see below). This was shown by Clemen (1979), who extirpated the vomer (in part or completely) of larval *S. salamandra*, which resulted in an incomplete adult vomer. The herein presented total preparations and CT images of the mouth roof of *L. vulgaris*, which clearly show parts of the larval vomer still persisting during the formation of the vomerine bars, suggest similar conditions.

Ad 4. A further structure developing *de novo* during metamorphosis is the dentate vomerine bar, a posterior expansion of the vomer up to the level of the mandibular joint. Previous transplant and amputation experiments have shown that in *Salamandra salamandra* the vomerine bar develops from the connective tissue attached to the posterior inner (lateral) edge of the larval vomer. Its formation does not depend on the presence of a functional dental lamina, but generally the dental lamina runs very closely along the most interior tooth row (s) (Clemen, 1978 b, 1979). Undoubtedly the development from osteogenic connective tissue is to be expected also in other Salamandridae (see Smirnov *et al*., 2003). A similar extension of the vomer seen in some plethodontids is not homologous to the vomerine bar of salamandrids; it arises at the posteromedial edge of the larval vomer, which leads to a lateral position of the dental lamina (Wilder, 1925; Regal, 1966; Rose, 2003).

Looking, however, at the palate of larva 5 (stage V), it appears that the formation of the vomerine bar may be somewhat different in *L. vulgaris*. We will give here only some hints, but will deepen this aspect in a forthcoming article with some developmental stages of the newt *Ichthyosaura alpestris apuana*. In larva 5 we noticed (1) a transversal arrangement of the most anterior vomerine teeth, from which the innermost tooth represents the first tooth of the single tooth row of the vomerine bar and (2) the broad space between the the "larval" vomer that bears remnants of teeth, and the bicuspid tooth buds of developing vomerine bar; this space is occupied by some large teeth embedded in the connective tissue. The anterior transversal arrangement of teeth requires a previous slight curvature of the dental lamina away from the larval vomer and the large space suggests a shift of the dental lamina towards a more middle position. Shifting is indicated by the position of the straight row of non-ankylosed developing teeth of the vomerine bar. The non-established large teeth found in the area between the dental lamina and the previous *pars dentalis* of the larval vomer may represent teeth from previous dentitions that did not find any support because this area is not yet ossified. The osteogenic activity of this material that parallels the dental lamina, and the expansion of both beyond the posterior border of the larval vomer may create the vomerine bar with the typical straight row of teeth, which are arranged at an acute angle (measured from the long axis) typical for *L. vulgaris.*

Appearance of "vomeropterygopalatina"

Most striking, not described in normal development so far, and observed only in natural populations of *Lissotriton vulgaris* (this article) and the related *Ichthyosaura alpestris apuana* (unpublished) is the fusion of the (larval) vomer with the intact palatopterygoid at least on one side of the palate, and the very probably simultaneous extension of the vomerine dental laminae providing the palatine with teeth (as the palatinal dental lamina has been disintegrated already at the end of the early larva phase). The different appearance of the zone of fusion on both sides may indicate that separated tooth systems react independently to a certain extent.

In normal development the small gap between vomer and palatine may be bridged by vomerine teeth and the vomerine dental lamina may even provide the anterior edge of the palatine with teeth as discussed for *Salamandra salamandra* (Clemen, 1978 a) and *Lissotriton vulgaris* (Clemen & Greven, 1979). A further posterior growth of the vomerine dental lamina (indicated by our histological series), comparable to the extension of the dental lamina of the premaxilla to provide the maxillae with teeth, is probable. Moreover, dental laminae or parts of them from several "tooth systems" (of *Ambystoma mexicanum*), which were reciprocally exchanged, remained active in the foreign environment producing their specific teeth (monocuspid or bicuspid) in their specific arrangement (single row, tooth patch) (see Clemen, 1988 a, b). However, the elongation of the vomerine dental lamina requires the disappearance of the palatinal dental lamina (what has previously happened; see Clemen & Greven, 1979) and retention of the palatines, e.g. by a delay of development (see below). We think that this extension also takes place when vomer and palatinum are not entirely fused.

If so, grouping of palatinal teeth (as seen in larva 4 and 6) could be explained as follows: Assuming that the smaller teeth seen on the palatinal portion of the "vomeropterygopalatinum" have been produced at a time, when the palatinal dental laminae was still active (an assumption supported by the exterior position of the teeth), whereas the larger teeth positioned interiorly near to the dental lamina have been originated from the elongated vomerine dental lamina, and considering that teeth produced by the palatinal dental lamina are generally small monocuspids with no or only a trace of the annular dividing zone (early larva-type), and teeth produced by the vomerine dental lamina are larger monocuspids with a more prominent dividing zone (late larva-type) (see Clemen & Greven, 1979), we, therefore, should be able to recognize the origin of the teeth by their outer appearance.

In fact, we could see only two groups of teeth on the palatine that differ in size, but appearance and degree of the annular dividing zone was not clear in all cases, possibly due to the bad preservation of the specimens.

Principally, a fusion of (i) vomer and pterygopalatium or of (ii) vomer and palatine already separated from the pterygoid could happen as the growing bones approach each other already during undisturbed development (Clemen & Greven 1979; 2013; Jömann *et al*., 2005; Greven *et al*., 2006). Reilly (1986) even described an interdigitation of palatine and vomer in *Notopthalmus viridescens* on the basis of cleared and double stained preparations.

Marconi & Simonetta (1988) reported on dentate separate palatines close to the vomer in "neotenic" *Lissotriton vulgaris meridionalis*. Smirnov & Vassilieva (2003), who reared various larval stages of *L. vulgaris* in presence of TH, occasionally noted vomeres fused with remnants of the still dentate palatine (see Fig. 3 b in their article). Later, they described vomeropalatines and even vomeropalatopterygoids in TH-treated *Ambystoma mexicanum* larvae, noted that these fusions "depend upon the TH dosage and developmental stage at which hormone was applied" and considered these formations as artefacts caused by TH-treatment (Smirnov & Vassilieva, 2005; see their figure 4 a and p. 125). Previously, Stadtmüller (1936, p. 639) has mentioned in passing a "vomeropterygopalatium" in a neotenic *Ichthyosaura alpestris* very likely captured in the wild. We found further instances of "vomeropterygopalatina", not yet published, in a natural population of "paedomorphic" *L. vulgaris* with goitres (Kordges *et al*. 2012). More recently, a closer inspection of a small series of wild-captured *Ichthysaura alpestris apuana* in the collection of G.C. also reveals the presence of "vomeropterygopalatina" (unpublished).

We think that this structure is highly ephemeral and that vomer and pterygopalatinum will separate and the palatine including the "vomerine" dental lamina will disintegrate as soon as development continues. This assumption of a highly temporary appearance may be indirectly supported by the fact that "vomeropterygopalatina" are practically unknown from natural populations of newts and from ontogenetic studies undertaken in the lab. Also in studies that used large samples of *L. vulgaris* and *Ichthyosaura alpestris* from populations exhibiting facultative paedomorphosis and which were checked for skull transformation, remodelling and development of TH-mediated skull bones, "vomeropterygopalatina" were not mentioned, although namely in paedomorphs of *L. vulgaris* a considerable intraspecific variation was noted ranging from predominantly larval skulls with untransformed palatal bones, with variously developed maxillae, without nasal and prefrontal bones to almost entirely metamorphosed skulls (e.g., Djorović & Kalezić, 2000; Ivanović *et al*., 2014).

Delay of metamorphosis

We have described (with different levels in detail) only a few traits in the mouth roof of *Salamandra salamandra*, *Ranodon sibiricus* and *Lissotriton vulgaris* so far, which we consider indicative for a delayed metamorphosis (see Clemen, 1978 a; Clemen & Greven, 1979, 2013; Jömann *et al*., 2005; Greven *et al*., 2006). More recently we have suggested that organisation and remodelling of the palate may provide useful criteria for staging *S. salamandra* larva and (after some modifications) also for other salamandrids. In this context, we also listed some traits typical for larvae with delayed metamorphosis (named stage IIIa and IIIb). The present article not only broadens the spectrum of these traits for *L. vulgaris*, because we could take a closer look at the specimens, but also reveals some differences between the salamandrid species. It is noteworthy that all these larvae had reached the late larval stage (roughly characterised by the reduction of the palatinal teeth) before the retardation started.

Delay of metamorphosis obviously affects relative growth (not only increase of body size) that continued albeit slowly (see introduction) and differentiation processes that include TH-inducible events (e.g. premature appearance of new structures). We noticed (1) increase in perichondral ossifications of the replacement bones (e.g. quadrate and orbitosphenoid); (2) slight posterior expansion of the (larval) vomer and increase of the number of teeth (all three species), (3) anterior edentate expansion of the vomer (*L. vulgaris*); (4) widening of the *partes palatinae* of vomer and palatine (*L. vulgaris*), (5) further growth of the pterygoid approaching the quadrato-squamosal complex, which takes place normally during metamorphosis (all three species); (6) retention of the pterygopalatinal bony bridge, but regression of the palatinal tooth patch (all three species); in *S. salamandra* this leads to complete edentulism of the palatine; (7) ossification of maxillae, nasals and prefrontals (*L. vulgaris*); (8) retention and further growth of the coronoid in anterior direction and growth of dentaries in posterior direction including their tooth patches (all three species); and (9) retention or new formation of a "vomeropterygopalatinum" and expansion of the vomerine dental lamina into the palatinal area (*L. vulgaris*). The latter may be the reason, why in *L. vulgaris* the palatinal portion of the intact palatopterygoid does not become edentulous as in *S. salamandra*, because the vomerine dental lamina provides the palatine with teeth. The "true" still existing small palatinal teeth will, however, be degraded gradually. To give an exact order (if it ever existed) of the effects described herein, is not possible at the moment probably due to the incomplete series available.

Although the most of our larvae were classified as stage III (however with signs of delayed metamorphosis), their mouth roofs slightly differed from each other, e.g.

with regard to the the degree of the fusion of vomer and palatapterygoid, the onset of disintegration of the palatinal bony bridge (seen only in larva 4), the grouping of palatinal teeth (in larva 4 and 6), maxillae with teeth (larva 8 and 9), a very low number of teeth on the palate (larva 8), and a distinct reduction of the number of tooth rows together with a large field of previous tooth loci (7). These differences may be largely related to a certain individual variation.

The fact that all larvae classified as overwinteres showed a "vomeropterygopalatinum", suggests that this structure may be common in those larvae (unless their relatively high number in our sample is attributed to a fortunate coincidence). Therefore, we currently take the presence of a "vomeropterygopalatinum" in *L. vulgaris* and *Ichthyosaura alpestris* (see above) for a sign of delayed metamorphosis caused probably by low temperatures in a range that does not entirely and permanently arrest thyroid activity. That "vomeropterygopalatina" have been found also after TH treatment (see literature cited above) and in non-overwinterers may be caused by a general imbalance between growth and differentiation.

Treatment with thiourea that may mimic the effect of cold temperatures to a certain extent resulted in larger larvae (*L. vulgaris*, *Pleurodeles waltl*), but showed some effects not comparable with those described above for overwinterers. Effects were primarily seen around metamorphosis and their degree depended on the larval stage treated with the goitrogen. For example, application of thiourea produced a persistent large pterygopalatine with a large, toothless palatinal portion. Several cranial elements that ossify prematurely after TH-treatment developed further after treatment with thiourea indicating certain TH-independence; resorption of bones proceeded less intensive; development of dermal bones arising during metamorphosis (maxillae, prefrontal, and nasal) is severely or completely inhibited, and also the entire remodelling of the palate did not proceed (see also Smirnov & Vassilieva, 2002, 2003, 2005; Smirnov *et al*., 2011).

In conclusion, our observations may be of interest in some respects: (1) delay of metamorphosis in *Lissotriton vulgaris* (and other urodele) larvae is clearly reflected in the organisation of the palate; (2) this delay can be easily recognized in overwintered larvae; (3) especially in the latter the delay leads to a fusion of the larval vomer and the intact palatopterygoid forming an obviously ephemeral "vomeropterygopalatinum", a formation, which has not yet been observed in any natural populations of urodeles; and (3) response to agents that delay meiamorphosis (e.g. cold temperatures) may vary, depending not only on the "intensity" of the agens and the stage the larvae have reached at the time of treatment (see citations above), but also on the taxon studied. These suggestions await further studies.

Acknowledgements

The ANKA Synchrotron Radiation Facility is acknowledged for providing beamtime. We thank Profs Dr. H. and Dr. U. Kierdorf, Universität Hildesheim, for the use of the Keyence VHX 500F digital microscope.

References

Accordi, F. & Mazzarini, D. (1992): Tooth morphology in *Triturus vulgaris meridionalis* (Amphibia, Urodela) during larval development and metamorphosis. – Bolletino di Zoologia, **59**: 371 – 376.

Bell, G. & Lawson, J.H. (1975): The ecology of the eggs and larvae of the smooth newt (*Triturus vulgaris*). – The Journal of Animal Ecology, **44**: 393 – 423.

Beachy, C.K. (1995): Effects of larval growth history on metamorphosis in a stream-dwelling salamander (*Desmognathus ochrophaeus*). – Journal of Herpetology, **29**: 375 – 382.

Beneski, J.T. & Larsen, J.H. jr. (1989): Ontogenetic alterations in the gross tooth morphology of *Dicamptodon* and *Rhyacotriton* (Amphibia, Urodela, and Dicamptodontidae). – Journal of Morphology, **199**: 165 – 174.

Bizer, J.R. (1978): Growth rates and size at metamorphosis of high elevation populations of *Ambystoma tigrinum*. – Oecologia **34**: 175 – 184.

Clemen, G. (1978 a): Aufbau und Veränderungen der Gaumenzahnleisten beim larvalen und metamorphosierenden *Salamandra salamandra* (L.) (Salamandridae: Amphibia). – Zoomorphologie, **90**: 135 – 150.

Clemen, G. (1978 b): Beziehungen zwischen Gaumenknochen und ihren Zahnleisten bei *Salamandra salamandra* (L.) während der Metamorphose. – Wilhelm Roux's Archives, **185**: 19 – 36.

Clemen, G. (1979): Untersuchungen zur Bildung der Vomerspange bei *Salamandra salamandra* (L.). – Wilhelm Roux's Archives, **185**: 305 – 321.

Clemen, G. (1988 a): Experimental analysis of the capacity of dental laminae in *Ambystoma mexicanum* Shaw. – Archives de Biologie (Bruxelles), **99**: 111 – 132.

Clemen, G. (1988 b): Competence and reactions of early- and late-larval dental laminae in original and not-original dental systems of *Ambystoma mexicanum* Shaw. – Archives de Biologie (Bruxelles), **99**: 307 – 324.

Clemen, G. & Greven, H. (1979): Morphoplogische Untersuchungen an der Mundhöhle von Urodelen. V. Die Munddachbezahnung von *Triturus vulgaris* (L.) (Salamandridae: Amphibia). – Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere, **102**: 170 – 186.

Clemen, G. & Greven, H. (1994): The buccal cavity of larval and metamorphosed Salamandra salamandra: Structural and developmental aspects. – Mertensiella, **4**: 83 – 109.

Clemen, G. & Greven, H. (2013): Remodelling of the palate: an additional tool to classify larval salamandrids through metamorphosis. – Vertebrate Zoology, **63**: 207 – 216.

Corsin, J. (1966): The development of the osteocranium of *Pleurodeles waltlii* Michahelles. – Journal of Morphology, **119**: 209 – 216.

Davit-Béal, T., Allizard, F. & Sire, J.-Y. (2006): Morphological variations in a tooth family through ontogeny in *Pleurodeles waltl* (Lissamphibia, Caudata). – Journal of Morphology, **267**: 1048 – 1065

Davit-Béal, T., Chisaka, H., Delgado, S. & Sire, H.-Y. (2007): Amphibian teeth. Current knowledge, unanswered questions, and some directions for future research. – Biological Reviews, **82**: 49 – 81.

Dingerkus, G. & Uhler, D. (1977): Enzyme clearing of Alcianblue stained whole small vertebrates for demonstration of cartilage. – Stain Technology, **53**: 229 – 232.

Djorović, A. & Kalezić, M.L. (2000): Paedogenesis in European newts (*Triturus*: Salamandridae): Cranial morphology during ontogeny. – Journal of Morphology, **243**: 127 – 139.

Eggert, B. (1934): Zur Überwinterung der Larven von *Molge alpestris* (Laur.) unter besonderer Berücksichtigung des Verhaltens der Schilddrüse. – Zeitschrift für wissenschaftliche Zoologie, **145**: 399 – 424.

Erdmann, K. (1933): Zur Entwicklung des knöchernen Skelets von *Triton* und *Rana* unter besonderer Berücksichtigung der Zeitfolge der Ossifikationen. – Zeitschrift für Anatomie und Entwicklungsgeschichte, **101**: 566 – 651.

Gabrion, J. & Chibon, P. (1973): Morphologie dentaire et disposition des dents chez des tritons neoteniques (*Tritrus helveticus* Raz.). – Comptes Rendus des Seances de la Societe de Biologie et de ses filiales, **167:** 50 – 54.

Greven, H. (1989): Teeth of extant amphibians: morphology and some implications. – Fortschritte der Zoologie, **35**: 451 – 455.

Greven, H. & Clemen, G. (1990): Effect of hyophysectomy on the structure of normal and ectopically transplanted teeth in larval and adult urodeles. – Acta Embryologiae et Morphologiae Experimentalis n.s., **11**: 33 – 43.

Greven, H., Jömann, E. & Clemen, G. (2006): Dentigerous bones, dentition and tooth systems in the hynobiid salamander *Ranodon sibiricus* Kessler, 1866 (Urodela). – Acta Biologica Benrodis, **13**: 195 – 217.

Greven, H., Richter, A. & Clemen, G. (2013): The formation of the secondary tongue in *Hynobius leechi* and *Ambystoma mexicanum* (Amphibia: Urodela). – Vertebrate Zoology, **63**: 217 – 232.

Günther, R. (ed., 1996): Die Amphibien und Reptilien Deutschlands. – Gustav Fischer, Stuttgart, Jena, pp. 826.

Haller-Probst, M. & Schleich, H.H. (1994): Vergleichende osteologische Untersuchungen an einigen Urodelen Eurasiens (Amphibia: Urodela, Salamandridae, Proteidae). – Courier Forschungsinstitut Senckenberg, **173**: 23 – 77.

Ivanović, A., Cvijanović, M., Denoël, M., Slijepčević, M. & Kalezić, M.L. (2014): Facultative paedomorphosis and the pattern of intra- and interspecific variation in cranial skeleton: lessons from European newts (*Ichthyosaura alpestris* and *Lissotriton vulgaris*). – Zoomorphology, **133**: 99 – 109

Iwasaki, F. & Wakahara, M. (1999): Adaptable larval life histories in different populations of the salamander, *Hynobius retardatus*, living in various habitats. – Zoological Science, **16**: 667 – 674.

Jömann, N., Clemen, G. & Greven, H. (2005): Notes on cranial ontogeny and delayed metamorphosis in the hynobiid salamander *Ranodon sibiricus* Kessler, 1866 (Urodela). – Annals of Anatomy, **187**: 305 – 321.

Kanki, K. & Wakahara, M. (2001): The possible contribution of pituitary hormones to the heterochronic development of gonads and external morphology in overwintered larvae of *Hynobius retardatus*. – International Journal of Developmental Biology, **45**: 725 – 732.

Kordges, A., Thiesmeier, B., Hornberg, C., Möller, U. & Greven, H. (2008): Kropfbildung und Pädomorphose in einer Population von Teichmolchen (*Lissotriton vulgaris*) bei Hattingen, NRW. – Jahrbuch für Feldherpetologie, **15**: 1 – 13.

Lebedkina, N.S. (2004): Evolution of the amphibian skull (translated by S.V. Smirnow). – Advances in Amphibian Research in the former Soviet Union, **9**: 1 – 265.

Marconi, M. & Simonetta, A.M. (1988): The morphology of the skull in neotenic and normal *Triturus vulgaris meridionalis* (Boulenger) (Amphibia, Caudata, Salamandridae). – Monitore zoologico italiano, **22**: 365 – 396.

Moriya, T. (1983a): The effect of temperature on the action of thyroid hormone and prolactin in larvae of the salamander *Hynobius retardatus*. – General and Comparative Endocrinology, **49**: 1 – 7.

Moriya, T. (1983b): Cytological changes induced by low temperature in the thyroid gland of larvae of the salamander *Hynobius retardatus*. – General and Comparative Endocrinology, **49**: 8 – 14.

Nishikawa, K. & Masafumi, M. (2008): A comparative study on the larval history in two populations of *Hynobius boulengeri* from Kyushu, Japan (Amphibia: Urodela). – Current Herpetology, **27**: 9 – 22.

Petranka, J.W. (1984): Soures of interpopulational variation in growth responses of larval salamanders. – Ecology, **65**: 1857 – 1865.

Regal, P.J. (1966): Feeding specialization and the classification of terrestrial salamanders. Evolution, **20**: 392 – 407,

Reilly, S.M. (1986): Ontogeny of cranial ossification in the eastern newt *Notophthalmus viridescens* (Caudata: Salamandridae) and its relationship to metamorphosis and neoteny. – Journal of Morphology, **188**: 215 – 326.

Reilly, S.M. (1987): Ontogeny of the hyobranchial apparatus in the salamanders *Ambystoma talpoideum* (Ambystomatidae) and *Notophthalmus viridescens* (Salamandridae): the ecological morphology of two neotenic strategies. – Journal of Morphology, **191**: 201 – 214.

Romeis, B. (1968): Histologische Technik. – Oldenbourg, München, pp. 757.

Rose, C.S. (1995a): Skeletal morphogenesis in the urodele skull: I. Postembryonic development in the Hemidactyliini (Amphibia: Plethodontidae). – Journal of Morphology, **223**: 125 – 148.

Rose, C.S. (1995b): Skeletal morphogenesis in the urodele skull: II. Effect of developmental stage in thyroid hormone-induced remodeling. – Journal of Morphology, **223**: 149–166.

Rose, C.S. (1995c): Skeletal morphogenesis in the urodele skull: III. Effect of hormone dosage in TH induced remodelling. – Journal of Morphology, **223**: 243 – 261

Rose, C.S. (1999): Hormonal control in larval development and evolution – Amphibians. – In: Hall, K. & Wake, M.H. (eds.): The origin and evolution of larval forms. – Academic Press, San Diego, pp. 167–217.

Rose, C.S. (2003): The developmental morphology of salamander skulls. – In: Heatwole, H. & M. Davies (eds.): Amphibian Biology, Vol. 5. Osteology. – Surrey Beatty & Sons, Chipping Norton, pp. 1684–1781.

Rose, C. & Reiss, J.O. (1993): Metamorphosis and the vertebrate skull: Ontogenetic patterns and developmental mechanisms. – In: Hanken, J. & Hall, B.K. (eds.): The skull, Vol. 1. – University of Chicago Press, Chicago, London, pp. 289–346.

Rumph, J.A. & Turner, W.J. (1998): Critical point drying for soft-bodied insect larvae. – Annals of the Entomological Society of America, **9**: 693–699.

Smirnov, S.V. & Vassilieva, A.B. (2001): The role of thyroid hormones in skull bone development in the ribbed newt *Pleurodeles waltl* (Urodela: Salamandridae). – Doklady Biological Sciences, **379**: 396–398.

Smirnov, S.V. & Vassilieva, A.B. (2003): Skeletal and dental ontogeny in the smooth newt, *Triturus vulgaris* (Urodela: Salamandridae): Role of the thyroid hormone and its regulation. – Russian Journal of Herpetology, **10**: 93 – 110.

Smirnov, S.V. & Vassilieva, A.B. (2005): Skull development in normal, TH-exposed, and goitrogen treated axolotls, *Ambystoma mexicanum*. – Russian Journal of Herpetology, **12**: 113–126.

Smirnov, S.V., Vassilieva, A.B. & Merkulova, K.S. (2011): Thyroid hormone mediation in skull development of Siberian newt, *Salamandrella keyserlingi* (Urodela: Hynobiidae), with comparison to other species. – Russian Journal of Herpetology, **18**: 203–209.

Stadtmüller, F. (1924): Studien am Urodelenschädel. I. Zur Entwicklungsgeschichte des Kopfskeletts der *Salamandra salamandra*. – Zeitschrift für Anatomie und Entwicklungsgeschichte, **75**: 149–225.

Stadtmüller, F. (1936): Kranium und Visceralskelett der Stegocephalen und Amphibien – In: Bolk, L., Göppert, E., Kallius, E. & Lubosch, W. (eds.): Handbuch der vergleichenden Anatomie der Wirbeltiere, Bd. 4. – Urban und Schwarzenberg, Berlin, Wien, pp. 501–698.

Trueb, L. (1993): Patterns of cranial diversity among the Lissamphibia. – In: Hanken, J. & Hall, B.K. (eds.): The skull, Vol. 2. – University of Chicago Press, Chicago, London, pp. 255 – 343.

Weitkamp, T., Haas, D., Wegrzynek, D. & Rack, A. (2011): ANKA phase: software for single-distance phase retrieval from inline X-ray phase-contrast radiographs. – Journal of Synchrotron Radiation, **18**: 617–629.

Wells, K.D. (2007): The ecology and behavior of amphibians. – The University of Chicago Press, Chicago, London, pp. 1148.

Wilder, I.W. (1925): The morphology of amphibian metamorphosis. – Smith College Publications, Northampton, **1**: 1–100.

Wintrebert, P. (1922): La voûte palatine des Salamandridae. Son évolution avant pendant et après la métamorphose suivant les conditions biologiques. – Bulletin biologique de la France et de la Belgique, **56**: 275–426.