

Vomeropterygopalatina in larval *Ichthyosaura alpestris apuanus* (Amphibia: Urodela) and comments on the formation of the definite vomer in the Salamandridae

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Abstract

We studied the mouth roof of some larval stages of the Alpine newt *Ichthyosaura alpestris apuanus*, collected in summer 1977 in the field, using total preparations stained with Alizarinred, X-ray computed microtomography, and scanning electron microscopy. In all specimens we found traits indicating a delayed metamorphosis, i.e. a temporarily slowed down or even halted development of traits dependent on thyroid hormones (TH), and a slight further growth of probably TH-independent traits. Especially noteworthy is the temporary partial fusion of vomer and palatine forming a vomeropterygopalatinum. Presence of vomeropterygopalatina was recently shown in overwintering larvae of the related species *Lissotriton vulgaris*. On the basis of these findings we suggest that vomeropterygopalatina appear to be a quite frequent side effect of delayed metamorphosis. Further, we hypothesize that large parts of the larval vomer become incorporated into the definite (transformed) vomer. Three larvae in the series showed developmental stages of the vomerine bar, a posterior outgrowth of the vomer, typical for salamandrids, which is formed around metamorphosis. Development of the vomerine bar begins on the front end of the larval vomer and not (as generally assumed) on its posterior inner edge. These findings support our previous view that the formation of the vomerine bar in *L. vulgaris* and *I. a. apuanus* is more complex than literature assumes. However, due to some shortcomings regarding number and preservation of the available larvae a deeper study is needed using standardized conditions and a more complete developmental series of these (protected) or related species to substantiate the observations reported here.

Kurzfassung

Wir haben die Munddächer einiger Larven des Bergmolchs *Ichthyosaura alpestris apuanus*, die im Jahre 1977 im Freiland gesammelt worden waren, mit Hilfe Alizarinrot gefärbter Totalpräparate, Röntgen-Computermikrotomographie und rasterelektronenmikroskopischer Aufnahmen untersucht und bei allen Larven Anzeichen einer verzögerten Metamorphose gefunden, d.h. einer zeitlich begrenzten Verlangsamung oder sogar Unterbrechung der Entwicklung von Elementen, die von Schilddrüsenhormonen (TH) abhängig sind, und eines, wenn auch verlangsamt, weiteren Wachstums weitgehend TH-unabhängiger Elemente. Besonders bemerkenswert ist die temporäre teilweise Fusion von Vomer und Palatinum. Solche Vomeropterygopalatina haben wir kürzlich auch bei überwinternden Larven der verwandten Art *Lissotriton vulgaris* nachgewiesen. Aufgrund dieser Befunde sind wir der Meinung, dass Vomeropterygopalatina eine durchaus häufige Begleiterscheinung verzögerter Metamorphosevorgänge sind. Zudem vermuten wir, dass der larvale Vomer weitgehend in den definitiven Adultvomer übernommen wird. Bei drei Larven war auch der Beginn der Entwicklung der für Salamandriden so typischen Vomerspange zu sehen. Offenbar beginnt sich diese am vorderen Teil des larvalen Vomer zu entwickeln und nicht, wie generell für Salamandriden angenommen wird, an seinem Hinterende. Dies unterstützt unsere frühere Hypothese, dass die Bildung der Vomerspange bei *L. vulgaris* und *I. a. apuanus* komplexer ist als in der Literatur angegeben wird. Aufgrund einiger Unzulänglichkeiten (Anzahl, Erhaltungszustand) des zur Verfügung stehenden Materials sind aber weitergehendere Untersuchungen, möglichst unter standardisierten Bedingungen und mit einer vollständigeren Entwicklungsreihe dieser (geschützten) oder verwandter Arten notwendig, um die hier mitgeteilten Beobachtungen zu bekräftigen.

Key words

Delayed metamorphosis, vomeropterygopalatinum, vomerine bar, salamandrids.

Introduction

Metamorphosis in Amphibia is characterized by the temporal sequence of processes, based on tissues having differential sensitivities to thyroid hormones (TH) and/or responding to different concentrations of TH. Further epigenetic tissue interactions and environmental cues may play a significant role. In transforming Urodela (= Caudata) most noticeable changes take place in the skull, which include loss of several skeletal elements, new formations and extensive remodelling of already existing elements (summarized in ROSE & REISS, 1993; ROSE, 1999, 2003).

Such changes are impressively seen in the tooth or dental systems. In fully developed larvae ('typical larvae') of Salamandridae dental systems in the mouth roof include the premaxilla (in this stage the paired anlagen are already fused in newts, whereas they remain separated in most salamanders), the vomers, and the palatopterygoids (palatine + pterygoid); each bone is accompanied by a dental lamina; the dental lamina of the palatine begins to disintegrate in this stage. Premaxillae or premaxilla (when fused) bear a single continuous row of non-pedicellate, monocuspid teeth, whereas each vomer and each palatine (palatal portion of the palatopterygoid) bear a tooth patch ("Zahnfeld") of likewise non-pedicellate, monocuspid teeth. Maxillae may start development or are already present, but incompletely.

The sequence of cranial ossification in salamandrids, especially of early skull bones that also include dentigerous bones varies among species (e.g., REILLY, 1986; SMIRNOV & VASSILIEVA, 2003, 2005; LEBEDKINA, 2004; VASSILIEVA & SERBINOVA, 2013 and table 3 there) and may also be affected by several environmental variables and intraspecific plasticity (SHELL *et al.*, 2015).

However, starting with a 'typical larva' the further development seems to take place in a more or less fixed order. Without taking in account that developmental events may overlap in part they include (1) the formation, ossification and elongation of maxillae and their colonization with teeth; (2) separation of the palatine and the pterygoid; (3) complete resorption of the palatal tooth patch and the palatine itself, which is preceded by the degradation of its dental lamina; (4) remodelling of the vomer, i.e. formation of a toothless anterior expansion that articulates with premaxilla and maxillae, and a postero-lateral expansion that supports the posterior margin of the choana as well as resorption of the vomerine tooth-patch and the larval vomer; (5) replacement of the tooth patch by a single tooth row that elongates posteriorly along the parasphenoid forming the dentate vomerine bar; and (6) gradual replacement of the larval non pedicellate monocuspid teeth by bicuspid pedicellate teeth via subpedicellate monocuspid teeth. Timing and chronological order of these events have been, more or less detailed and under different aspects, repeatedly described in several salamandrids (e.g., ERDMANN, 1933; Corsin, 1966; CLEMEN 1978 a, 1979; CLEMEN & GREVEN, 1979, 2013; GREVEN & CLEMEN, 1985, 1990; REILLY, 1986; ACCORDI

& MAZZARINI, 1992; SMIRNOV & VASSILIEVA, 2001, 2003; LEBEDKINA, 2004; DAVIT-BÉAL *et al.*, 2006; VASSILIEVA & SERBINOVA, 2013; GREVEN *et al.*, 2015).

Concerning dental bones in *Lissotriton vulgaris* (as a representative of newts) coronoid, palatine, dentary and vomer are early appearing not TH-inducible bones, whereas the late appearing maxillae are TH-inducible as well as the metamorphic skull remodelling, i.e. disintegration of the palatine, the anterior expansion of the vomer and the outgrowth of the vomerine bar (e.g., SMIRNOV & VASSILIEVA, 2003). Dentition reacts in a similar way, i.e. early larval events are TH-independent, whereas the gradual transition from mono- to bicuspid teeth is TH-dependent (see GABRION & CHIBON, 1973; GREVEN & CLEMEN, 1990; SMIRNOV & VASSILIEVA, 2003).

In case of an artificially or naturally induced acceleration or delay of metamorphosis, deviations from this pattern occur. There are several studies on various Urodela including Salamandridae, in which authors treated larvae of different ages either with TH to accelerate metamorphosis or with the goitrogen thiourea to retard it. These studies showed that the extent of such deviations depends on the developmental stage, on which the decelerating or accelerating agent operates (e.g., ROSE, 1995 b; SMIRNOV & VASSILIEVA, 2001, 2003, 2005; SMIRNOV *et al.*, 2011). In midlarval *L. vulgaris* TH-treatment accelerates processes normally occurring at metamorphosis, among other things the transition from monocuspid to bicuspid teeth, whereas thiourea-treatment for example decelerates resorption of bones that normally fully disintegrate, but the metamorphic skull remodelling generally does not proceed. Further, the transition from monocuspids to bicuspid fails to appear, but the disintegration of the palatal dental lamina proceeds (SMIRNOV & VASSILIEVA, 2003).

Based on such observations we previously hypothesized that in case of a delayed metamorphosis development of primarily TH-dependent traits will slow down or even temporarily stop, i.e. the development is "frozen" to a certain extent. The same may apply to TH-independent elements that may continue to growth albeit at a slower pace. Differences in the speed of development and growth may result in a mixture of further grown (largely TH-independent) and more or less unchanged (largely TH-dependent) traits. The latter may tell us something about the developmental stage, in which the retarding agent has started to affect TH-production provided that timing of events which characterize metamorphosis is not infinitely variable (see CLEMEN & GREVEN, 2013; GREVEN *et al.*, 2015).

Studying the dental systems of the mouth roof in larvae of the smooth newt *L. vulgaris* captured in the wild (see GREVEN *et al.*, 2015), we found that more than the half of the specimens examined showed clear signs of a delayed metamorphosis. As most of them were classified as overwinterers, we attributed this delay to low temperatures. One of the most striking features seen in the palate was the presence of a vomeropterygopalatinum, i.e. an at least partial fusion of the intact palatopterygoid (the fully dentate palatine connected with the pterygoid by a bony

bridge) with the vomer, which was hitherto considered as a very rare exception or even as a lab artifact caused by the external application of TH (e.g., SMIRNOV & VASSILIEVA, 2005). In the same series we found some stages suggesting that the development of the definite vomer consisting of the anterior toothless vomer plate, the vomer body, and the vomerine bar differs from what can be read in the literature on this matter (GREVEN *et al.*, 2015).

In the present study we examine a small number of larval *Ichthyosaura alpestris apuanus* collected 1977 in the field. Due to some more favourable developmental stages in this series, we are able (1) to broaden the spectrum of salamandrids forming a vomeropterygopalatinum, (2) to support our previous suggestion that the presence of a vomeropterygopalatinum may be ascribed to a delay of metamorphosis, (3) to give some further evidence that the vomeropterygopalatinum is a temporary phenomenon, and (3) to support our hypothesis concerning the formation of the definite (adult) vomer in the two species.

Material and methods

Specimens

Larvae of *Ichthyosaura alpestris apuanus* were collected by the late FRITZ REHBERG (Münster/Westf.) in the last week of June 1977 in two standing water bodies in the area of the Monte Maggiorasca (Italy) at approx. 1500 m above sea level. Unfortunately, details on the location are not available. Eight specimens were anesthetized and fixed in 80 % ethanol on-site by the gatherer. Later they were donated to one of us (G.C.), measured, decapitated, and transferred in buffered formalin according to Lillie for a while (see ROMEIS, 1968). Following removal of the lower jaw, specimens were stained with Alizarinred (some crystals in 30% ethanol) and then stored for years in 70% ethanol.

Total preparations

For the present study larvae were staged (according to CLEMEN & GREVEN, 2013; see also GREVEN *et al.*, 2015), restained with Alizarinred, if necessary, and transferred in glycerine. Photos were taken with a Keyence VHX 500F digital microscope. In some cases the oral mucosa was removed with fine forceps after the first inspection.

Scanning electron microscopy (SEM)

To assess mono- or bicuspidity of teeth more precisely than under a binocular, the stained heads were dehydrated in ethanol, air dried, sputtered with gold and examined with a SEM (Hitachi S-530). In a further step these preparations were rehydrated, treated with a KOH

solution to remove some of the soft tissue (to remove all was not possible, but generally replacement teeth, not yet attached, were not conserved), rinsed in aqua dest. dehydrated with Hexamethyldisilazane (HMDS) (RUMP & TURNER, 1998), sputtered again with gold and viewed in a SEM Leo 1430 (Fa. Zeiss). Due to the general poor preservation and contamination of the samples only a few results are pictured.

Synchrotron-based microtomography (μ CT)

An indirect detector system composed of a 12 μ m LSO:Tb scintillator, diffraction limited optical microscope (Optique Peter) and 12 bit pco.dimax high speed camera (2016 x 2016 pixels resolution) was employed to capture 3000 projections per tomographic scan with an exposure time of 40 ms each.

Specimens were scanned with optical magnifications of 1.8 \times (larva 7) and 3.6 \times (larvae 2 and 6), resulting in effective pixel sizes of 3.06 μ m and 1.53 μ m, respectively.

Prior to volume reconstruction, all projection images were processed with the phase retrieval ImageJ plugin ANKAphase (WEITKAMP *et al.*, 2011). Volume reconstruction was done by the PyHST software developed by the European Synchrotron Radiation Facility, Grenoble, France, and KIT (CHILINGARYAN *et al.*, 2011)

Terminology and abbreviations used in the figures

Terminology largely follows TRUEB (1993) and ROSE (2003). Teeth of the first tooth generation of a given dentigerous bone are called primary teeth. As in our previous article (GREVEN *et al.*, 2015) position of the heads in the text and legends refers to the anatomical side.

Abbreviations: ch = *choana* (choana); ey = *eye*; m = *maxillare* (maxilla); im = *glandula intermaxillaris* (intermaxillary gland); n = *nasale*; os = *orbitosphenoidium* (orbitosphenoid); pdp = *processus dorsalis praemaxillaris* (*pars praenasalis, pars dorsalis, pars frontalis*, frontal spine, alary process of premaxilla); pf = *praefrontale* (prefrontal); pfm = *processus facialis maxillaris*; pl = *palatinum* (palatine); pm = *praemaxillare* (premaxilla); pp = *pars palatina* (lingual palatal shelf); ps = *parasphenoidium* (parasphenoid); pt = *pterygoideum* (pterygoid); ptp = *pterygopalatinum* (palatopterygoid); tvb = *teeth of the vomerine bar*; v = *vomer* (vomer); vb = *vomerine bar*; vpl = *vomerine plate*; vpp = *vomeropterygopalatinum*.

Results

The following description focuses on the tooth bearing bones of the mouth roof, i.e. the upper jaw consisting

Tab. 1: Various parameters, preparations, putative stage, in which the delay of metamorphosis has started (“frozen” stage), and further growth and development beyond the “frozen” stage of the eight (1-8) larvae of *Ichthyosaura alpestris apuanus* examined. TL = total length, SV = snout-vent length. For further details see text.

	TL and SV (cm)	Stage (St) and gross morphology (M)	Preparation	Stage (St), in which the causal agents probably started to act, and organisation of the mouth roof with traits typical for the respective stage (TT) and traits indicating further growth and development (FD)	Fig.
1	TL: 4.8 SV: 2.5	St: I–II M: Lip fringes, gills and primary tongue	Alizarin, SEM	St: Slightly before a typical young larva TT: Teeth monocuspid; dentate premaxilla, incomplete edentate maxillae; large tooth-patch on the palatal portion of the palatopterygoid, FD: Vomeropterygopalatina with large tooth-patches of similar size on both parts;	1 A, 2 A
2	TL: 3.9 SV: 2.0	St: II M: As in larva 1	Alizarin, μ CT,	St: Typical larva TT: Teeth monocuspid; number of teeth on the vomer and palatine similar FD: Maxillae overlap premaxilla; vomeropterygopalatina with vomerine teeth on the palatal portion; common tooth patch of vomer and palatine (right side)	3 A–C
3	TL: 3.5 SV: 1.8	St: II–III M: As in larva 1	Alizarin, SEM	St: Slightly beyond typical larva TT: Teeth monocuspid; maxillae elongated, not joined with the premaxilla; vomers and palatines have similarly-sized tooth patches FD: Vomeropterygopalatina; vomerine replacement teeth up to the level of the palatal tooth patch	1 B 2 B, C
4	TL: 4.6 SC: 2.4	St: III M: Small glandular anlage of the secondary tongue in front of the primary tongue	Alizarin, SEM	St: Late larval stage TT: Teeth monocuspid: maxillae with some teeth; anterior und lateral expansion of the vomer; palatal replacement teeth absent, palatine with few teeth FD: Relatively large maxillae, bicuspid vomerine replacement teeth	1 C 2 D, E
5	TL: 5.6 SV: 2.9	St: IV M: Somewhat larger and slightly elevated glandular anlage of the secondary tongue	Alizarin, SEM	St: Larva at the onset of metamorphosis TT: Teeth monocuspid; broad pars palatina of premaxilla; anterior maxillae joined to the premaxilla and bear teeth; degradation of the pterygopalatal bony bridge; reduced palatal tooth patch, palatal replacement teeth absent FD: Posteriorly elongated maxillae; posteriorly enlarged vomerine tooth patches; growth of the vomerine tooth plate; start of formation of the prospective tooth row of the vomerine bar; bicuspid vomerine teeth	1 D, E 2 F–I
6	TL: 3.9 SV: 1.9	St: V M: The glandular area of the secondary tongue is elevated and clearly separated from the primary tongue	Alizarin, μ CT,	St: Midmetamorphic larva TT: Mono- (upper jaw, larval vomer, prospective vomerine bar) and bicuspid (prospective vomerine bar) teeth; maxillae near the premaxilla; maxillae posteriorly elongated; short tooth row on the prospective vomerine bar FD: Large vomerine plates, vomer posteriorly elongated, Dentate bony islets between vomer and palatine (degrading vomeropterygopalatinum); field of old replacement teeth between the larval vomer und the matrix of vomerine bar	1 F, G 3 D–F
7	TL: 4.1 SV: 2.1	St: V M: As larva 6	Alizarin, μ CT	St: Midmetamorphic larva TT: Mono- (upper jaw, larval vomer, prospective vomerine bar) and bicuspid (prospective vomerine bar) teeth; maxillae near the premaxilla; maxillae posteriorly elongated; short tooth row on the prospective vomerine bar FD: Large vomerine plates, vomer posteriorly elongated, Dentate bony islets between vomer and palatine (degrading vomeropterygopalatinum); field of old replacement teeth between the larval vomer und the matrix of vomerine bar	1 H 4 A–E
8	TL: 3.8 SV: 1.9	St: V–VI M: Gill stumps, glandular field of the secondary tongue at the height of the primary tongue	Alizarin, SEM	St: Larva slightly beyond midmetamorphosis TT: Teeth monocuspid (upper jaw, vomer, prospective vomerine bar, palatine) and bicuspid (prospective vomerine bar; few teeth on the maxillae; maxillae join the premaxilla; vomerine plates articulate with the partes palatinae of the premaxilla; FD: Elongation of the maxillae, expansion of the vomerine plate, bony islets between vomer and palatine, non-anklosed teeth between the matrix of the vomerine bar and the larval vomer.	1 I

of the already fused premaxillae (therefore referred to as premaxilla hereafter) and the paired maxillae (so far present), and the palate, i.e. the paired vomers and the paired pterygopalatina. Unless otherwise specified, teeth and teeth numbers refer to monocuspid established teeth. Some of the comments added to the descriptions will be examined in greater detail in the discussion.

Table 1 summarizes the main data of the larvae examined, i.e. their treatment, their size and external appearance, their stage according to traits in the mouth roof typical for this stage (TT) and supposed deviations of largely TH-dependent or TH-independent traits.

Larva 1

(Stage I – II; Fig. 1 A, 2 A)

Upper jaw: The premaxilla bears 32 teeth and two non ankylosed teeth beyond its ossified ends; posterolateral to the premaxilla maxillae are present as small toothless ossifications extending approximately at the height of each choana (Fig. 1 A).

Palate: On both sides dentate vomers and dentate palatines are fused in the area of their *partes dentales* forming vomeropterygopalatina, as palatines are still connected with the pterygoid by a broad bony bridge. Replacement

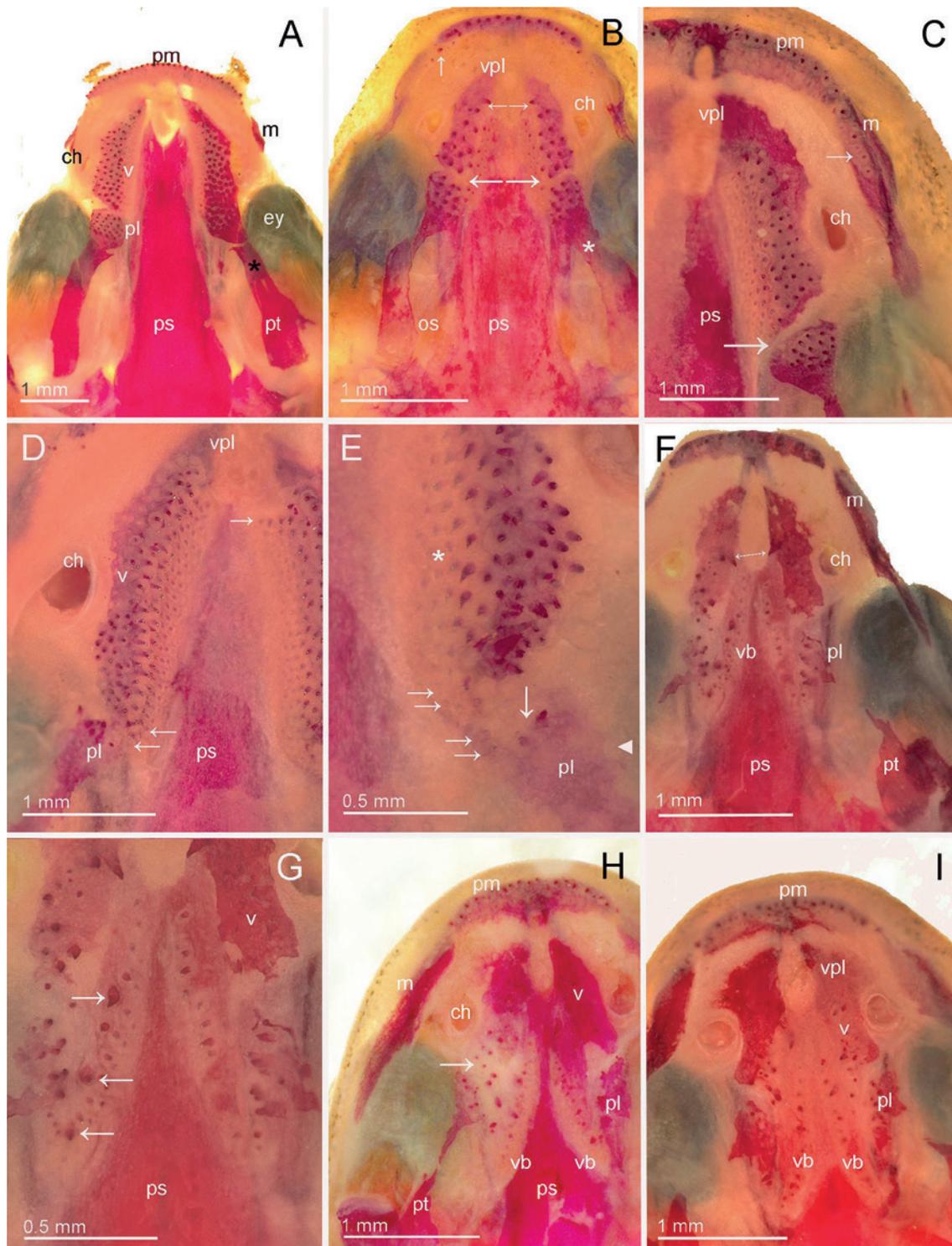


Fig. 1 A–I. Mouth roof of larval stages of *Ichthyosaura alpestris apuanus* stained *in toto* with Alizarinred. **A** Larva 1 (stage I–II); note edentate maxillae (m), the fused vomer (v) and palatine (pl) with a single tooth patch (left). **B** Larva 3 (stage II–III); note primary teeth of maxillae (arrow), the separation of vomerine and palatinal tooth patches (large arrows), and the large most anterior teeth (small arrows). **C** Larva 4 (stage III; left side); primary teeth of the maxilla (arrow) terminate before the choana (ch); tooth patches of the vomer and the palatine are separated (large arrow). **D** Larva 5 (stage IV); large vomerine (v) and small palatine (pl) with degrading tooth patch. Note the transversal arranged teeth (arrow). Replacement teeth extend beyond the posterior edge of the vomer (two arrows). **E** Larva 5 (stage IV; detail left); note the degrading (arrowhead) palatine (pl) and the large teeth on its anterior part. Vomerine replacement teeth (asterisk) bridge the gap between vomer and palatinum (small arrows). **F** Larva 6 (stage V); note the long edentate maxilla (m), the larval portion of the vomer (v) with large teeth anteriorly (double arrow), and the degrading palatine (pl) are largely edentate; tooth row of the prospective vomerine bar (vb). **G** Ditto, note the large larval tooth on each side (upright arrows) followed by the tooth row of the prospective vomerine bar (vb); large monocuspid teeth on the buccal side of the vomerine bar (horizontal arrows). **H** Larva 7 (stage V); note the largely edentate vomer (v), the large larval teeth on the buccal side of the right vomerine bar (arrow), the row of smaller teeth of the vomerine bar (vb), and partly dentate “islets” of the degrading palatine (pl), the separation of the palatine (pl) and the vomer (v). **I** Larva 8 (stage V–VI); note the vomerine plate (vpl); the largely edentate “larval” vomer (v) separated from the degrading palatine. For further abbreviations see above.

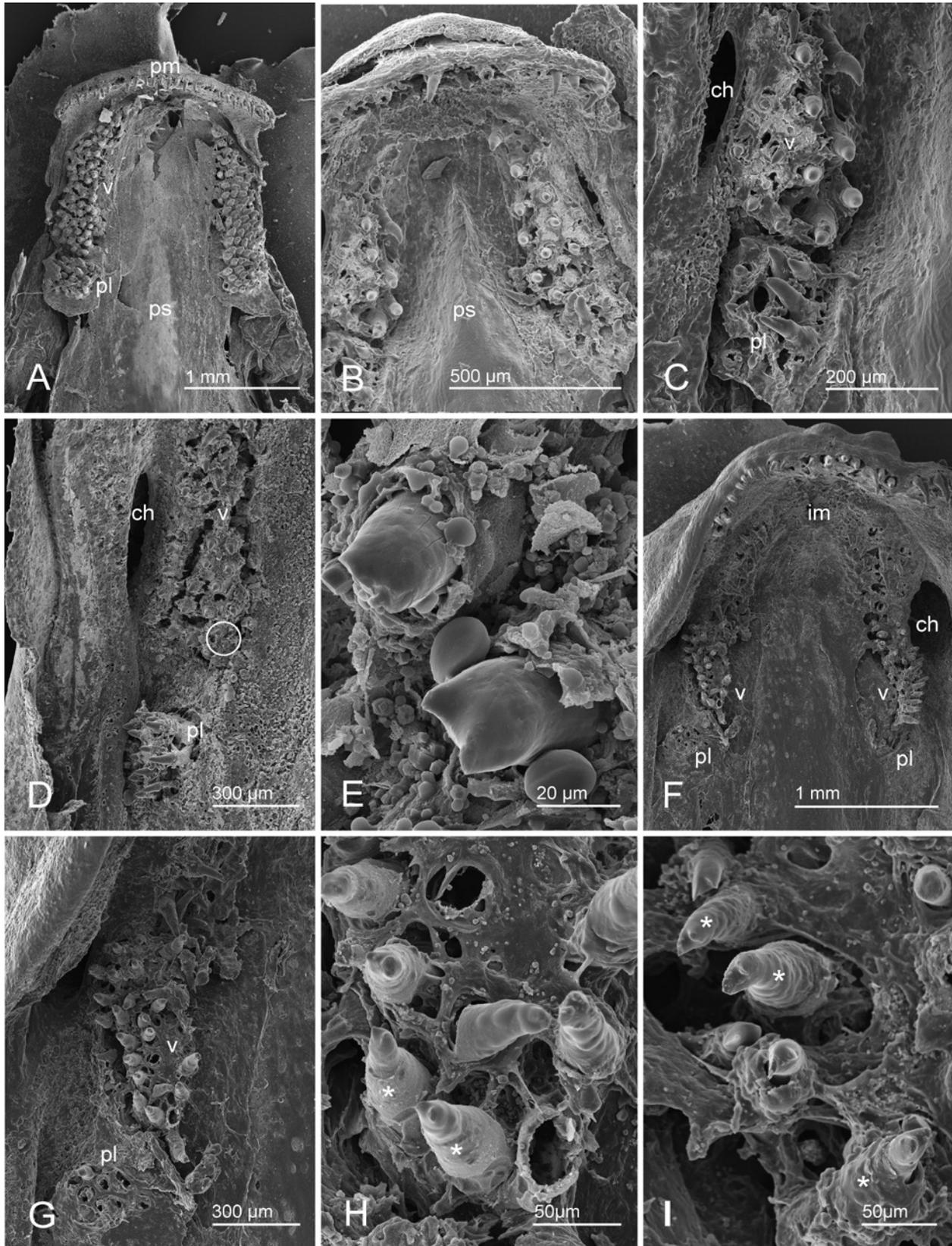


Fig. 2 A–I. SEM–images of the dental systems in the mouth roof of larval *Ichthyosaura alpestris apuanaus*, ventral view. **A** Larva 1 (stage I–II; see Fig. 1 A). **B** Larva 3 (stage II–III; see Fig. 1 B). **C** Ditto; right half of the palate. **D** Larve 4 (stage III; see Fig. 1 C), right palate. The circle labels the bicuspid teeth shown in E. **E** Ditto; bicuspid teeth at the posterior edge of the vomer. **F** Larva 5 (stage IV; see Fig. 1 D, E). **G** Ditto; right palate. **H** Ditto; tooth patch of bicuspid teeth (asterisks) on the posterior end of the vomer (right side). **I** Ditto; left vomer; bicuspidity of teeth is more pronounced (asterisks). For abbreviations see above.

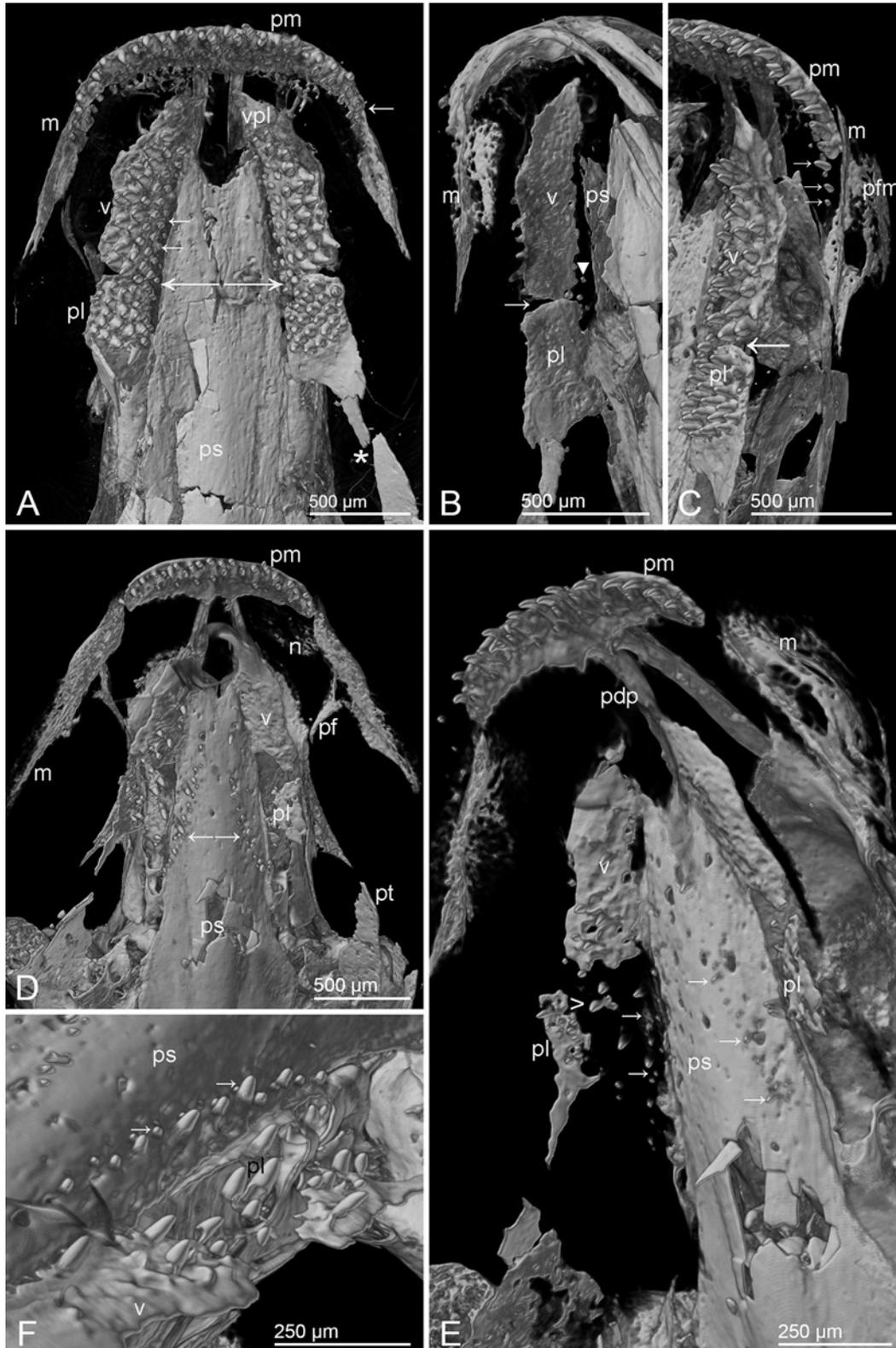


Fig. 3 A–F. μ CT images of the mouth roof of *Ichthyosaura alpestris apuanus* larva 2 (stage II; A – C), and larva 6 (stage V; D–F; see Fig. 1 F). **A** Ventral view; maxillae join the premaxilla, fusion (arrows) of the vomer (v) and palatine (pl); replacement teeth of the vomer (small arrows); bony bridge of the palatopterygoid artificially broken (asterisk); vomer plate (vpl). **B** Left side, dorsal view; maxilla (m), fused (arrow) vomer (v) and palatine (pl), vomerine replacement teeth (arrowhead). **C** Ventral view, left side slightly tilted; dentate premaxilla (pm) and primary non ankylosed teeth (small arrows) of the future maxilla (m); vomer (v) and palatine (pl) fused (arrow). **D** Ventral view; note the elongated maxillae (m) and the tooth row of the prospective vomerine bar (arrows); pterygoid (pt), prefrontal (pf), ossifying nasale (n). **E** Ventral view, right side, slightly tilted; closer moved premaxilla (pm) and maxilla (m), non ankylosed teeth of the vomerine bar and between vomerine bar and larval vomer (arrows); teeth of the prospective vomerine bar (larger arrows); remains of the palatine (pl); putative degradation of a former connection (arrowhead) of vomer (v) and palatine (pl); *processus dorsalis praemaxillaris* (pdp). **F** Detail of the palate, ventral view, right side; vomer (v) and palate (pl) are separated. Bicuspid and (buccally) large monocuspid teeth of the prospective vomerine bar (arrows). For further abbreviations see above.

teeth are seen along the inner face of all dentigerous teeth. Along the palatines their number is reduced and only older developmental stages are present.

Right side: The vomer bears approx. 53 teeth (23 in the posterior part, i.e. behind the posterior edge of the choana) in up to six rows (posteriorly) (Fig. 1 A). Its tooth-patch is clearly separated from the palatinal tooth-patch that bears 25 teeth. Vomer and palatine are fused at their inner (lingual) face leaving a wide notch at the opposite side (Fig. 1 A, 2 A).

Left side: Vomer and palatine appear completely fused possessing a 'common' tooth-patch of approx. 79 teeth in up to six rows (posteriorly) (Fig. 1 A). Approximately 30 teeth are beyond the putative zone of fusion (Fig. 1 A, 2 A).

Comments: We classify this larva as stage slightly before a "typical young" larva on the basis of the largely monocuspid established teeth, the dentate premaxilla, the incomplete and edentate maxillae and the large tooth-patch on each palatine. Further growth and development are indicated by the presence of vomeropterygopalatina with larger tooth-patches on the vomerine portion. Absence of younger stages of replacement teeth along the palatine suggests the resorption of its dental lamina. Therefore, the established monocuspid teeth and the older replacement teeth on the inner margin of the palatine represent the last tooth generations of this element, whereas the teeth between the vomer and palatine and on the anterior part of the palatines must be provided by the vomerine dental lamina.

Larva 2

(Stage II; Fig. 3 A–C)

Upper jaw: The premaxilla with approx. 17 teeth is close to the maxillae. Each maxillae extending beyond the vomer has three non-established primary teeth anteriorly; their *partes faciales maxillares* are large, but not completely ossified (Fig. 3 A - C).

Palate: The vomers are expanded medio-anteriorly (parts of the prospective edentate vomerine plate. The dentate parts of the vomers and the palatopterygoids are fused on both sides to some extent (Fig. 3 A, B). Palatines are broadly connected with the pterygoid.

Right side: The vomer bears 15 teeth (6 in the posterior part; see above) in up to three rows. At least five large and approx. 13 small replacement teeth are present (Fig. 3 A, C). The vomerine tooth patch is not clearly separated from the palatinal tooth patch that has 18 established teeth arranged in four rows. In the putative zone of fusion seven and along the palatine four replacement teeth are present (Fig. 3 A).

Left side: Vomer and palatinum are fused lingually leaving a small notch on the buccal side. Tooth patches of both elements are clearly separated from each other (Fig. 3 A, C). The vomer has 17 teeth (8 in the posterior part) and 4 large as well as approx. 15 small replacement teeth that reach the middle of the palatine. The palatinal portion bears five rows of altogether 19 teeth and has 3

replacement teeth. In the zone of fusion approx. 5 replacement teeth are present (Fig. 3 A).

Comments: We classified this larva as 'typical' on the basis of the monocuspid teeth (for further details see larva 1) and the similar number of teeth on the vomer and on the palatine (in younger larvae the number of palatinal teeth is higher). The low number of replacement teeth along the palatine indicates the further degradation of its dental lamina. Further growth and development are indicated again by the presence of vomeropterygopalatina, by replacement teeth from the vomerine dental lamina that extend beyond the zone of fusion, the maxillae overlapping the premaxilla and the first primary teeth of maxillae.

Larva 3

(Stage II–III; Fig. 1 B, 2 B, C)

Upper jaw: The premaxilla bears 15 teeth; maxillae extend to the level of the anterior edge of the eye. On both sides there is a gap between the premaxilla and the anterior ossified part of maxillae. Here 3 primary teeth are present. Two of these teeth are seen at the height of the ossifying anterior part of maxillae; a single tooth occupies the gap (Fig. 1 B).

Palate: Vomers and palatopterygoids are fused partially; palatines are connected with the pterygoid. Parts of the prospective edentate vomerine plate approach the intermaxillary gland medio-anteriorly. Each vomer bears approx. 15 teeth anteriorly in up to four rows (Fig. 1 B). Number of teeth on the vomerine parts before and behind the choanae is similar. The vomerine tooth patches are clearly separated from the palatinal tooth patches, but bones are fused at their inner (lingual) face (Fig. 1 B, 2 B). Vomerine replacement teeth extend beyond the zone of fusion. On the right side are 2 larval replacement teeth very probably derived from the vomerine dental lamina, as younger palatinal replacement teeth are missing (Fig. 1 B, 2 C). Palatines bear up to 4 rows of teeth with 14 established and 3 replacement teeth on the left side and 15 teeth plus 1 replacement tooth on the right side provided from the palatinal dental lamina. These replacement teeth are seen in the middle part of the palatine (Fig. 1 B).

Comments: We classified this larva as 'typical' on the basis of the monocuspid teeth, the gap between premaxilla and maxillae, and the similarly sized tooth-patches on the vomerine and palatinal portion. Further growth and development are indicated by the posterior elongation of the maxillae, the presence of vomeropterygopalatina and replacement teeth of vomerine origin at the level of the palatinal tooth patch. The palatinal dental laminae appear largely resorbed as posteriorly replacement teeth are missing.

Larva 4

(Stage III; Fig. 1 C; 2 D, E)

Upper jaw: The premaxilla bears 30 teeth. The anterior part of each maxilla bears at least 4 established and some

non-established (primary) teeth. Posteriorly the maxillae extend to the height of the anterior margin of the eye (Fig. 1 C).

Palate: Vomers and pterygopalatines are clearly separated from each other. The posterior margins of the vomers are fissured. The posterior inner part the vomer extends up to the palatines and is accompanied by a broad area of bicuspid replacement teeth extending up to the first third of the palatine (Fig. 1 C). The sample preparation does not allow to decide definitely, whether the bicuspid teeth in Figure 2 D (encircled) and E are already established or replacement teeth. However, their position and size speaks for the latter, although most newly developing teeth, not yet attached, usually were not conserved during the preparation procedure. Vomers are enlarged antero-medially and laterally extending up to the choanae. Palatines are fully connected with the pterygoid (Fig. 2 D, E).

The right vomer bears 38 (20 in the posterior part), the left vomer 33 teeth (16 in the posterior part), which are posteriorly arranged in 5 and anteriorly in 1 or 2 two rows. Each palatine bears approx. 15 teeth; replacement teeth are missing (Fig. 1 C).

Comments: We classified this larva as ‘late larval stage’ on the basis of the teeth that are still largely monocuspid, the maxillae bearing some teeth, the expansion of the edentate vomerine plate, the still existing palatinal tooth patches, the absence of palatinal replacement teeth, and the small, but still intact pterygopalatinal bony bridge. Further growth and development are indicated by the anteriorly more elongated maxillae, and the bicuspid vomerine replacement teeth.

Larva 5

(Stage IV; Fig. 1 D, E, 2 F–I)

Upper jaw: The praemaxilla bears approx. 37 teeth. Maxillae join the premaxilla bearing some monocuspid primary teeth anteriorly; posteriorly they extend to the level of the centre of the eye (Fig. 1 D).

Palate: Parts of the edentate vomerine plate have continued to grow. Replacement teeth along the palatines are missing.

Right side: The vomer bears approx. 81 teeth in up to six rows. With 47 largely bicuspid teeth; the posterior part is clearly enlarged (Fig. 1 D, 2 H). The vomer is accompanied by a broad field of bicuspid replacement teeth (Fig. 1 D, E). The regressive palatine is close to the vomer, and seems to be connected with it (Fig. 2 F, G). The palatine bears 7 degrading teeth anteriorly; it is still connected with the pterygoid, but margins of the bony bridge are fissured (Fig. 1 D).

Left side: The vomer bears 72 largely bicuspid in up to six rows (42 in the posterior part). The broad area of replacement teeth extends to the height of the palatinal teeth (Fig. 1 E). In front of the vomerine tooth patch are four transversally arranged monocuspid teeth (Fig. 1 D). Some small replacement teeth are in the gap between

vomer and palatine. The degrading palatine bears 2 teeth. It is entirely separated from the pterygoid (Fig. 1 D, 2 I).

Comments: We classified this larva as “larva at the onset of metamorphosis” on the basis of the broad *pars palatina* of the premaxilla, the maxillae joined to the premaxilla with a few teeth anteriorly, degradation of the pterygopalatinal bridge, the small palatinal tooth patches and the absence of palatinal replacement teeth. Further growth and development are indicated by the posteriorly elongated maxillae, the largeness of the vomerine tooth patch, by the growth of the vomerine plate, and the putative separation from vomer and palatine of a previous vomeropterygopalatinum, the onset of the formation of the vomerine bar indicated by the transversally arranged teeth and the enlargement of the area of the vomerine replacement teeth. Noteworthy but somewhat enigmatic are the bicuspid teeth on the larval vomer (see discussion). This larvae seems to be caught by the metamorphic stimulus responsible for the development of several generations of bicuspid teeth (established and replacement teeth) and further indicated by the degrading bony bridge.

Larva 6

(Stage V; Fig. 1 F, G, 3 D–F)

Upper jaw: The premaxilla bears 16 teeth. Maxillae extend to the posterior margin of the eye, but do not join the premaxilla anteriorly. On the right side three primary teeth are located in the gap between premaxilla and maxilla. The large *partes faciales maxillares* are incompletely ossified (Fig. 1 F, 3 E).

Palate: Vomers show a broad expansion medio-anteriorly on both sides (Fig. 1 F, G, 3 E). Their dentate part and remnants of the palatines are widely separated from each other and also palatines are separated from the pterygoid (Fig. 1 F, 3 E). The posterior border of the larval vomer shows large resorption lacunae (Fig. 1 G, 3 E, F). Anteriorly in front of the vomerine tooth patch large transversally arranged teeth are located (Fig. 1 F, G).

Right side: The larval part of the vomer bears a reduced tooth patch (approx. 10 teeth) and some degrading teeth. The prospective vomerine bar has a row of 12 bicuspid teeth and some large monocuspid teeth with a dividing zone; these teeth are not attached to a bony substrate. Between this row and the larval vomer a few small monospids are seen that likewise are non ankylosed (Fig. 1 G, 3 F).

Left side: Anteriorly the left vomer bears a single large monocuspid tooth at the inner margin (Fig. 1 F, G). The prospective vomerine bar is indicated by a row of 8 bicuspid non ankylosed teeth; some of the largest teeth are monocuspid. Between the vomer and the tooth row are few small non ankylosed monospids (Fig. 1 G, 3 F).

In the gap that separates (larval) vomers and palatines, dentate or edentate bony “islets” and degrading teeth are present (Fig. 3 E). The palatinal tooth patches disintegrate (Fig. 3 E, F).

Comments: We classified this larva as “midmetamorphic” on the basis of the still monocuspid established teeth, the *partes faciales maxillares* ossified only in part, the degradation of the pterygopalatal bony bridge, the wide separation of the vomer and the still not fully degraded palatine, the few large monocuspid degrading teeth of the larval vomer as well as the mono- (upper jaw, larval vomer, prospective vomerine bar) and bicuspid (prospective vomerine bar) teeth. Further growth and development are indicated by the posterior elongation of the still edentate maxillae, the further expansion of the vomerine plate, the bony islets between vomer and palatine indicating perhaps the previous existence of a vomeropterygopalatinum.

Larva 7

(Stage V; Fig. 1 H, 4 A–E)

Upper jaw: Premaxilla and maxillae are not joined; the former bears 15 teeth and has a small *pars palatina*. The long edentate maxillae reach up to the posterior margin of the eye; they are clearly separated from the premaxilla. In the gap between both elements few primary non-established teeth are seen on the right side (Fig. 1 H, 4 A, B).

Palate: On both sides the vomerine plate extends beyond the choanae and surrounds the intermaxillary gland anteriorly. The (larval) vomerine tooth patches bear only a few degrading teeth. A row of predominantly bicuspid non ankylosed teeth indicates the course of the prospective vomerine bar. This row parallels the larval portion of the vomer at a slight distance and extends beyond the palatines, where they are slightly curved outward (Fig. 1 H, 4 C, D). Between the prospective vomerine bar and the larval vomer some monocuspid non ankylosed divided teeth and some smaller monocuspid crowns are present. Larval vomers are separated from palatal remnants (Fig. 4 C, D); the latter are separated from the pterygoids.

Right side: The larval vomer portion bears approx. 7 degrading teeth. In the front of the former larval tooth patch 2 two large divided teeth are transversally arranged. The innermost is followed by primary bicuspid teeth not attached to substrate and arranged in one row (Fig. 1 H, 4 A, C, D). The gap between vomer and palatine contains bony pieces with degrading teeth. The palatine bears six degrading teeth (Fig. 1 H, 4 E). It is separated from the pterygoid, but residues of the pterygoidal bridge are still present (Fig. 4 B, E).

Left side: Anteriorly the larval vomer bears a single, largely complete tooth and some remnants of teeth followed by a row with of small bicuspid teeth, similar as on the right side (Fig. 4 A, B). The palatine shows large resorption pits and bears 2 degrading teeth. Some teeth are ankylosed on a small piece of bone between palatine and vomer (Fig. 1 H, 4 B).

Comments We classified this larva as “midmetamorphic larva” on the basis of the monocuspid (upper jaw) and bicuspid teeth (of the prospective vomerine bar), the proximity of premaxilla and maxillae, the small *pars palatina*

of premaxilla, the *partes faciales maxillares* that reach far in dorsal direction, a short tooth row of non ankylosed bicuspid and monocuspid teeth in the area of the future vomerine bar, and the still dentate larval vomer. Further development is indicated by bony fragments between vomer and palatinum, which may indicate a secondary separation of a previously existing vomeropterygopalatinum. The few non ankylosed teeth between the larval vomer and the not yet ossified matrix of the vomerine bar seem to represent previous replacement teeth.

Larva 8 (Stage V-VI) (Fig. 1 I)

Upper jaw: The premaxilla bears approx. 17 teeth; maxillae joining the premaxilla bear two primary monocuspid teeth at each side; maxillae reach the posterior border of the eye. Both elements have broad *partes palatinae* (Fig. 1 I).

Palate: Vomers are anteriorly and medially expanded enclosing the choana anteriorly. The dentate portion bears a few largely non degraded teeth and some more degraded teeth. The most anterior teeth are at the height of the anterior margins of the choana (left side) (Fig. 1 I). A few monocuspid non ankylosed teeth are present between the larval vomer and the row of teeth of the prospective vomerine bar. This row contains large monocuspid and smaller bicuspid teeth, extends beyond the palatal tooth patches and is curved posteriorly. The right (very large) palatine bears anteriorly 2 teeth; the left one has 8 degrading teeth. In the gap between vomer and palatine there are some bony islets bearing teeth. Palatines are separated from the pterygoid (Fig. 1 I).

Comments: We classified this larva as “slightly beyond midmetamorphosis” (see also larva 7) based on the still monocuspid teeth on the upper jaw, and the non ankylosed monocuspid and bicuspid teeth on the prospective vomerine bar, the poorly dentate maxillae and the large vomer. Further growth and development is indicated by various bony remnants, dentate in part.

Discussion

Our previous article (GREVEN *et al.*, 2015) and the present study show again that it is hardly possible, if any, to infer any discrete metamorphic change in the mouth roof of urodele larvae alone from their external appearance (see also LEBEDKINA, 2004). Most changes in the mouth roof are relatively easy to recognize allowing a more precise staging at least of certain periods in the life of a urodele species (e.g., CLEMEN & GREVEN, 2013; GREVEN & CLEMEN, 2015 and further literature therein).

Articles studying developmental sequences of the urodele skull and dental systems are relatively selective (see citations in the introduction) and are often scarcely comparable either because of unclear criteria for the

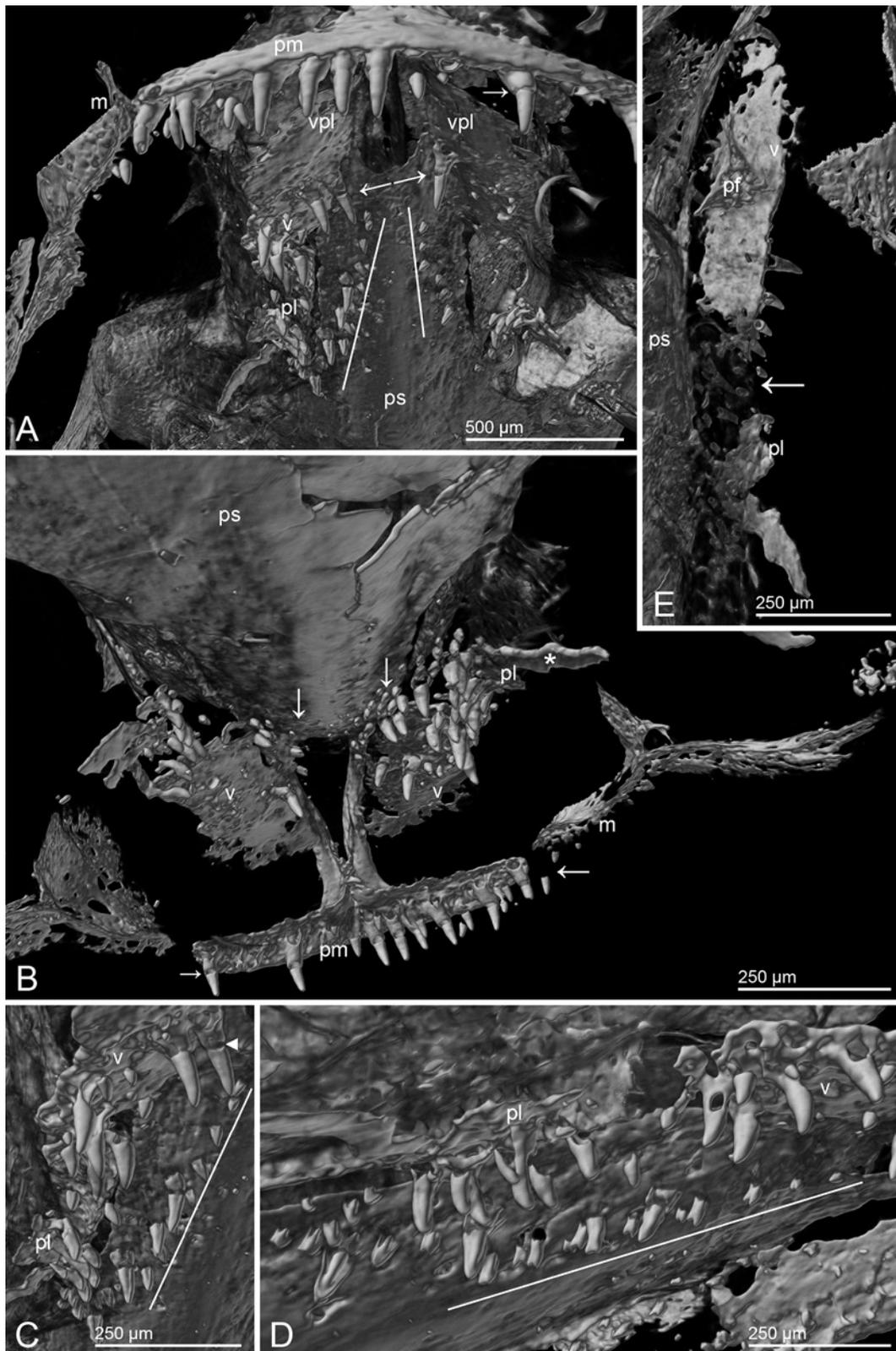


Fig. 4 A–D. μ CT images of parts of the palate (mouth roof) of *Ichthyosaura alpestris apuanus* larva 7 (stage V; C–F; see Fig. 1 H). **A** Ventral view; premaxilla (pm) with subpedicellate teeth (small arrow), maxilla (m) and vomer plate (vpl). Note the large transversally arranged anterior vomer teeth (arrows), the teeth of the prospective vomerine bar (lines), and large pedicellate teeth and some further teeth between vomer and prospective bar. Note the different degree of tooth patch degradation on the two largely intact vomers (v). **B** Ventral view; note non ankylosed primary teeth (arrow) between premaxilla (pm) and maxilla (m); fragmented palatine (pl); pedicellate monocuspid non ankylosed replacement tooth of the prospective vomerine bar (large vertical arrows); remains of the palatal bony bridge (asterisk). **C** Detail of the right vomer (v) and palatine (pl) with pedicellate monocuspids (arrowhead) in front and teeth of the prospective vomerine bar (line, see figure A). **D** Detail of the vomer (v) and the palatine (pl), both with some teeth; tooth row of the vomerine bar mit bicuspid, and some larger monocuspids (line). **E** Palate, dorsal view; right side; vomer (v) and palatine (pl); note bony islets between the two bones (arrow). For further abbreviations see above.

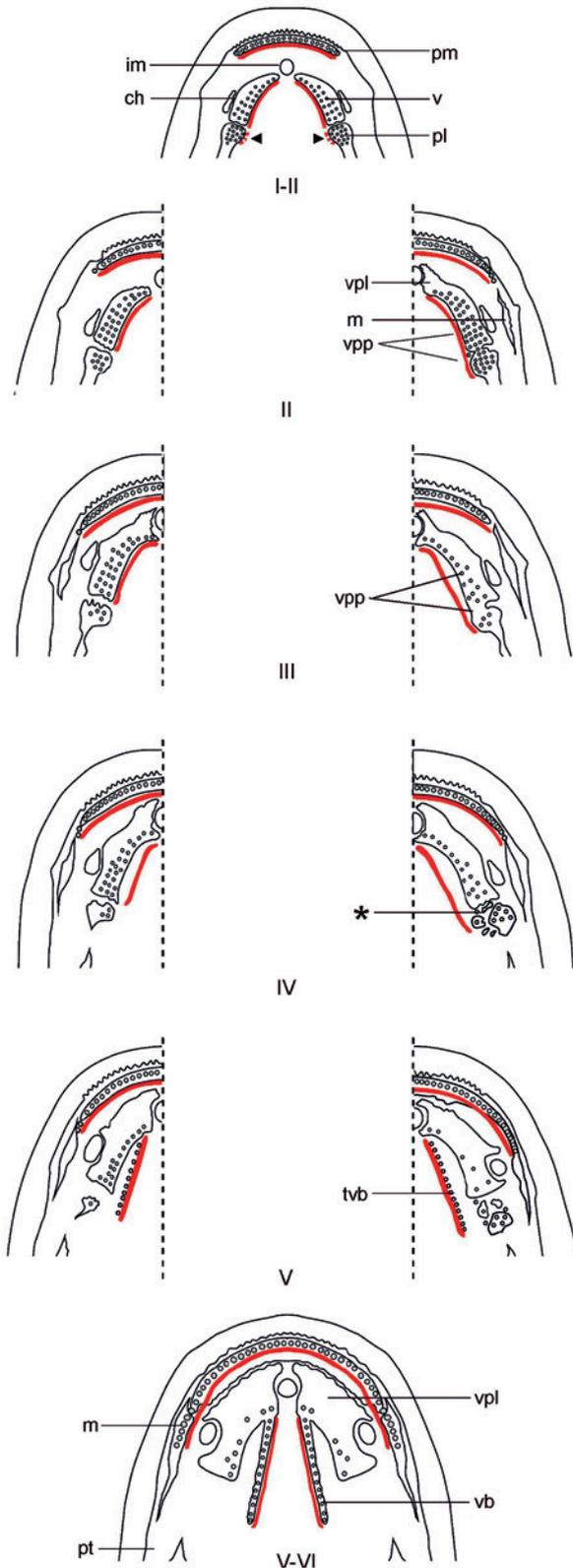


Fig. 5. Suggested changes in the mouth roof during normal (left side) and delayed (right side) metamorphosis of *Ichthyosaura alpestris apuanus* starting with a larva slightly before the ‘typical’ (stage I–II; on the top) with a degrading palatinal dental lamina (arrowheads) and ending with larva (stage V–VI; on the bottom). Note the degrading area between vomer and palatine (asterisk) indicating an earlier connection between the two bones (stage IV right side) and the tooth row of the prospective vomerine bar (tvb). Red lines = dental laminae; arrowhead = regressive dental lamina. For further explanations see text, for further abbreviations see above.

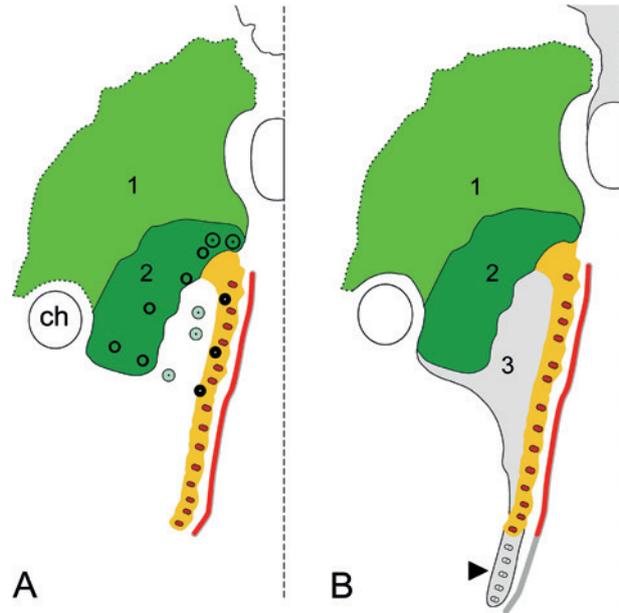


Fig. 6. The palate (right half) of *Ichthyosaura alpestris apuanus* illustrating the suggested development of the definite vomer and the vomerine bar: (1) Newly formed edentate latero-anterior extension (vomerine plate; green); (2) incorporation of the previously dentate larval vomer (dark green); (3) subsequent completion of the definitive vomer between the matrix of the prospective vomerine bar (yellow) and the larval vomerine portion (2), which area may contain non-established larval teeth (light blue), especially when metamorphosis is delayed; further elongation of the tooth row plus dental lamina of the vomerine bar (arrowhead) and ossification of the matrix of the latter. Red = dental lamina; open circles = monocuspid teeth on the larval vomer, black circles = non ankylosed large monocuspid teeth on the buccal side of the prospective vomerine bar; two circles fused together, red or grey = non ankylosed bicuspid teeth on the matrix of the vomerine bar, which become fixed when the matrix bar ossifies.

identification of the first appearance of a given element, of use of field-caught and lab-reared specimens that may not have the same developmental pattern, of a hitherto unknown intraspecific plasticity of the species considered, and of environmental and other influencing variables (see the discussion in SHELL *et al.*, 2015). This and the incompleteness of our series make an adequate comparative analysis of our findings difficult.

Actually, we do not yet understand all details described above (see below) and a larger series of developmental stages and histological serial sections would have been advantageous to reach more definitive conclusions. Therefore we discuss only some selected topics. Noteworthy observations are: (i) all larvae of *Ichthyosaura alpestris apuanus* showed changes in the mouth roof, which deviate from the assumed normal development (see introduction) and may be attributed to a delay of metamorphosis; (ii) three larvae (larva 1, 2, 3) developed vomeropterygopatatina, two larvae (larva 6 and 7) might have had vomeropalatina; (iii) in all stages examined the larval vomer was clearly visible, and (iv) three larvae (6, 7, 8) showed developmental stages of the vomerine bar allowing to specify our hypothesis concerning the final organisation of the definite vomer.

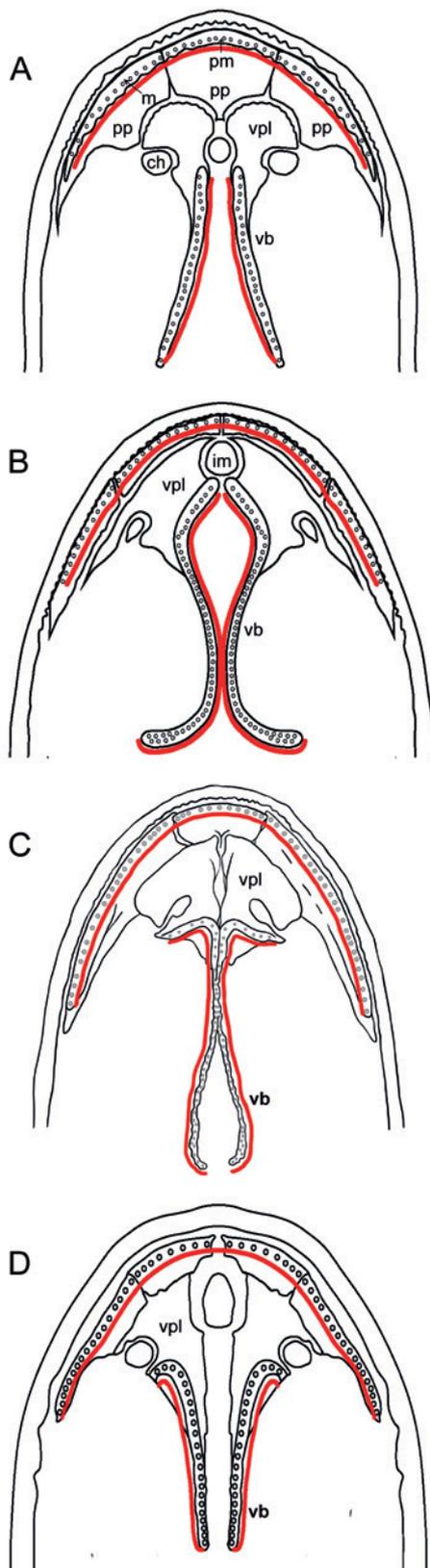


Fig. 7 A–D. Dentigerous bones with their dental laminae (red) of several transformed urodele taxa with a vomerine bar. Drawings are not in scale. Small circles = teeth. Red line = dental lamina. **A** Salamandridae: *Lissotriton vulgaris* and *Ichthyosaura alpestris*. **B** Salamandridae: *Salamandra salamandra*. **C** Plethodontidae: *Streococheilus marginatum* (modified after WAKE, 1966). **D** Hynobiidae: *Hynobius formosanus* (drawn after a photo of a cleared and stained specimen; see figure 7 B in VASSILIEVA *et al.*, 2015). Abbreviations see above.

Ichthyosaura alpestris apuanus and the delay of metamorphosis

Metamorphosis and growth are influenced by temperature. Increased temperatures may accelerate metamorphosis shortening the larval period, whereas low temperatures may lower or almost completely inhibit metamorphosis, prolonging the larval period. Delayed metamorphosis induced by low temperatures is common in overwintering larvae, i.e. in larvae from eggs laid in the first year, which may metamorphose in their second year or even later. Overwinterers show characteristic alterations in the mouth roof similar to those gained in specimens kept by low temperatures in the laboratory (*Salamandra salamandra*: CLEMEN, 1978 a; CLEMEN & GREVEN, 2013; *Lissotriton vulgaris*: CLEMEN & GREVEN, 1979; GREVEN *et al.*, 2015, and further literature therein). Such changes concern both, traits that are thought to be independent from thyroid hormones (TH), which therefore may continue growth to some extent, and characters dependent on TH that may slow down further development (for literature see introduction).

Ichthyosaura alpestris apuanus is found throughout much of Europe occurring from sea level to around 2,500 m in a broad range of habitats. Overwintering larvae are common in this species and the related *L. vulgaris*; both species are known for facultative paedomorphosis and paedomorphic and metamorphic specimens can exist simultaneously within populations (for review see DENOËL *et al.*, 2005). JOHNSON & VOSS (2013) and others discuss that paedomorphic taxa arise by selection for delayed metamorphic timing or increased/prolonged growth rate and insensitivity to TH.

With regard to Italy many paedomorphic populations of *I. a. apuanus* are known primarily from sites in Tuscany and Emilia-Romagna, which are mainly located between 500 and 2000 m above sea level (DENOËL *et al.*, 2001). The area, in which our animals were collected, lies on the border between Liguria and Emilia-Romagna. It is quite possible therefore that our specimens are derived from such populations.

Paedomorphic populations are characterized by a high phenotypic plasticity and paedomorphic individuals often have skull morphologies ranging from a mosaic of larval and metamorphic characters to a completely larval condition, which shows that there exists a notable variation in the timing of events which characterize metamorphosis. This variation is ascribed to genetic and environmental influences (e.g., DENOËL *et al.*, 2005; see also DJOROVIĆ & KALEZIĆ, 2000; IVANOVIĆ *et al.*, 2014). According to IVANOVIĆ *et al.* (2014) the skull of paedomorphic alpine newts resembles that of late larval stages (partially developed maxillae and partially transformed vomers, resorbed palato-pterygoids to name only the dentigerous bones). Obviously larvae exhibit a high variation in the level of ossification (DJOROVIĆ & KALEZIĆ, 2000).

Unfortunately, we do not have exact data on the locality, where our specimens were caught (e.g. climatic conditions, depth and largeness of the water bodies, tem-

perature, accompanying fauna including metamorphic and/or paedomorphic conspecifics etc.). So we can not say anything about the possible further development and the organisation of the mouth roof in adult specimens. We can only state that (i) all specimens collected were clearly larvae covering at least four stages and caught within a few days, suggesting a relative high variability within a relatively narrow time window; and (ii) changes seen in the mouth roof are very similar to those found in larval *L. vulgaris* from a non paedomorphic population (GREVEN *et al.*, 2015).

Vomer, palatine and vomeropterygopalatinum

The posterior edge of the larval vomer and anterior edge of the palatopterygoid are very close to each other, which is a favourable condition for a possible fusion. However, ontogenetic and experimental studies on various wild- and lab-reared urodeles (SMIRNOV & VASSILIEVA, 2001, 2003, 2005; SMIRNOV *et al.*, 2011) as well as large series of *Lissotriton vulgaris* and *Ichthyosaura alpestris* from populations exhibiting facultative paedomorphosis (e.g., DJOROVIĆ & KALEZIĆ, 2000; IVANOVIĆ *et al.*, 2014) have shown that vomeropterygopalatina were practically unknown from natural populations. They have been found extremely seldom in animals treated with thyroxin (SMIRNOV & VASSILIEVA, 2005; summarized in GREVEN *et al.*, 2015).

Therefore, its frequency in a very limited sample of specimens as shown herein, and in our previous article (GREVEN *et al.*, 2015) is striking. This may be simply explained by the fact that vomeropterygopalatina have been overlooked, because this structure is hard to see without any handling of the animals. Anyway, our findings suggest that the existence of a vomeropterygopalatinum may be common in the two species examined, but may occur also in other urodele taxa, e.g., in the paedomorphic *Eurycea rathbuni* (Plethodontidae) (CLEMEN *et al.*, 2009). Certainly, vomerpterygopalatina in metamorphic urodeles are ephemeral structures; vomer and pterygopalatinum separate again as soon as development continues, simply because the palatine disintegrates in the normal course of metamorphosis (see GREVEN *et al.*, 2015). We are quite sure that the bony islets between the vomer and the palatopterygoid seen in larvae 6 and 7 indicate degradation of the fusion zone of these elements (Fig. 5).

The suggestion that the palatinal portions may be provided with teeth by the outgrowing vomerine dental laminae comes from the observation that in these cases the number of palatinal replacement teeth was reduced or even absent, that the palatine itself showed signs of degradation, and that the area of vomerine replacement teeth extended beyond the posterior edge of the vomer. This may occur during 'normal' development, but seems to be enhanced during delayed metamorphosis (see also CLEMEN, 1978; CLEMEN & GREVEN, 1979; SMIRNOW & VASSILIEVA, 2003; GREVEN *et al.*, 2015).

Previous studies (e.g., CLEMEN & GREVEN, 1979; SMIRNOV & VASSILIEVA, 2003) suggested that palatinal dental laminae produce only early larval teeth, i.e. non pedicellate monocuspids, as the formation of pedicellate bicuspid teeth is highly TH-dependent (e.g., GABRION & CHIBON, 1973; GREVEN & CLEMEN, 1990). TH in turn accelerates the degradation of the palatinal dental lamina and the palatinum itself, i.e. the palatine is resorbed before pedicellate teeth are formed. More recently VASSILIEVA & SERBINOVA (2013) stated that pedicellate teeth are generally missing on the palatine (and coronoid), but described subpedicellate teeth on the coronoid in late larval *Mertensiella caucasica* (Salamandridae).

The definite vomer

The studied larvae, especially larvae 5–8, provided further information regarding the development of the definite (adult) vomer. Contrary to a commonly held doctrine, that the larval vomer becomes almost completely or completely resorbed in transforming Urodela (e.g., SMIRNOV & VASSILIEVA, 2003; LEBEDKINA, 2004; ROSE, 2005; VASSILIEVA & SERBINOVA, 2013) and that the definite (adult) vomer develops entirely *de novo* (e.g., ROSE, 2005), we suggest that parts of the toothless larval vomer become incorporated in the definite vomer.

The *de novo*-formation of the lateral and anterior edentate expansions of the vomer is not questioned, but the new formation of large parts of the adult vomer body is at least arguable. Robust (experimental) data supporting the 'incorporation'- or the 'degradation'-hypothesis are missing at least for *Ichthyosaura alpestris apuanus* and *Lissotriton vulgaris*. The statement that the complete larval vomer is replaced comes from studies examining Alizarinred labelled developmental stages of several species (for summary see LEBEDKINA, 2004; ROSE, 2005). LEBEDKINA (2004) reported that in *Ranodon sibiricus* (Hynobiidae) a small strip of the larval vomer overlaps the newly formed vomer, and more recently VASSILIEVA & SERBINOVA (2013) noted that in *Mertensiella caucasica* resorption of the larval vomer proceeds from its posterolateral margin. CLEMEN (1979) showed experimentally that in *Salamandra salamandra* at least parts of the larval vomer were used to form the definite vomer, but here the incorporation may be related to the different formation of the definite vomer (see below).

Currently, our only argument supporting the 'incorporation'-hypothesis is the fact, that the largely intact larval vomer was visible in all larvae studied herein and did not show conspicuous signs of degradation except the resorption of its teeth (see also GREVEN *et al.*, 2015). The postulated degradation from the posterolateral margin might leave a large area (from the parasphenoid to the developing vomerine plate) of the palate unprotected to some extent, unless the new vomerine plate would also grow out posteriorly simultaneously.

We think that the vomer of midmetamorphic and newly transformed *L. vulgaris* and *I. a. apuanus* (at this age

the mouth roof is not yet fully developed; see ACCORDI & MAZZARINI, 1992; LEBEDKINA, 2004) and related species is composed of (1) the newly formed edentate antero-lateral expansion (vomere plate), whose anterior median part develops already during a delay of metamorphosis, (2) the larval vomer that becomes edentate, (3) the newly formed edentate area between the vomerine bar and the newly formed dentate part of the vomer including the vomerine bar (see Fig. 6).

The formation of the dentate part of the definite vomer, i.e., the tooth row on the vomer body and its posterior elongation on the vomerine bar, is not entirely clear. A further commonly held doctrine is that the vomerine bar of salamandrids is a posterior bony outgrowth of the vomer accompanied by the elongated vomerine dental lamina (e.g., ROSE, 2003; LEBEDKINA, 2004). According to SMIRNOW & VASSILIEVA (2003) the vomerine bar in *L. vulgaris* has an “uncalcified predecessor” (called matrix by us), the anterior part of which ossifies in continuation with the vomer, whereas the posterior portions develop by fusion of several ossification centres, thus, resembling the development of the vomerine bar in Plethodontidae (SMIRNOW & VASSILIEVA, 2003; see also VASSILIEVA & SERBINOVA, 2013).

However, as previously noted for *L. vulgaris* (GREVEN *et al.*, 2015; see also Fig. 6) and also suggested herein for *I. a. apuanus* we assume that the formation of the vomerine bar takes place in a different way. In contrast to the palatine, where the dental lamina, the teeth and the palatine itself are completely resorbed in course of metamorphosis, the larval vomer and its dental lamina do not disintegrate, but only the larval teeth (see above). The dental lamina that first closely follows the vomer on its inner side (the anterior end of the tooth row and the dental lamina is marked by transversally arranged monocuspid teeth) elongates beyond the posterior edge of the larval vomer. At the same time the dental lamina produces teeth and becomes slightly shifted from front to rear to the middle leaving a more or less wide non-ossified area between itself and the larval vomer. The dental lamina provides the prospective vomerine bar with teeth resulting in a row of largely bicuspid teeth still not attached to a bony substrate, as the matrix of the vomerine bar ossifies later.

The small non ankylosed monocuspid teeth (crowns) in the connective tissue between the inner edge of the larval vomer and the prospective vomerine bar have been produced by the shifting dental lamina before it has arrived at his final position, but they did not found a proper substrate to settle. The large monocuspid, non-transformed larval teeth with a dividing zone closest to the buccal side of the prospective vomerine bar may represent the first teeth of the shifted dental lamina already arrived at its final position.

However, the putative shifting of the dental lamina and the production of teeth that differ in size and shape sizes deserve further study, the more so, as vomers of larvae 4 and 5, which do not show any traces of a developing vomerine bar, either reveal bicuspid vomerine replacement teeth (larva 4) or a vomerine tooth patch

with bicuspid teeth indicating that the vomerine dental lamina in this larvae was able to produce bicuspid teeth before the supposed shifting of the dental lamina and the transformation of the vomerine tooth patch in a single row of (bicuspid) teeth. As the development of bicuspid teeth is clearly TH-dependent (GABRION & CHIBON 1970; GREVEN & CLEMEN, 1990), the presence of bicuspid in an otherwise largely unchanged palate remains unanswered. In the literature we did not find clear statements that larval vomers (normally characterized by a tooth patch of monocuspids) may also bear bicuspid teeth. Primary teeth of the vomerine bar have been reported as subpedicellate monocuspid (*M. caucasica*: VASSILIEVA & SERBINOVA, 2013) or bicuspid (*L. vulgaris*: SMIRNOW & VASSILIEVA, 2003)

Despite such caveats, we can say with some certainty that the formation of the vomerine bar in *L. vulgaris* and *I. a. apuanus* (and very probably in related newts) is clearly distinguished from that observed in the salamandrid *Salamandra salamandra*. As experimentally proved the vomerine bar of this species rises from a much defined area of connective tissue at the posterior inner (lateral) edge of the larval vomer. In presence of matrix material of the vomerine bar the dental lamina elongates and the tooth row of the vomerine bar appears to be a mere elongation of the tooth row on the vomer body (the larval tooth patch is reduced to single row) (CLEMEN, 1978b, 1979). This pattern fits very well in the general opinion.

The vomerine bar in Salamandridae and other Urodela

The definite vomer in all transformed salamandrids so far studied has a long, slender row of ankylosed teeth that extends posteriorly over nearly the entire length of the parasphenoid (see Fig. 7 A) called often vomerine bar (e.g., STADTMÜLLER, 1936; CORSIN, 1966; CLEMEN & GREVEN, 1994; HALLER-PROBST & SCHLEICH, 1994; ROSE, 2003; LEBEDKINA, 2004; SMIRNOW & VASSILIEVA, 2003; VASSILIEVA & SERBINOVA, 2013; WU *et al.*, 2012). Obviously, however, dentition and configuration of the definite vomer show at least two distinct patterns in salamandrids: Either, the dentigerous ridge is largely straight, begins in the lower third of the vomer body (see above) and continues on the vomerine bar as shown in *Lissotriton vulgaris* and *Ichthyosaura alpestris apuanus* (Fig. 7A) or is more or less S-shaped with its anterior part on the vomer body and the larger posterior part on the vomerine bar as in *Salamandra salamandra* (Fig. 7 B). In both, the tooth rows (and the accompanying dental lamina) are not interrupted. A preliminary literature search and own studies (unpublished) indicate that the S-pattern is realized only in ‘true’ salamanders, i.e. *Salamandra*, *Lyciasalamandra*, *Chioglossa* and *Mertensiella* (unpublished). At first glance, in both configurations teeth of the vomerine bar appear to be a mere extension of the lateral end of the row of larval vomerine teeth (e.g., LEBEDKINA, 2004; ROSE, 2003). This is actually the case in *S. sala-*

mandra (see above), but not in *Lissotriton vulgaris* and *Ichthyosaura a. apuanus* (see above). We had not expected to find such striking differences within salamandrids and think that the mode of development and the configuration of the vomer in *Salamandra* represent an ancient trait.

A structure comparable, but probably not homologous with the vomerine bar of Salamandridae is present in some Plethodontidae (Fig. 7 C) and Hynobiidae (Fig. 7 D). The vomerine bar of Plethodontidae appears to arise at the posteromedial edge of the larval vomer, which finally leads to a lateral position of the dental lamina (WILDER, 1925; REGAL, 1966; ROSE, 1995a, 2003). Early growth of the bar is assumed to be sustained by osteogenesis in the vomer and later growth by the ossification of tooth bases (see ROSE, 1995 a). In some species the posterior part of the bar bearing tooth patches becomes separated from the anterior part. In the few Hynobiidae, in which such a structure was described, the medial end of the vomerine tooth row extends posteriorly; the length of this bar varies depending on the species. Developmental pattern and arrangement relative to its dental lamina is said to be the same as in Plethodontidae (REGAL, 1966; ROSE, 2003; VASSILIEWA *et al.*, 2015).

Conclusions

Our observations confirm previous findings suggesting that the organisation of the mouth roof in Urodela indicates more sensitively the impact of metamorphosis-affecting (environmental) stressors than external morphological features. Further, findings demonstrate that the formation of a vomeropterygopalatinum appears to be a common phenomenon, which obviously reflects some perturbations during development (in the cases described in consequence of a delayed metamorphosis), which will be, however, repaired when development proceeds. Fusion of these bones is certainly favoured by the general closeness of the larval vomer and the palatopterygoid. There is some evidence that in newts the tooth row and its bony substrate of the definite vomer develop newly. This is different from the related *Salamandra salamandra*, where the vomerine bar develops from the posterior inner (lateral) edge of the larval vomer and the innermost larval tooth row is involved in the final dentitional pattern. However, these differences within salamandrids have to be substantiated using a larger growth series of specimens preferably raised under constant condition to monitor the development step by step.

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