VERTERBRATE ZOOLOGY

63 (2): 207 – 216
11.9.2013
SENCKENBERG

Remodelling of the palate: an additional tool to classify larval salamandrids through metamorphosis

Günter Clemen [1] & Hartmut Greven [2]

[1] Doornbeckeweg 17, 48161 Münster, Germany; gclemen(at)web.de — [2] Institut für Zoomorphologie und Zellbiologie der Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany; grevenh(at)uni-duesseldorf.de

Accepted 02.v.2013.
Published online at www.senckenberg.de/vertebrate-zoology on 29.viii.2013.

Abstract

Schematic drawings as well as some cleared and stained preparations of the upper jaw and the palate (mouth roof) of larval (before and during metamorphosis) and transformed specimens of the larviparous *Salamandra salamandra* are presented to illustrate changes especially of the palate through metamorphosis. We distinguished seven stages ranging from early larvae until fully transformed specimens by using characters easily to see in preserved and anesthetized living specimens by means of a dissection microscope at various magnifications and reflected light. Distinctive characters for classification were growth of the maxillae, the anterolateral expansions and posterior outgrowths (vomerine bar) of the vomeres, complete degradation of both, the bony "bridge" connecting the pterygoid, and the palatine and the palatine itself. Larger specimens (length of ca. ≥ 3 cm) can be inspected non-invasively by fixing them in a simple holder. As we used elements that are always present in metamorphic salamanders either exclusively in their larvae (palatine) or in larvae as well as in transformed specimens (premaxillae, maxillae and vomeres), the classification proposed herein appears to be applicable not only to the type form of *S. salamandra*, but also to other Salamandridae and, appropriately modified, very probably even to urodele taxa that may considerably differ for instance in the shape of the vomeres and dentition. Obviously metamorphic Urodela appear to be constrained by a largely similar developmental sequence with regard of growth and remodelling of the palate, which may be categorized in a standard manner.

Kurzfassung

Wir stellen Schemazeichnungen und einige Aufhellungspräparate vor, welche die zeitliche Abfolge von weitgehend metamorphosebedingten Veränderungen des Oberkiefers und besonders des Gaumens larvaler (vor und während der Metamorphose) und metamorphosierter Exemplare des larviparen Feuersalamanders *Salamandra salamandra* illustrieren. Wir unterscheiden sieben Stadien (von der frühen Larve bis zum vollständig metamorphosierten Tier) anhand von Merkmalen, die sowohl bei Museumsexemplaren als auch betäubten lebenden Exemplaren leicht mit einer Stereolupe und Auflicht bei verschiedenen Vergrößerungen erkannt werden können. Die für eine Klassifikation markanten Merkmale sind das Auswachsen der Maxillaria, die Ausdehnung der Vomeres nach vorn und seitlich, das posteriore Auswachsen von Vomerspangen sowie der vollständige Abbau der „pterygopalatinen Knochenbrücken" und der Palatina. Größere Individuen (Länge etwa ab 3 cm) können in einem einfachen Halter fixiert auch nicht-invasiv betrachtet werden. Da wir für unsere Klassifikation Elemente benutzt haben, die bei allen zur Metamorphose befähigten Salamandern vorkommen, und dort entweder nur bei den Larven (Palatinum) oder in Larven und metamorphosierten Individuen (Prämaxillaria, Maxillaria und Vomeres), kann die hier vorgestellte Klassifizierung wohl nicht nur auf *Salamandra*-Arten (die sich von allen anderen Urodelen durch ihren einzigartigen Fortpflanzungsmodus unterscheiden), sondern auch auf Salamandriden insgesamt angewendet werden. Unter Berücksichtigung spezifischer Besonderheiten, z. B. der Form der zahntragenden Knochen, vor allem der Vomeres, und der Anordnung der Zähne, kann sie, entsprechend modifiziert, sehr wahrscheinlich auch zur Klassifizierung andere Urodelentaxa, deren Arten zur Metamorphose befähigt sind, benutzt werden. Offenbar ist die Abfolge der Wachstums- und Umbaueireignisse der hier behandelten Systeme bei Urodeln so ähnlich, dass diese weitgehend standardisiert werden können.

Key words

Urodela (= Caudata), (delayed) metamorphosis, upper jaw arcade, palate.

Introduction

Metamorphosis is a concentrated period of postembryonic development, in which the thyroid hormone is involved (Alberch, 1989; Rose & Reiss, 1993), and is characteristic of the life history of the majority of Urodela (= Caudata). In salamanders metamorphic changes appear to be less drastic than in Anura, but their assessment by a categorical classification is useful for both embryological and life-history studies. Numerous developmental staging series (= normal tables) of various urodele taxa exist, which are largely based on external morphological characters. Examples for such series are found in any textbook of embryology and herpetology. Most tables, however, do not encompass the entire development, i.e. through metamorphosis, ending after hatching or when larvae begin to feed (e.g. Gallien & Durocher, 1957; Epperlein & Junginger, 1981/1982; and the summarizing table 5–6 in Duellman & Trueb, 1986). Developmental tables that include metamorphic stages are rare (for Salamandridae see for example *Pleurodeles waltl*: Gallien & Durocher, 1957; "*Triturus" helveticus*: Gallien & Bidaud, 1959). However, such stages occasionally can be found in life history studies, but are likewise based upon external criteria (e.g. several morphometric parameters, body length, presence, reduction and absence of external gills, reduction of fins etc; for Salamandridae especially *Salamandra salamandra* see Erdmann, 1933; Gasche, 1939; Juszczyk & Zakrzewski, 1981; Thiesmeier, & Günther, 1996; Buckley et al., 2007). Depending on temperature, oxygen, food supply etc., and even on variations between populations, such external traits may vary considerably and some also appear to be decoupled from internal traits (e.g. Reilly & Lauder, 1990). Thus, the metamorphic condition of salamanders has to be judged by "internal" traits emerging for instance during the transformation of the cranium and the hyobranchial apparatus (e.g. Reilly, 1986, 1987; Reilly & Altig, 1996). However, visualization of those traits requires invasive and time-consuming procedures.

In Urodela the tooth bearing bones in the upper and lower jaw as well as in the palate, their accompanying dental laminae and their teeth ("tooth systems" according to Clemen & Greven, 1994) are conspicuously affected in course of larval growth and metamorphosis. Especially in course of metamorphosis larval elements (e. g. the palatinal and coronoid systems) are lost, vomeres and pterygopalatines change their shape, size and position and even new structures (vomerine bars) may appear (for a thorough description of the skull and the palate, the relations of the various bones in Urodela see Stadtmüller, 1924, 1936; Wilder, 1925; Duellman & Trueb, 1986; Trueb, 1993; Rose, 2003). In addition, a shift from monocuspid teeth in larvae to bicuspid teeth in transformed specimens can be observed and mostly the number of tooth rows changes (reviewed in Clemen & Greven, 1994; Davit-Béal *et al*., 2006, 2007).

Over the years we used in combination with external features the organisation and remodelling especially of the vomeres and pterygopalatines, and the arrangement and appearance of the teeth to classify larval stages of various salamanders (reviewed in Clemen & Greven, 1994; see also Jömann *et al*., 2005; Greven *et al*., 2006) and found that this approach proved to be a reliable and simple tool to stage larvae before, during and after metamorphosis. The resulting classification was most consistently used so far by Amend & Greven (1996), who studied the ossification of the skeleton in larval and metamorphosing *Salamandra salamandra*. However, apart from a short note concerning this classification (Greven & Clemen, 1985) and four drawings showing a small selection of the different stages distinguished in this species (see Clemen & Greven, 1994), we never illustrated and commented the above mentioned changes and the resulting classification in detail.

In the present article we therefore complete the set of drawings for *S. salamandra* considering primarily changes of the dentate palatinal bones and the upper jaw arcade and arbitrarily distinguish seven stages based on characters easily to be seen in preserved and living specimens. Although designed primarily for *S. salamandra*, we think that this classification may be applicable to all metamorphic salamandrids, and, after some modifications, even to all metamorphic Urodela.

Material and methods

Source of the specimens. Data presented here come from specimens collected many years before at a time, when we studied dentition and reproduction of the larviparous *Salamandra salamandra* (for definition of the reproductive mode see Greven, 2003), and pregnant females could be legally imported from the former Yugoslawia by Fa. Stein (Lauingen) (e.g. Clemen, 1978; Clemen & Greven, 1974; 1994; Amend & Greven, 1996; Greven 1977, 1998; Greven & Clemen 1985; Greven & Guex, 1994).

Inspection. We examined living and preserved (4 % buffered formalin or 70% ethanol) larvae excised from the uterus in autumn, growing larvae and various stages of metamorphosing as well as transformed specimens already obtained from or used in previous studies (see literature cited above).

Larvae of approx. 3 cm in length and longer can be inspected non-invasively. For this purpose we used a wax-filled Petri-dish (diameter 14 cm), in which specimens covered with water or water containing 0.03 % MS 222 (Fa. Sigma) were fixed in a supine position directly before the forelegs and the behind the hind legs by two cramps. The anterior end of a thread loop was winded around the tongue and the posterior end of the loop was pulled back until the mouth was sufficiently opened to enable inspection. Then the loop was fixed with a pin behind the specimen (Fig. 1). Depending on their size,

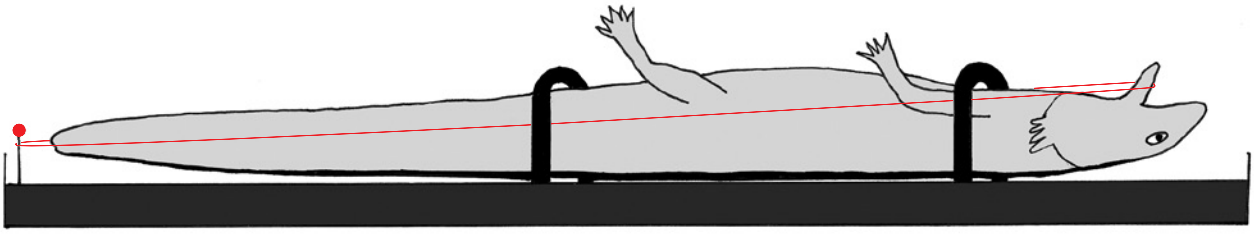


Fig. 1. Fixing a larva with two cramps in a wax-filled Petri-dish (grey) and opening the mouth by means of a thread loop (red) for non-invasive inspection of the palate.

specimens were examined using a hand magnifier or a dissecting microscope at low magnifications. For illumination we used direct incident (e.g. a cold light source with a gooseneck light guide). If preserved specimens are slightly stained with Alizarin red, the identification of structures important for staging is facilitated (Fig. 3 F). In living, anaesthetized specimens details could be recognized more clearly due to the relative transparency of tissues. Anterolateral expansions of vomeres were not fully seen and therefore their real size in the drawings should be accepted with reservation.

Figures. Drawings are freehand sketches, while maintaining the proportions. The different stages are not pictured to scale. For comparison, some specimens cleared and stained with Alcian blue/Alizarin-Red (see Dingerkus & Uhler, 1977) and stored in glycerol were available from two previous studies (Greven & Clemen 1985; Amend & Greven, 1996). Dental laminae are not visible without further processing and therefore are not marked in the drawings. Photos were taken with Keyence VHX 500F digital microscope.

Abbreviations and symbols used in the figures

ch = choana; im = intermaxillary gland; m = maxilla; ps = parasphenoid; pl = palatine; pm = premaxilla; pt = pterygoid; ptp = pterygopalatinum; asterisk = “pterygopalatinal bony bridge”; v = vomer; vb = vomerine bar; tooth patch (“Zahnfeld”) describes dentate areas with more than one tooth row; therefore tooth patches comprise several tooth rows (“Zahnzeilen”) and tooth families (“Zahnfamilie”), i.e. first generation tooth and all its successors at a given position; rhomb = groove in the oral mucosa; arrows and arrowheads = most distinctive traits. Dotted lines = edges from elevations or depression in the mouth epithelium; solid lines = contours of the mouth roof and in advanced stages fully developed and reduced lip fringes; small circles = “tooth loci” that include large replacement teeth and established teeth as both can not be easily distinguished in untreated preparations (counts refer to all tooth loci); broken lines = contours of the (dentigerous) bones gleaming through the more or less translucent tissue.

Results

Figures 2.1–2.7 illustrate the characteristics defined in each stage by showing the ventral view of the upper jaw and palate by the inspection of preserved and living specimens. At the left side of each drawing characters are seen that can be identified by inspecting the specimens as described. At the right size details are delineated, which can be occasionally recognized in living specimens and especially in cleared and stained preparations (see Fig. 3 A–F). For some slight discrepancies between the untreated and the cleared and stained specimens see discussion.

Stage I: “young larva”

Fig 2.1

The premaxillae (pm), paired in younger stages, are fused and usually bear a single row of teeth (= monostichous dentition), but have not reached their final size. Posteriorly of the premaxillae some tooth buds (arrow) are buried in the connective tissue; maxillae are missing. Vomeres appear elongate; each vomer bears approximately 26 teeth. Number of tooth rows increase from anterior (1–2) to posterior (up to 4) forming altogether a tooth patch (v). The posterior edge of this patch approaches the posterior border of the bean-like choanae. The relatively broad edentate parts of the vomeres reach the anterior edge of the choanae. A groove (rhomb) in the oral mucosa extends from choana to choana spanning the opening of the intermaxillary gland (im).

The palatinal portion (pl) of the pterygopalatinum bears approximately 12 teeth in three to four rows. This area closely approaches the vomerine tooth patch giving sometimes the impression of a single, large toothed area. The posterior part of the palatine lacks teeth, narrows (= “pterygopalatinal bridge”; asterisk) and merges with the pterygoid (not drawn).

Stage II: "Typical larva"

Fig. 2.2

Premaxillae (pm) extend posteriorly beyond the vomerine tooth patches; maxillae (m) are present approaching the height of the choanae. On the right side of figure 2.2 a piece of the lip fringe, very well developed in this stage, is excised to expose the maxillary teeth row (arrow). Premaxillae and maxillae are separated (not easily recognizable), but bear a single continuous tooth row without a gap. Posteriorly one or two replacement teeth indicate further growth of the maxillae in caudal direction (not drawn).

The lateral edentate vomerine margins vary in size due to the enlargement of the tooth patch. Number of teeth (> 30) and tooth rows (4–5) per vomer has increased. Vomerine (v) and palatinal (pl) tooth patches are separated by a distinct gap, in which some tooth buds (from the vomerine dental lamina!) are present. The bony bridge (asterisk) connecting the palatinum and the pterygoid appears intact.

Stage III: „Late larval specimen"

Fig. 2.3, 3 A

Premaxillae (pm) are approximately twice as long as maxillae. Maxillae (m) extend beyond the anterior edge of the choane (ch). Labial fringes are reduced and do only cover the most posteriorly located maxillary teeth. The transversal groove (rhomb) extending between the choanae is broad.

On the vomeres number of anterior tooth loci (approx. 26–28 teeth) has slightly decreased. The same holds for the tooth loci (6–7 teeth or loci) of the palatinum (pl). The gap between vomerine and the palatinal tooth patches is noticeably broad. The width of the "pterygopalatinal bridge" seems to be reduced, but their margins appear unaffected.

In larvae kept at low temperatures (approx. 12 °C) and/or with reduced food supply (see Clemen, 1978) we found some deviations from stage 3, which obviously depended on the duration, the animals were kept under these conditions.

***Stage III a*:** "Late larval specimen with slightly delayed development" (Fig. 2.3 a, 3 B). The tooth row of the upper jaw arcade (the paired premaxillae anteriorly and maxillae posteriorly) has elongated ending slightly behind the the choanae (ch). The edentate anterolateral expansions of the vomer are partly located under the mucosal groove (rhomb). Number of palatinal tooth loci is noticeably reduced. The "pterygopalatinal bridge" appears relatively broad.

***Stage III b*:** "Late larval specimen with an obviously delayed metamorphosis" (Fig. 2.3 b). Maxillae with approx. 14 tooth loci are longer than the praemaxillae reaching the posterior end of the palatina. The anterolateral expansions of the vomeres appear considerably larger approaching the intermaxillary gland medio-rostrally. Especially in the anterior vomer number of tooth loci is reduced (whole number of loci approx. 20). Vomerine tooth patches extend beyond the posterior margins of the choanae. Palatinal tooth patches are absent (arrowhead). The palatinal bone itself and the relatively broad "palatinal bony bridge" appear unaffected.

Stage IV: „Larva at the onset of metamorphosis"

Fig. 2.4, 3 C

Posteriorly, the upper jaw arcade, premaxillae (pm) and maxilllae (m), reaches the midst of the elongate choana (ch). Anterolateral vomerine expansions appear rather small compared to those in the stained and cleared specimen shown in Figure 3 C. Posteriorly from the lingual edge of the vomeres small outgrowths (the prospective vomerine bars; arrowhead) develop that initially are seen as slight bulges of the oral mucosa.

The gap between vomerine and palatinal tooth patch is broad. The palatinal tooth patch is further reduced showing only remnants of previously established teeth. Anteriorly and lingually palatines reveal distinct indentations indicating degradation processes. Most conspicuously these indentations are seen in the "pterygopalatinal bridge" (asterisk). Somewhat later the pterygoid and palatinum are entirely separated (Fig. 3 C).

Stage V: „Midmetamorphic larva "

Fig. 2.5, 3 D

The tooth row of the premaxillae (pm) and maxillae (m) extends beyond the slightly oval choanae (ch). The groove of the oral mucosa is very broad (rhomb).
Below this groove the anterolateral expansions of the vomer (v) are further grown reaching rostrally the intermaxillary gland (im). At the height of the choanae there are 3–4 rows of vomerine teeth. On the vomerine bars (vb) that are increased in length, number of rows is reduced to a single row. Here most posteriorly a second row of teeth may be present. The tip of the vomerine bars is slightly bent laterally. Palatinal tooth patches are strongly reduced bearing up to three remnants of teeth (arrowhead). The "pterygopalatinal bridge" is entirely missing or only a few punctuate remnants are present.

Stage VI: „Recently metamorphosed specimen"

Fig. 2.6, 3 E

The single row of teeth on the premaxillae (pm) and maxillae (m) extends far beyond the round choanae (ch).

The edentate anterolateral expansions of the vomeres approach the partes palatina of the praemaxillae and maxillae forming a broad articulation with both. Posterolateral vomerine expansions support the posterior margin of the chonae. Teeth are located on the postero-medial part of the vomeres. Vomerine bars (vb) have the shape of a slightly curved S. They carry a single tooth row; posteriorly, however, two rows are present. Palatines and "pterygopalatinal bridges" are entirely missing.

Stage VII: „Fully transformed and adult specimen"

Fig. 2.7; 3 F

The single row of teeth of the premaxillae (pm) and maxilla (m) extends far beyond the posterior margin of the choanae (ch). Vomeres (v) have their definite shape and articulate broadly with the premaxillae and maxillae anteriorly (= vomerine plate). Edentate vomerine expansions also form most of the margin of the choanae. The largely monostichous dentition of the vomer and the thin vomerine bar (vb) continues posteriorly ending near the esophagus. The most posterior portions of the bars always bear two to three rows.

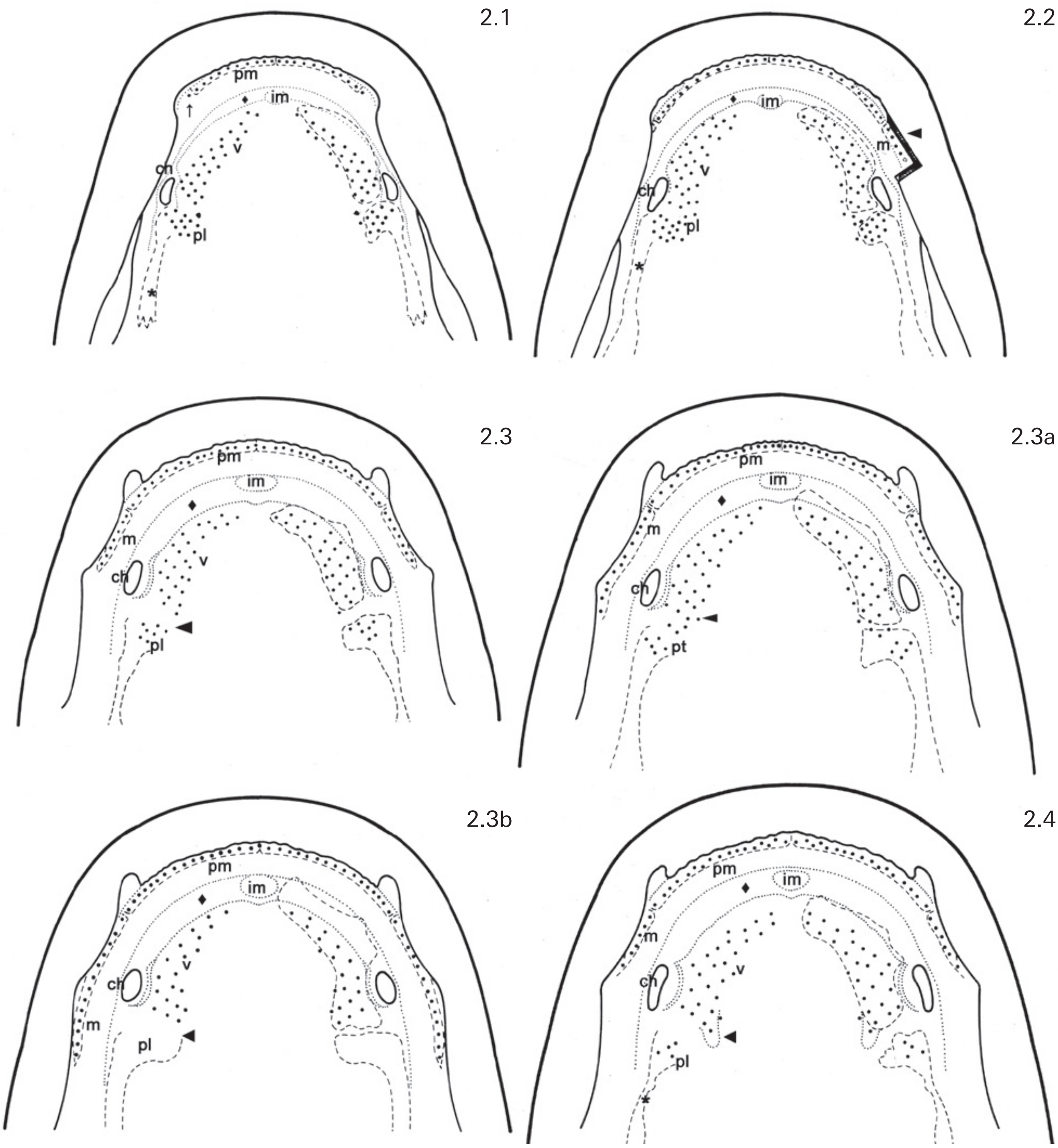
Discussion

The present note gives a diagrammatic overview of growth and remodelling of the dentigerous bones in the palate (and upper jaw) of the larviparous *Salamandra salamandra* from "typical" larvae to fully transformed specimens, which allows the distinction of seven arbitrary stages. In the "typical" larva already all the dermal bones considered herein are present with the exception of the maxillae. "Key events" used for staging and visible in preserved and living specimens (changes of the dentition are given in brackets) are: (1) appearance and growth of the maxillae (and increase of their tooth loci), (2) the anterolateral growth of the vomeres (and increase of the number of teeth on the tooth patches until the late larval stage), (3) posterior outgrowths of the vomeres (= vomerine bars) (with teeth situated in the postero-medial part), (4) splitting of the pterygopalatines in palatines and pterygoids, and (5) complete resorption of the palatines and their tooth patches. At proper magnifications also the characteristic shift from non-pedicellate monocuspids to pedicellate bicuspids can be recognized (e.g. Clemen & Greven, 1994; Davit-Béal *et al*., 2006, 2007).

In the stained and cleared preparations the anterolateral expansions of the vomeres that finally form large shelf-like plates (see point 2) appear to develop earlier and are larger than stated onto the drawings. Either the degree of the expansions varies considerably or, more likely, staining reveals their size more accurately. In any case, plates increase remarkably in size during development.

The "key events" although susceptible, e.g. to temperature (as growth in general) (see below) do not vary distinctly in their temporal relations. The classification presented herein is especially suited to judge the metamorphic condition of salamanders on the basis of "internal" features without using invasive techniques (see Introduction). Although "metamorphosis is an arbitrarily determined and primarily quantitative phenomenon" (Rose & Reiss, 1993, p. 291), a key feature indicating the onset of metamorphosis is the disintegration of the palatopterygoid (Wilder, 1925; Reilly, 1986, 1987; Reilly & Altig, 1996; Rose, 2003).

Albeit limited to the larviparous *S. salamandra* in the present article, the classification proposed herein may be applicable to other metamorphic salamandrid species and, after some modifications, even to other metamorphic urodele taxa as suggested previously (Clemen & Greven, 1994). So far known from the literature and own observations the above mentioned "key events" may be considered as typical for metamorphic salamandrids in general, even so more as the vomerine bar growing from the posterior edge of the vomer is a typical character of this taxon (for some other salamandrids see Stadtmüller, 1936; Clemen, 1979; Haller-Probst & Schleich, 1994; Lebedkina, 2005). A similar dentigerous process of plethodontids, which grows from the medial edge of the vomer forming the so-called "parasphenoid tooth patch" obviously has been evolved independently (Wilder, 1925). In fact, slight modifications may be observed in other salamandrids such as the presence of unpaired premaxillae instead of paired premaxillae, the direction in which maxillae develop, e.g., from posterior to anterior in "*Triturus*" sp. (own observation) etc., but these do not affect the above mentioned "key events", among others as our classification does not cover early stages. Its application may be broadened to other Urodela, as the chronology of ossification (along with the manifestation of dermal bones) and the resorption of some elements generally reveal a relatively stable pattern in Urodela (e.g. Lebedkina, 1979, 2005; see the summarizing table 1 in Rose, 2003). This suggests that, despite considerable differences in the organisation of the skull and palate, metamorphic urodeles are constrained by similar developmental sequences that may be categorized in a standard manner. Further, it should be noted that all dermal bones considered herein are present in larvae and remodelled present in transformed specimens of all metamorphic Urodela. The single exception is the pterygopalatine that either loses its palatinal portion or disintegrates entirely (e.g. in plethodontids) during metamorphosis. If necessary, taxon-specific traits might be added when other taxa have to be staged such as various modes of lateral and posterior expansions of the vomeres and their dentition pattern (lateral, transverse, curved etc.), only a partial loss of the palatine during metamorphosis and, if present, variations derived from heterochrony such as a delayed appearance of the maxillae etc. (for such differ-



ences between taxa see among others text and figures in Regal, 1966; Duellman & Trueb, 1986; Trueb, 1993; Rose, 2003).

Not all structural traits mentioned above may express a distinct metamorphic response. Such putative largely metamorphosis-independent responses may be recognized in newts and salamanders showing a ”delayed” metamorphosis, a phenomenon repeatedly documented in field studies from temperate zones (e.g. in “*Triturus*”-species, and *Salamandra salamandra*, e.g. Schreiber, 1912; Eggert, 1934; Gasche, 1939; Clemen & Greven, 1979; Juszczyk *et al*., 1984; Günther, 1996; and further literature therein). Generally, hibernating larvae become larger than larvae that undergo metamorphosis before the onset of winter in the year they had hatched. In *S. salamandra* the offspring arising from summer-mating are ready for birth already in autumn, but they are generally born in the subsequent spring. During this time intrauterine development is retarded (Amend & Greven, 1996). Larvae born in autumn, inhabiting cold water bodies or suffer from lack of food also transform in the next spring (see Thiesmeier & Günter, 1996). This “delayed” metamorphosis is obviously reflected in the organisation of the palate, but this phenomenon has little attracted interest so far. Some observations very similar to that reported herein are noticed for “*Triturus” vulgaris* (Clemen & Greven, 1979) and *Ranodon sibiricus* (Jömann *et al*., 2005; Greven *et al*., 2006). Hypophysectomized lar-

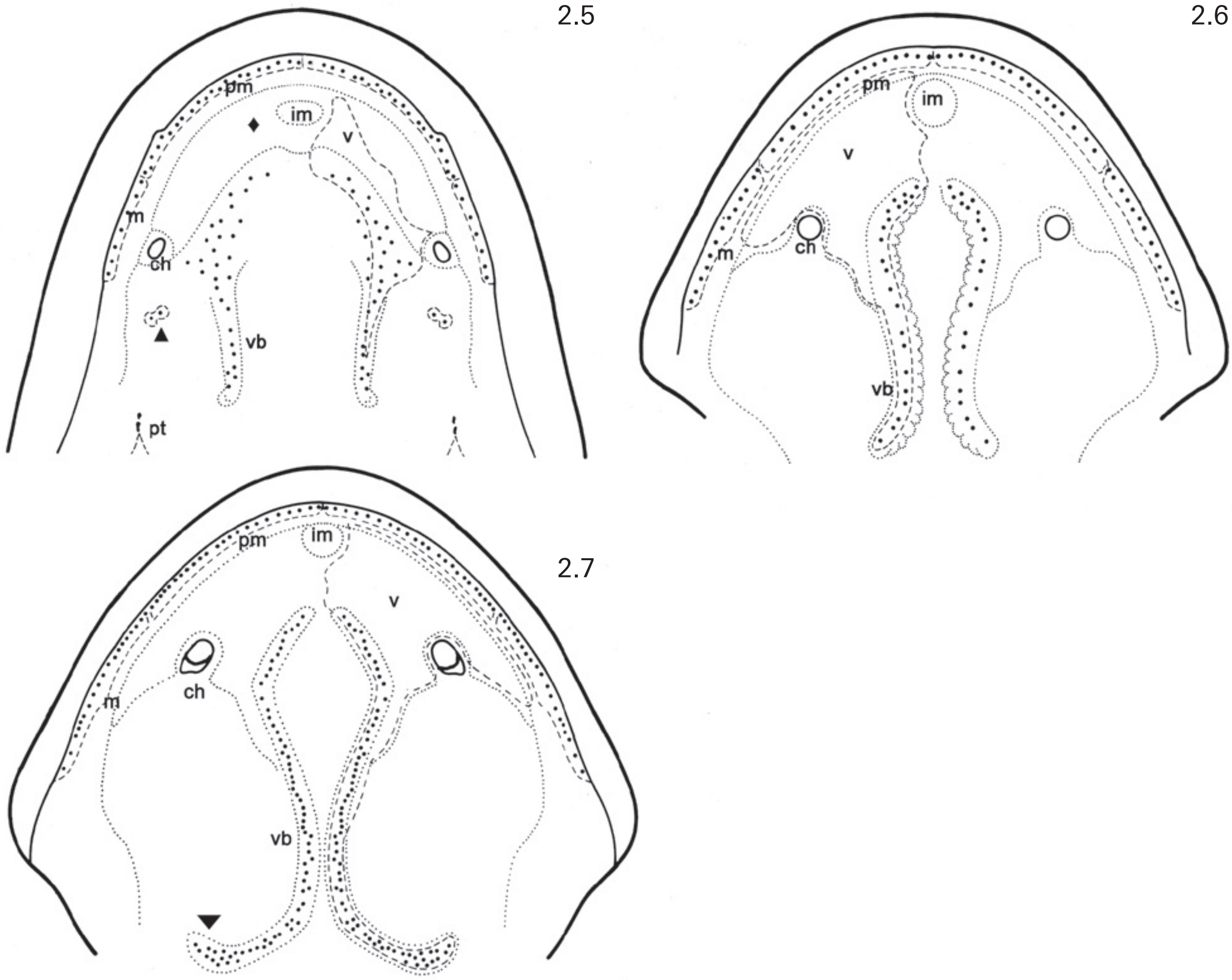


Fig. 2.1. Stage I: young larva. For further explanation, abbreviations and symbols see text.
Fig. 2.2. Stage II: Typical larva. For further explanation, abbreviations and symbols see text.
Fig. 2.3. Stage III: Late larval specimen. For further explanation, abbreviations and symbols see text.
Fig. 2.3a. Stage IIIa: Late larval specimen with slightly delayed development. For further explanation, abbreviations and symbols see text.
Fig. 2.3b. Stage IIIb: Late larval specimen with an obviously delayed metamorphosis. For further explanation, abbreviations and symbols see text.
Fig. 2.4. Stage IV: Larva at the onset of metamorphosis. For further explanation see text.
Fig. 2.5. Stage V: Midmetamorphic larva. For further explanation, abbreviations and symbols see text.
Fig. 2.6. Stage VI: Recently metamorphosed specimen. For further explanation, abbreviations and symbols see text.
Fig. 2.7. Stage VII: Fully transformed specimen. For further explanation, abbreviations and symbols see text.

vae of *S. salamandra* and larvae kept at relatively low temperatures and limited food rations become larger (e.g. Clemen, 1978). Interestingly, in both the palatinal tooth patches were entirely resorbed, whereas the palatinal bone itself and the relatively broad "palatinal bony bridge" appeared intact. In the hypophysectomized specimens a gradual regression of the palatinal dental lamina (from posterior to anterior) was demonstrated by histology (Clemen, 1978).

We think that the palatinal dental lamina becomes regressive, when larvae enter the "late larval" stage. A delay of development at this time, caused for instance by low temperatures that slow down the activity of the thyroid (see also Eggert, 1934; Gasche, 1939) – in hypophysectomized specimens this activity is further reduced –, leads to the resorption of the palatinal teeth in a typical order, i.e., from buccal to lingual and from posterior to anterior keeping the palatine and the "pterygopalatinal bridge" unchanged. Smirnov & Vassilieva (2003) achieved comparable results when studying a large number of stained and cleared developmental stages of "*Triturus*" *vulgaris* that were treated either with thyroxine or with thiourea (that inhibits the thyroxine synthesis). Authors showed that the disintegration of the palate was highly thyroxine-dependent and, therefore, that application of thiourea produced a persistent large pterygopalatine with a large, toothless palatinal portion. Gradual loss of teeth inevitably resulted from the degeneration of the dental lamina, i.e. degradation of teeth is simply age-related. We will deepen this aspect in a forthcoming article.

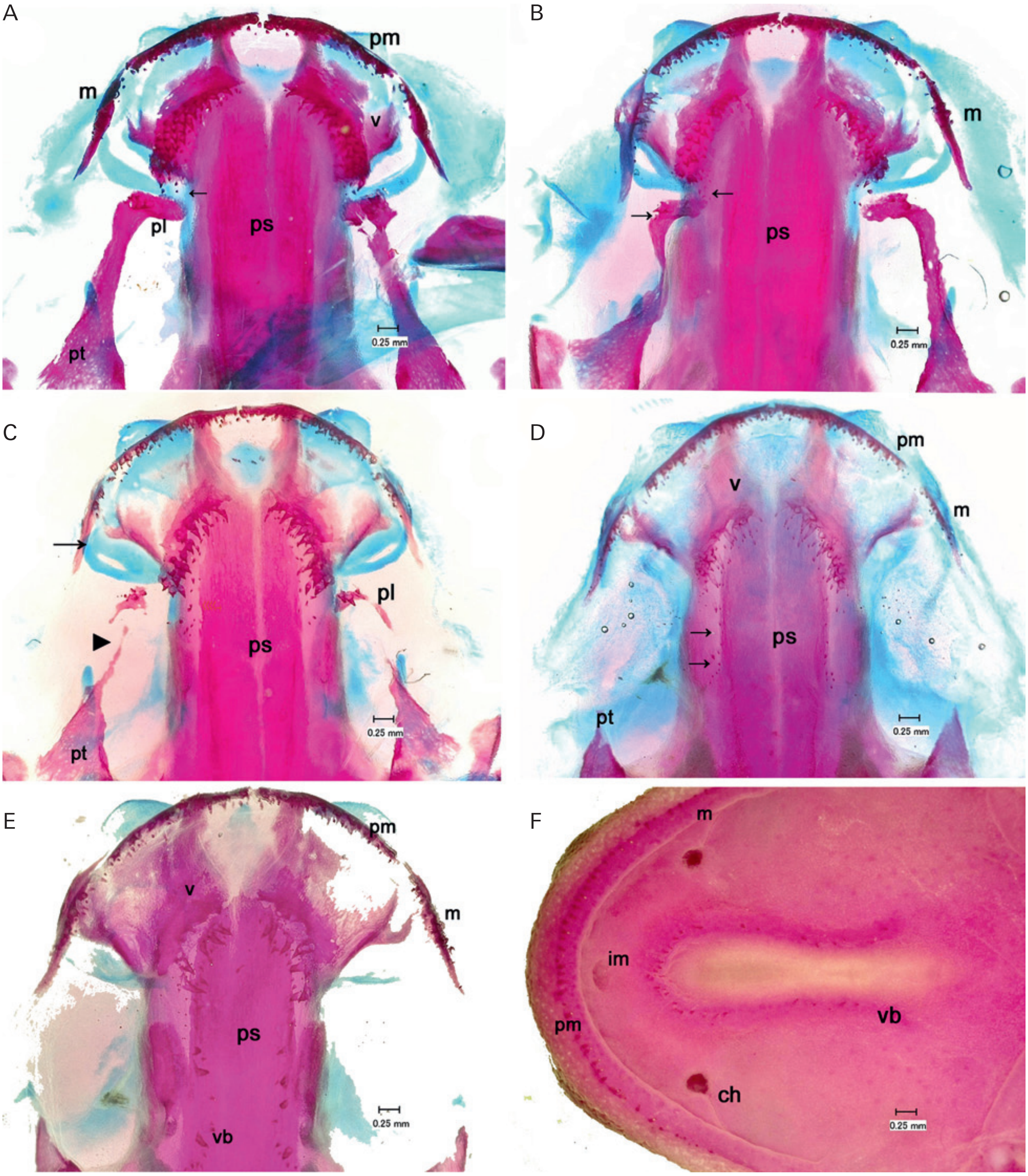


Fig. 3 A–F. Cleared and stained developmental stages. **A** Stage III: Maxillae edentate with tooth buds anteriorly; vomeres expanding anterolaterally (v) bear numerous teeth (tooth patches); gap with vomerine tooth buds between vomer and palatine (arrow); palatines bear tooth patches; the "pterygopalatinal bridge" is intact, but artificially affected on the right side. **B** Stage III a: Maxillae anteriorly dentate; vomeres with tooth patches; vomerine tooth buds fill the gap towards the palatine (right arrow); the palatine becomes regressive (left arrow); palatinal tooth patches are largely resorbed. **C** Stage IV: Maxillary dentition has proceeded; anterior vomeres monostichously dentate; anterolateral vomerine expansions (slightly stained) are larger, most posterior vomerine tooth buds beyond the remnants of the palatines; palatines highly regressive with remnants of the "pterygopalatinal bridge" (arrowhead). **D** Stage V: maxillae comparably short; vomeres with one and two (anteriorly) tooth rows; tooth buds indicate the course of the growing, largely non-ossified vomerine bars (arrows); palatines and "pterygopalatinal bridge" fully resorbed. **E** Stage VI: maxillae fully dentate; vomeres monostichously dentate and expansions ossified; vomerine bar (vb) with established teeth and tooth buds. **F** View on the upper jaw and palate of a juvenile specimen preserved in ethanol and slightly stained with alizarin red.

Acknowledgements

We are indebted to Profs Dr. H. und Dr. U. Kierdorf, Universität Hildesheim, for the use of the Keyence VHX 500F digital microscope.

References

Alberch, P. (1989): Development and the evolution of amphibian metamorphosis. – In: Splechtna, H, & Hilgers, H (eds.): Trends in Vertebrate Morphology. – Gustav Fischer Verlag, New York, pp. 163 – 167.

Amend, R. & Greven, H. (1996): Zur Ossifikation des Skeletts intra- und extrauteriner Larven des Feuersalamanders *Salamandra salamandra* (L.) (Amphibia, Urodela). – Abhandlungen und Berichte für Naturkunde, Magdeburg, **29**: 31 – 67.

Buckley, D., Alcobendas, M., García-París, M. & Wake, M.H. (2007): Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. – Evolution & Development, **9**: 105 – 115.

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

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

Clemen, G. & Greven, H. (1974): Morphologische Untersuchungen an der Mundhöhle von Urodelen. I. Die Gaumenzähne von *Salamandra salamandra* (L.) (Salamandridae: Amphibia). – forma et functio, **7**: 249 – 280.

Clemen, G. & Greven, H. (1979): Morphologische 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.

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., Cisaka, H.,Delgado, S. & Sire, J.-Y. (2007): Amphibian teeth: current knowledge, unanswered questions, and some directios for future research. – Biological Reviews, **82**: 49 – 81.

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

Duellman, W.E. & Trueb, L. (1986): Biology of Amphibians. – McGraw-Hill, New York, pp. 670.

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.

Epperlein, H.H., & Junginger, M. (1981/1982): The normal development of the new, *Triturus alpestris* (Daudin). – Amphibia-Reptilia, **2**: 295 – 308.

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.

Gallien, L. & Bidaud, O. (1959): Table chronologique du développement chez *Triturus helveticus* Razoumowsky. – Bulletin de la Société zoologique de France, **84**: 22 – 231.

Gallien, L. & Durocher, M. (1957): Table chronologique du development chez *Pleurodeles waltlii* Michad. – Bulletin biologique de la France et de la Belgique, **9**: 97 – 114.

Gasche, P. (1939): Beitrag zur Kenntnis der Entwicklung von *Salamandra salamandra* L. mit besonderer Berücksichtigung der Winterphase, Metamorphose und des Verhaltens der Schilddrüse (Glandula thyreoidea). – Revue Suisse Zoologique, **46**: 403 – 548.

Greven, H. (1977): Comparative ultrastructure investigations of the uterine epithelium in the viviparous *Salamandra atra* Laur. and the ovoviviparous *Salamandra salamandra* (L.) (Amphibia, Urodela). – Cell and Tissue Research, **181:** 215 – 237.

Greven, H. (1998): Survey of the oviduct of salamandrids with special reference to the viviparous species. – Journal of Experimental Zoology, **282**: 507 – 525.

Greven, H. (2003): Larviparity and pueriparity. – In: Sever, D.M. (ed.), Reproductive Biology and Phylogeny Vol. 1, Reproductive Biology and Phylogeny of Urodela. – Science Publisher, Inc. Enfield, pp. 447 – 475.

Greven, H. & Clemen, G. (1985): Metamorphosebedingte Veränderungen der Zähne und zahntragenden Knochen im Munddach von *Salamandra salamandra* (L.) (Amphibia, Urodela). – Verhandlungen der Deutschen Zoologischen Gesellschaft, **78**: 162 (1985)

Greven, H. & Guex, G. D. 1994. Structural and physiological aspects of viviparity in *Salamandra salamandra*. – Mertensiella, **4**: 139 – 160.

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.

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.

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.

Juszczyk, W. & Zakrzewski, M. (1981): External morphology of larval stages of the spotted salamander, *Salamandra salamandra* (L.). – Acta Biologica Cracoviensia, Series Zoologia, **23**: 1127 – 1135.

Juszczyk, W., Zakrzewski, M. & Swierad, J. (1984): The hibernation and survival of larvae of the Spotted Salamander *Sala-*

mandra salamandra (Linnaeus, 1758) in natural water reservoirs of the Carpathian Uplands and West Beskids in Poland. – Acta Zoologica Cracoviensia, **27**: 61 – 72.

Lebedkina, N.S. (1979): Evolution of the amphibian skull. Moskau, Nauka (in Russian)

Lebedkina, N.S. (2005): Evolution of the amphibian skull (translated by S.V. Smirnow). – Advances in Amphibian Research in the former Soviet Union, **9**: 1 – 265.

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.

Reilly, S.M. & Lauder, G. (1990): Metamorphosis of cranial design in tiger salamanders (*Ambystoma tigrinum*). A morphometric analysis of ontogenetic change. – Journal of Morphology, **204**: 121 – 137.

Reilly, S.M. & Altig, R. (1996): Cranial ontogeny in *Siren intermedia* (Caudata: Sirenidae): paedomorphic, metamorphic, and novel patterns of heterochrony. – Copeia, **1996**: 29 – 41.

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.

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.

Schreiber, E. (1912): Herpetologia europaea. Eine systematische Bearbeitung der Amphibien und Reptilien welche bisher in Europa aufgefunden sind (2. Auflage). – Gustav Fischer, Jena, pp. 1 – 960.

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

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.

Thiesmeier, B. & Günther, R. (1996): 6.2. Feuersalamander – *Salamandra salamandra* (Linnaeus, 1758). – In: Günther, R. (ed.): Die Amphibien und Reptilien Deutschlands. – Gustav Fischer, Stuttgart, Jena, pp. 82 – 104.

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.

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