

Long-term effects of arrested metamorphosis on dental systems in *Salamandra salamandra* (Salamandridae: Urodela)

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Abstract

We describe the dental systems of six larvae of *Salamandra salamandra* hypophysectomized in 1976 under an earlier project to arrest metamorphosis. Larvae cover roughly three developmental stages from early (intrauterine) larval stage to early metamorphosis. Animals survived surgery and lived up to 16 months until fixation. One specimen was studied by histological serial sections. In the period until fixation larvae grew from approximately 3 cm to 7 cm in length (dependent on the developmental stage before surgery) retaining their larval appearance. Changes in the dental systems depended on the stage the larvae had reached before surgery. Generally, after surgery some traits had started or continued development, such as appearance of maxillae as well as resorption of the palatinal tooth patches and the coronoids in the youngest larva examined, whereas other traits had been largely retained and even continued to grow (e. g. larval dentate vomers). In three larvae, the anterior part of the palatines was covered by the posterior margin of the vomer giving the impression of a ‘vomeropterygopalatinum’, known from some newts as temporary fusion of vomer and palatine in consequence of a delayed metamorphosis. However, in the *Salamandra*-larvae both elements appeared to be connected syndesmoticly rather than to be fused by bone tissue. The oldest larva (metamorphic stage IV, approximately at the onset of metamorphosis), had lost the palatinal portion of pterygopalatina and had reduced the tooth patches of the vomers to a single row, but outgrowth of the vomerine bar had not taken place.

Kurzfassung

Wir beschreiben die Zahnsysteme von sechs *Salamandra salamandra*-Larven, denen im Rahmen einer im Jahre 1976 durchgeführten Studie die Hypophyse entnommen worden war, um die Metamorphose zu hemmen. Die Larven befanden sich vor dem Eingriff in verschiedenen Entwicklungsstadien, i. e. frühe (intrauterine) Larve bis Larve Anfang der Metamorphose. Die Larven wurden nach dem Eingriff bis zu 16 Monate später fixiert und bis zur gegenwärtigen Analyse aufbewahrt. Ein Exemplar wurde histologisch untersucht. In der Zeit bis zur Fixierung waren die Larven je nach Stadium vor dem Eingriff von etwa 3 auf bis fast 7 cm herangewachsen, hatten aber ihr larvales Äußeres beibehalten. Veränderungen in den Zahnsystemen waren zum Teil von dem Stadium abhängig, das die Tiere vor dem Eingriff erreicht hatten. Generell bildeten sich nach der Operation einige Merkmale neu, andere setzten ihre Entwicklung fort (z. B. Bildung der Maxillaria sowie Abbau der palatinalen Zahnfelder und der Coronoiden bereits bei der jüngsten untersuchten Larve), während andere Strukturen beibehalten und sogar größer wurden, z. B. die larvalen bezahnten Vomer. Bei drei weiteren Larven war 16 Monate p.o. der Vorderrand der Palatina vom Hinterrand des Vomers überdeckt. Dies erinnert an ein Vomeropterygopalatinum, das bei manchen Salamandriden temporär in Folge einer verzögerten Metamorphose auftreten kann, doch waren im vorliegenden Fall beide Knochen nicht fusioniert, sondern wohl nur syndesmoticly miteinander verbunden. Bei der ältesten Larve war der palatinal Anteil der Pterygopalatina abgebaut und die vomerale Bezahnung auf eine Zeile reduziert; das Auswachsen der Vomerspange war aber gehemmt.

Key words

Urodela, hypophysectomy, TH-deficiency, TH-induced processes, remodelling of the palate.

Introduction

During metamorphosis the skull of Urodela (=Caudata) shows remarkable changes. Many of these processes are induced by the increasing concentration of circulating thyroid hormone (TH), the master regulator of amphibian metamorphosis. Release of TH by the thyroid is under complex control of the hypothalamic–pituitary–thyroid axis (e. g., SHI, 2000; DENVER *et al.*, 2002). However, metamorphic events may also be affected by epigenetic interactions and various environmental factors (e. g., ROSE, 1999; SHEIL *et al.*, 2014). TH-induction – dependency is likely, if larval traits disappear, and structures appearing later in development are formed precociously during accelerated metamorphosis (induced for example by application of exogenous TH), or larval traits are retained and appearance of new typical adult characters is prevented during arrested metamorphosis (TH deficiency caused by hypophysectomy, extirpation of the thyroid, or application of goitrogens such as thiourea (TU), or low temperature). A relative TH independency may be present, if during arrested metamorphosis certain developmental processes would proceed as in the case of growth.

Studies on the skull development of urodeles showed that bones differ in TH dependency and TH responsivity. Early-appearing bones (i. e. bones existing already prior to metamorphosis) do not react to changes in the TH level, whereas late-appearing bones (i. e. bones forming “around” metamorphosis) are highly TH-inducible. Further it was demonstrated that remodelling activity depends on the TH-dosage applied, and that interspecific and individual variation in TH sensitivity of bone remodelling may occur (for a review of previous articles see ROSE, 1999, 2003; for more recent studies see SMIRNOV & VASSILIEVA, 2001, 2003, 2005; SMIRNOV *et al.*, 2011; for a general broad range of variation in the relative timing of appearance or ossification of bones in amphibians see SHEIL *et al.*, 2014).

TH dependency and TH responsivity also apply to the dental or ‘tooth’ systems, i. e. the dermal dentigerous bones, and their accompanying dental laminae. Dental systems appear relatively early in development, except for the maxillae that normally appear late (for salamandrids see STADTMÜLLER, 1924, 1936; SMIRNOV & VASSILIEVA, 2001; LEBEDKINA, 2004). In the past we distinguished three paired systems in larvae (premaxilla + maxilla in the upper jaw; vomer + palatine in the palate; dentary + coronoid in the mandible) (see CLEMEN & GREVEN, 1994). However, based on the number and course of dental laminae it might be appropriate to differentiate between (1) the dental upper jaw arcade consisting of the bipartite or unipartite premaxilla and the paired maxillae, which is accompanied by a single continuous dental lamina; (2) the lower jaw consisting of the paired dentaries and the paired coronoids, each with its own dental lamina; (3) the palate formed by the paired vomers, and the paired palatinal portion of the pterygoid (=palatine),

each with its own dental lamina separated from each other, and to consider bones with an own dental lamina as specific subsystems that may react relatively independently and at different times to some extent during development and metamorphosis. An example is the delayed development of bicuspid teeth on the vomers or the variation in the reduction of the palatines. Around metamorphosis the coronoids are lost and the palate is remodelled. Remodelling involves the formation of the *pars palatina* of the premaxillae and maxillae, the reabsorption of the palatines, and the formation of the vomer. The latter process includes partial or complete reabsorption of the larval vomer, and development of new dentate elements (parts of the definite vomer and (in some taxa) caudal outgrowths (vomerine bar) and edentate parts (the anterolateral vomer plate, posterolateral choanal processes) (summarized in ROSE, 1999, 2003).

Further, the larval dentition, polystichous on some bones, becomes monostichous in most taxa, and non-pedicellate monocuspid larval teeth are replaced by pedicellate (= teeth exhibiting a dividing zone or zone of “weakness” between crown and pedicel) bicuspid teeth. Formation of the dividing zone takes place progressively throughout intermediate stages (‘subpedicellate’ condition according to BENESKI & LARSEN, 1989), whereas the change from monocuspidity to bicuspidity takes place abruptly (e. g., DAVIT-BÉAL *et al.*, 2006). This abrupt change is clearly TH-induced (e. g., CHIBON, 1972; GABRION & CHIBON, 1973; CLEMEN, 1988; GREVEN & CLEMEN, 1990; SMIRNOV & VASSILIEVA, 2003). The complete (coronoid, palatine, vomer) or partial (vomer?) disintegration of larval dentigerous bones is accompanied by degradation of their functional teeth, and regarding dentate coronoids and palatines this process is preceded by the degradation of the respective dental laminae (CLEMEN, 1978).

Within the Salamandridae the members of the genera *Salamandra* and *Lyciasalamandra* are viviparous (summarized in GREVEN, 2003). After a gestation period of several months *Salamandra salamandra* bears larvae (larviparity) that show the developmental stage that they had reached prior to ‘hibernation’, as the growth and ossification processes of intrauterine larvae slow down or are even stopped during the winter period (see GASCHÉ, 1936; SCHINDELMEISER, 1985; AMEND & GREVEN, 1996). In the newborn larvae all dental systems are present except for the maxillae (AMEND & GREVEN, 1996).

In a previous histological study CLEMEN (1978) described teeth and dental laminae of the palate of *S. salamandra* comparing normally developing larvae, hypophysectomized larvae and larvae reared under low temperature conditions. During this study several successfully hypophysectomized larvae were kept for some months post operationem before they were fixed and stored in the private collection of GC. In course of our studies on the mouth roof of Urodela and our interest in the morphology of delayed metamorphosis, we re-examined six of these specimens that revealed some details of the organisation and transformation of the mouth roof not documented so far, as well as some questions still to be answered.

Material and methods

For details on keeping the animals and the operation procedure see CLEMEN (1978). In brief, in January and March 1976 larvae were removed from pregnant females of *Salamandra salamandra* purchased from the company Stein (Lauingen/Donau; today no longer existent). Larvae were kept individually in small plastic containers at 17–18 °C and with 12 hours light on and 12 hours light off. At different times larvae were anaesthetised with MS 222, measured, and examined without any further treatment under a binocular to note the main external characters and traits of the mouth roof, at that time mainly the presence or absence of maxillae, appearance of teeth and presence of the vomerine bar. These larvae were originally roughly classified as representing “early”, “typical”, and “metamorphic” developmental stages. However, later we refined their classification considering additional morphological traits such as the length of the growing maxillae, the number of teeth, the position of the vomer, and the growth of the vomerine bar (see CLEMEN & GREVEN, 2013). Immediately after inspection, the pituitary gland was removed (see CLEMEN, 1978). The hypophysectomized larvae were reared under the same conditions as before surgery, euthanized after different times post operationem by an overdosis of MS 222, and examined immediately (CLEMEN, 1978); but six successfully operated specimens were for the first time examined in the present study. Before fixing the specimens, it was checked under a binocular, whether the hypophysis, seen as a whitish knob-like structure through the transparent parasphenoid, has recovered. In addition, the success of the operation in larva 1 was checked by histology and was in general indirectly confirmed by the fact, that clear TH-dependent structures failed to develop (see below) One specimen (larva 1), removed from the uterus in November and hypophysectomized on the same day, was fixed in Bouin, embedded in paraplast, and sectioned serially (ca. 8 µm). Sections were stained with Azan (Heidenhain) (see ROMEIS, 1968). The heads of the specimens 2, 3, 4 and 5 were fixed in buffered formalin after Lillie (ROMEIS, 1968), skinned, cleared, stained with Alizarin red and stored in glycerol (collection of G.C) until further processing. The head of larva 6 was stored without the lower jaw in 70% ethanol and processed recently.

Table 1 summarizes these data, the time the larvae were kept alive after hypophysectomy, and the main characters of larvae before (a.o.) and after (p.o.) surgery.

Heads were examined under a binocular. Photos were taken using a Keyence VHX 500F digital microscope, a dissecting microscope (Olympus SZH), or a Vanox-T AH-2-microscope equipped with a digital camera (Olympus C-3030-Z) using transmitted light or interference contrast. To judge details, e. g. bicuspidity or monocuspidity of teeth, tooth bearing bones were carefully excised, rinsed in 70% ethanol, and examined and photographed under the Vanox microscope. For each figure up to six images were combined using PICOLAY (www.picolay.de).

Abbreviations

ch = *choana* (choana); co = *coronoideum* (coronoid); d = *dentale* (dentary); ep = oral epithelium; ey = eye; m = *maxillare* (maxilla); Me = Meckels cartilage; pl = *palatinum* (palatine); pm = *praemaxillare* (premaxilla); ps = *parasphenoideum* (parasphenoid); pt = *pterygoideum* (pterygoid); v = *vomer* (vomer); vb = vomerine bar; vpl = vomerine plate.

Results and discussion

Before surgery the six larvae presented herein covered three developmental stages. The youngest intrauterine larva 1 (classified as stage 0; this stage was not included in CLEMEN & GREVEN, 2013) and 2 (approx. stage I) were in an “early larval” period, larvae 3, 4 and 5 were “typical larvae” (approx. Stages II–III), i. e. these five larvae were premetamorphic, whereas larva 6 was metamorphic (approx. stage IV). After surgery larvae had grown further, did not metamorphose and retained their larval appearance.

In the following, we describe the dental systems of these larvae one by one and make comparison mainly with other Salamandridae (see literature cited above), especially with *Lissotriton vulgaris*. From this salamandrid the most comprehensive experimental data concerning the effects of arrested and accelerated metamorphosis on dental systems are available (see SMIRNOW & VASSILIEVA, 2003). Special attention was given to the upper jaw and the palate, but some information is also given for the lower jaw (Tab. 1). We do not repeat all details listed in the table in the running text that mainly comments noticeable characters. In the schematic drawings (Fig. 1 A, 2 A, F, J; 3 A) the number of teeth (circles) does not correspond to their actual number and the marked anterolateral extensions of the vomer do not reflect their actual size, as they were not clearly seen in our preparations. Teeth and replacement teeth were monocuspid unless otherwise stated and classified as undivided (early larval) or divided (late larval) as soon as we recognized a zone of division, which term therefore also includes the subpedicellate condition (according to BENESKI & LARSEN, 1989).

Larvae 1 and 2 (a.o stage 0 and I)

Before surgery larvae 2, classified tentatively as stage I, was slightly further developed than larva 1 (classified as stage 0). All dental systems were present, but maxillae were absent. After surgery histology of larva 1 and whole mount preparation of larva 2 showed similar changes (see Tab. 1). The gap between premaxillae was bridged by a tooth (Fig. 1 A–C) obscuring the fact that premaxillae are non-fused (STADTMÜLLER, 1924, 1936). This gap and a gap between premaxilla and the short posterolaterally articulating maxilla was not conspicuous in all lar-

Tab. 1. Main characters of the six larvae before (a. o. = ante operationem) and various times after surgery (p.o. = post operationem) examined in the present study. TL = total length. Traits noted before surgery are less detailed, as larvae could not be inspected as closely as the stained specimens after surgery. For tentative staging a.o. see CLEMEN & GREVEN (2013). Teeth and replacement teeth were monocuspid unless otherwise stated and classified as undivided (early larval) or divided (late larval). The term 'replacement teeth' also includes tooth buds; row = linear series of teeth approximately parallel with the axis of the dentigerous bone. *Details before surgery (a. o.) are partly deduced from numerous intrauterine larvae examined during previous studies.

	Larva 1* (Fig. 1 A–D, sections)	Larva 2 (Fig. 1 E–I), whole mount	Larva 3 (Fig. 2 A–E), whole mount	Larva 4 (Fig. 2 F–I), whole mount	Larva 5 (Fig. 2 J, K), whole mount	Larva 6 (Fig. 4 A–G), whole mount
removal from the uterus	18.11.1976,	15.01.1976	01.01.1976	01.01.1976	01.01.1976	January 1976
date of surgery	18.11.1976	04.03.1976	04.03.1976	04.03.1976	04.03.1976	17.04.1976
stage a. o.	0 (intrauterine)	I (early larva)	II–III (mid- to late larval)	II–III (mid- to late larval)	II–III (mid- to late larval)	IV metamorphic (Fig. 4 H)
fixation p.o.	5 ½ months	11 months	16 months	16 months	16 months	10 months
TL, a. o.	3.0	2.8	3.4	3.3	3.3	5.0
TL, p. o.	5.0	5.9	6.8	6.9	6.6	6.8
coronoid a.o.; teeth a. o.	present; undivided, 1–2 rows	present; undivided, 1–2 rows	present; undivided, 2 rows	present; undivided, 2 rows	present; undivided, 2 rows	present, but reduced; un- divided, 2 rows
coronoid p.o.; teeth p.o.	reduced; undivided, a few	absent	absent	absent	absent	no data available
dentaries a.o.;	present;	present;	present;	present;	present;	present;
teeth a. o.	undivided, 1 row	undivided, 1 row	undivided + divided	undivided + divided	undivided + divided	divided + undivided (posteriorly)
dentaries p.o.; teeth p. o.	present; undivided, 1 row	present; undivided, 2 rows (posteriorly)	present; undivided, 2 rows (posteriorly)	present; undivided, 2 rows (posteriorly)	present; undivided, 2 rows (posteriorly)	no data available
premaxilla a. o.;	present (short);	present;	present;	present;	present;	present;
teeth a.o.	undivided, 1 row	undivided, 1 row	undivided + divided, 1 row	undivided + divided, 1 row	undivided and divided, 1 row	divided, 1 row
premaxilla p.o.; teeth p.o.	present; undivided, 1 row	present; undivided, 1–2 rows	present; divided, 1–2 rows	present; divided, 1–2 rows	present; divided, 1 row	present; divided, 1–2 rows
maxillae a.o.;	absent;	absent;	up to the middle of the choana;	up to the middle of the choana;	up to the middle of the choana;	beyond the posterior edge of the choana;
teeth a.o.	absent	absent	divided + undivided, 1 row	divided + undivided, 1 row	divided + undivided, 1 row	divided, 1 row
maxillae p. o.;	up to the posterior edge of the choana; undivided, 1 row	up to the posterior edge of the choana; undivided, 1 row	up to the anterior edge of the palatine; divided, 1–2 rows	up to the middle of the palatine; divided, 1–2 rows	up to the posterior end of the palatine; divided, 1–2 rows	beyond the choana (palatine degraded); divided, 1 row
dentition p.o.	present; 12–15 per vomer 1–3 rows	present; 21–23 per vomer 1–3 rows	present; 25–27 per vomer 1–4 rows	present; 25–27 per vomer 1–4 rows	present; 25–27 per vomer 1–4 rows	present; 26–28 per vomer 1–4 rows

Tab. 1. continued.

	Larva 1* (Fig. 1 A–D), sections	Larva 2 (Fig. 1 E–I), whole mount	Larva 3 (Fig. 2 A–E), whole mount	Larva 4 (Fig. 2 F–I), whole mount	Larva 5 (Fig. 2 J, K), whole mount	Larva 6 (Fig. 4 A–G), whole mount
vomer p.o.; teeth, p. o.	present; undivided	present; undivided, 52 (right), 40 (left), 5 rows, replace- ment teeth, 39 (right) 1–3 rows	present; divided 58 (right), 60 (left) 5–6 rows, replace- ment teeth, ca. 25 (right, left), 1–3 rows	present; divided, 60 (right), 57 (left) up to 7 rows, replacement teeth 37 (right), 30 (left), 1–4 rows	present; divided, 41 (right, left) in 4 rows, replacement teeth 39 (right), 35 (left) 1–3 rows	present; divided, posteriorly undi- vided, 26 (right), 25 (left), 1–2 rows, replacement teeth 19 (left) 1–2 rows
overlapping vomer, a. o.	no	no	no	no	no	no
overlapping vomer, p. o.	no	no	left and right	left and right	right	no (palatine degraded)
palatine a.o.; teeth a. o.; bridge	present; ca. 16 per patch; present	present; ca. 16 per patch; present	present; ca. 10 per patch; present	present; ca. 10 per patch; present	present; ca. 10 per patch; present	present; ca. 5 per patch; reduced
palatine p.o.; teeth, p.o.	present; a few, undivided, no re- placement teeth;	present; undivided, 8 (right), 4 (left), no replacement teeth;	present; no teeth, no replacement teeth;	present; no teeth, no replacement teeth;	degradings; 0 (left), 1 (right), no re- placement teeth;	absent; absent;
bridge p.o.	present	present	present	present	present	absent

vae examined (see Fig. 2 A, F, J, 3 A). Arrangement of teeth on the premaxillae suggests partial bistichy (Fig. 1 D). Established teeth and replacement teeth were undivided and monocuspid. This holds also for the maxillae (see Fig. 1 D, F). The vomerine tooth patch was relatively large (Fig. 1 A, B, E), whereas the palatinal tooth patches appeared reduced (Fig. 1 A, E, F). In the whole mount preparation the field of premaxillary replacement teeth was paralleled by a fibrous band that might represent the strong *lamina propria* at the (inner) unproductive site of the dental lamina (Fig. 1 C). Sections revealed an intact dental laminae and replacement teeth of dentaries (Fig. 1 G), premaxillae, maxillae, and vomers (see Fig. 1 H). In the coronoids (Fig. 1 G) and the palatines dental laminae and replacement teeth were missing (Fig. 1 D).

Noteworthy are (1) the appearance of the anterior portion of maxillae bearing undivided teeth, (2) the arrangement of some teeth on the premaxillae suggesting partial bistichy; (3) the large vomerine tooth patch (Fig. 1 D, E); (4) the absence of the palatinal dental laminae and reduction of the palatinal tooth patches, and (5) the absence of the dental laminae of the coronoid and reduction of the coronoid.

Ad 1. Contrary to the premaxillae, maxillae are relatively late appearing TH-inducible bones (see introduction). Generally, in salamandrids maxillae arise in the “late” larval or even metamorphic period (see STADTMÜLLER, 1924, 1936; CLEMEN & GREVEN, 1979; REILLY, 1986; SMIRNOV & VASSILIEVA, 2001; ROSE, 2003). After surgery both larvae possessed maxillae close to the premaxilla, which possessed undivided teeth typical for ‘early’ larvae. The formation of the dividing zone is well studied (DAVIT-BÉAL *et al.* 2006), but the causative factors for its development are unknown (GREVEN & CLEMEN 1990). Due to their relative late appearance, early undivided tooth generations of maxillary teeth may be skipped in some species (see the discussion in VASSILIEVA & SERBINOVA, 2013), but this is obviously not the case in *Salamandra salamandra*. *Pleurodeles waltl* larvae raised in TU developed small largely reduced maxillae without teeth, and it was suggested that TH may be involved in the regulation of bone growth and calcification rather than inducing the formation of the bone itself or some TH was produced despite TU-treatment (see SMIRNOV & VASSILIEVA, 2001). In *Lissotriton vulgaris* maxillae are considered as “highly TH-dependent”, as they developed precociously after TH application, but either did not appear, or developed with some delay and remained rudimentary in TH-deficient specimens (SMIRNOV & VASSILIEVA, 2003; SMIRNOV *et al.*, 2011). We do not think that the maxillae of larva 2 shown in figure 1 A had a rudimentary appearance.

Ad 2. Usually, larval (and adult) premaxillae and maxillae bear a single row of functional, i. e. attached teeth. In five of the examined larvae at least two teeth were arranged one behind the other in some places of either the premaxillae (larva 2), the premaxillae and maxillae (larvae 4, 5, 6) or the maxillae (larva 3) suggesting a kind of

bistichy. Generally, bistichy (and polystichy) results from the attachment of several teeth of different age arranged one behind the other. These teeth and their developmental stages (arising at a given position) belong to the same (tooth) family, which term also includes “the first-generation tooth at a given position in early larvae and all its successors until adulthood” (DAVIT-BEÁL *et al.*, 2006, p. 1049; see also Fig. 1 E). In salamandrids polystichy is the rule on the larval vomer, the palatine and the coronoid. On the dentary of *P. waltl* only first-generation teeth and their immediate successors are functional, i. e. are attached to the bone (DAVIT-BEÁL *et al.*, 2006). Local bistichy of premaxillae was also shown in larval *Ambystoma mexicanum* and *Lissotriton vulgaris* (CLEMEN & GREVEN, 1977, 1979) and a remarkable temporary polystichy is present on the upper and lower jaw of intratuterine larvae (Schwalbe stage 3) of *Salamandra atra* as an adaptation to intrauterine feeding (GUEX & CHEN, 1986; GREVEN, 2003). The presence of bistichy in our preparations indicates the general ability of the dental lamina of the upper jaw to produce polystichy. However, a direct effect of surgery or TH-deficiency cannot be excluded.

Ad 3. Vomers appear early in development. After surgery the larval vomer has grown further, and also the number of teeth has increased considerably. Position of replacement teeth extended slightly beyond the posterior end of the vomer. On the anterior and posterior end of the vomer (here only on one side), the putative *lamina propria* of the dental lamina (see above) describes a curve buccalwards preventing perhaps vomerine teeth to occupy the gap between vomer and palatine, which may occur in normogenesis (CLEMEN, 1978; see also GREVEN & CLEMEN 2015).

Ad 4. The palatines are early appearing bones, and are resorbed during metamorphosis. In larva 1 the palatal dental laminae and replacement teeth were missing after surgery (confirming indirectly the degradation of the dental lamina), but established teeth were still present. The same applied to larva 2, but here the number of established teeth was reduced. These changes were also shown in premetamorphic larvae reared at low temperatures and in hypophysectomized larvae 10 month after surgery, which lack palatal tooth patches (CLEMEN, 1978; CLEMEN & GREVEN, 2013). TU-treatment prevented the resorption of the palatines in *Pleurodeles waltl* (SMIRNOV & VASSILIEWA, 2001). In *Lissotriton vulgaris* overwintering larvae have lost their palatal dental laminae (CLEMEN & GREVEN, 1979). TU-treatment decelerated disintegration

of the palatine, whereas TH-treatment led to a precocious reduction of tooth patches (SMIRNOV & VASSILIEWA, 2003).

Ad 5. Coronoids (and dentaries) appear early in development. Generally, coronoids and palatines disintegrate at at metamorphosis (REILLY, 1986; SMIRNOV & VASSILIEWA, 2003; SMIRNOV & VASSILIEWA, 2001; SMIRNOV & VASSILIEWA, 2003). Degradation of these bones as well as their development is thought to be TH-induced (e. g. ROSE, 1999; SMIRNOV & VASSILIEWA, 2003). Therefore, the relative early reduction and even absence of the coronoid in premetamorphic larva of *Salamandra salamandra* was not expected. In *P. waltl* coronoids were retained in TU treated larvae (SMIRNOV & VASSILIEWA, 2001). In *L. vulgaris* exogenous TH leads to a precocious resorption of the coronoids (and palatines). After TU treatment disintegration of coronoids started slowly, but these bones became considerably reduced after extremely long treatment (570 d) (SMIRNOV & VASSILIEWA, 2003).

As a consequence of the early disintegration of the coronoids and palatines in *S. salamandra*, which obviously takes place earlier than previously suggested (see CLEMEN, 1978), their dental laminae neither may produce bicuspid nor (sub)pedicellate teeth and it was suggested that they even lack the potential to produce such teeth (see CLEMEN & GREVEN, 1994). This does not hold for divided (subpedicellate) teeth. In the salamandrid *Mertensiella caucasica* the late larval coronoids bear divided teeth, probably because in this species the early larval phase of the ontogeny of jaw dentition is reduced (see VASSILIEVA & SERBINOVA, 2013).

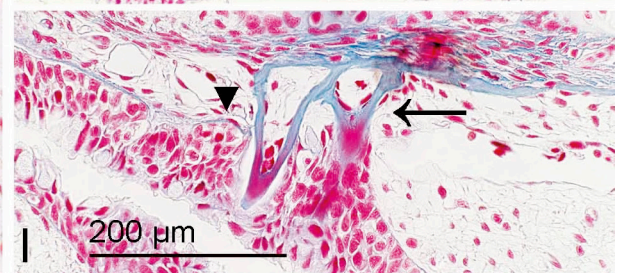
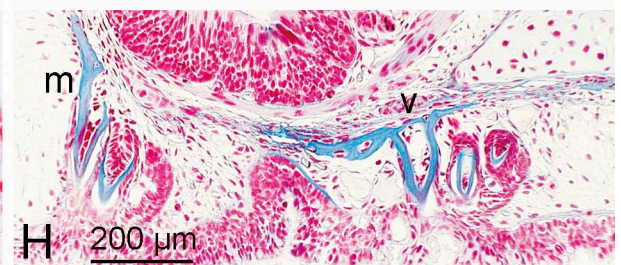
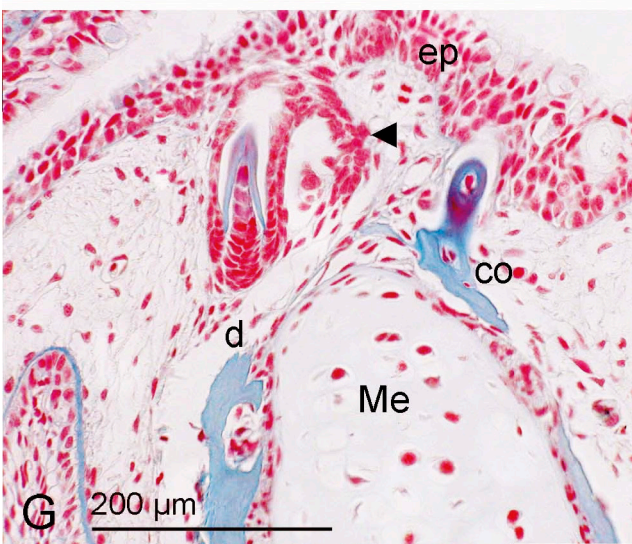
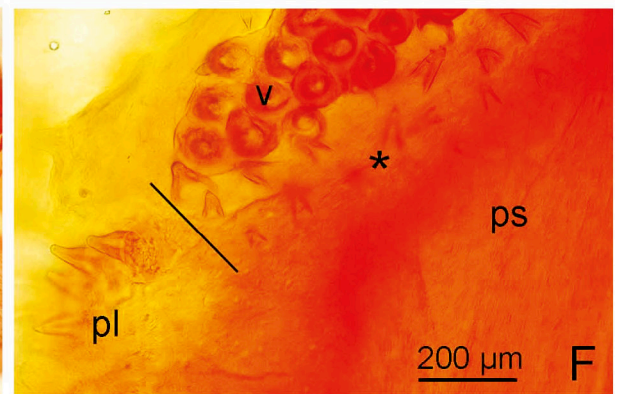
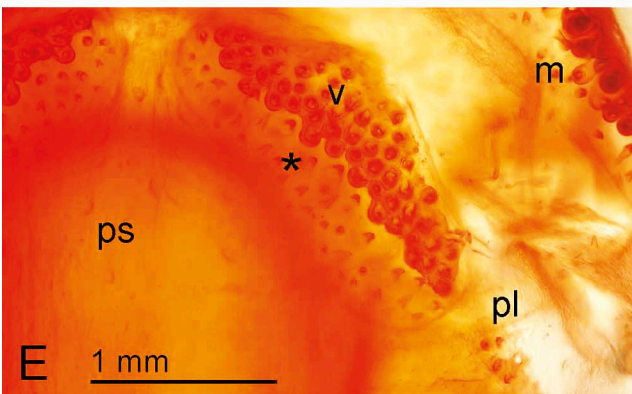
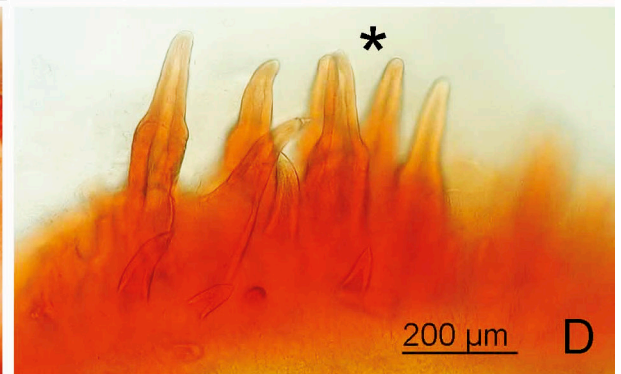
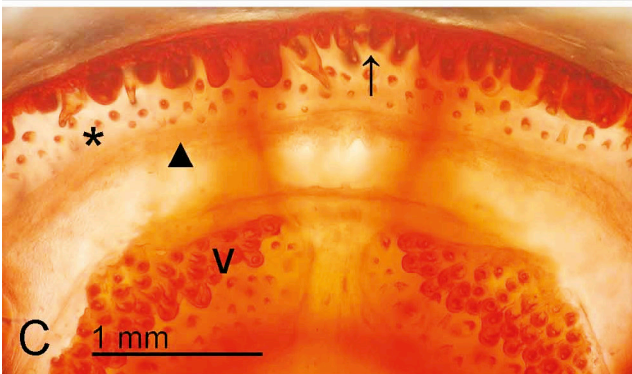
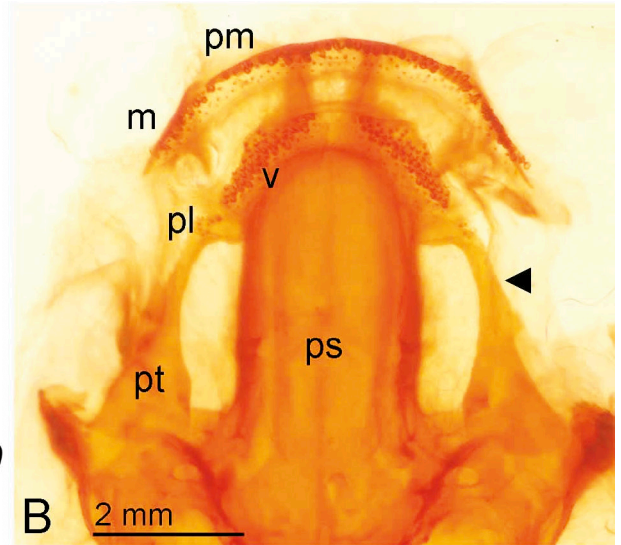
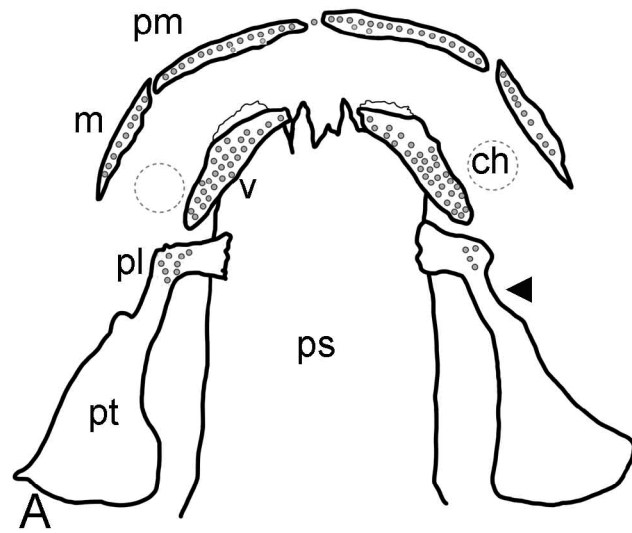
Larvae 3, 4 and 5 (Stage II–III a. o.)

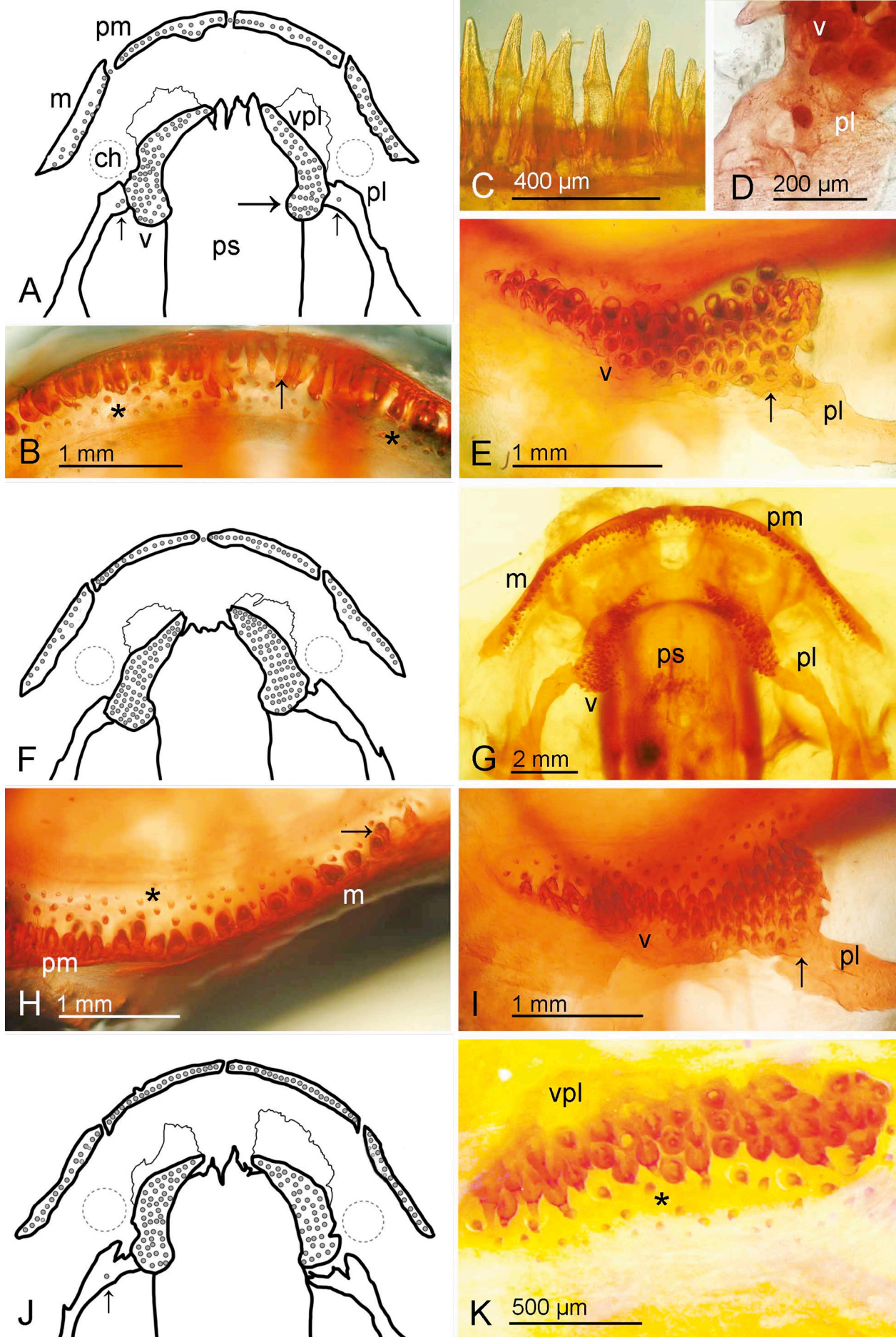
These larvae, classified as stage II–III before surgery, had the full set of dental systems with undivided and divided teeth (s. Tab. 1). Premaxillae and maxillae bear a continuous tooth row; maxillae appear slightly elongated (Fig. 2 A, B). There was no clear gap between these bones that may be bridged by a tooth (see Fig. 2 H). Local ‘bistichy’ (see above) was seen on premaxillae and maxillae in all larvae (Fig. 2 A, B, C, F, H). Dentalia showed large teeth posteriorly (not shown).

Noteworthy are (1) the broadened and highly dentate posterior end of the vomer that may overlap the anterior palatal portion; and (2) the edentate palatines.

Ad 1. In normogenesis vomer and vomerine tooth patches increase in size, i. e. grow in anterior and posterior (here in a slightly caudo-medial) direction. This process

→ **Fig. 1.** Mouth roof, ventral view, and details of the dentition of larva 2 11 months p.o. (A–F) and larva 1 (G–I) 3 months p.o. **A, B:** Overview; note the reduced palatal tooth patch and partial bistichy on the premaxilla; pterygoid bony bridge (arrowhead). **C:** The gap between the bipartite dentate premaxillae (pm) is bridged by teeth (arrow); strong lamina propria of the unproductive side of the dental lamina (arrowhead). **D:** Partial bistichy (asterisk) on the premaxilla, lingual view. **E:** Left vomer (v); reduced tooth patch on the palatine (pl). **F:** Right vomer (v) and palatine (pl). Note the non-ankylosed tooth between vomer (v) and palatine (p). Line = tooth family. **G:** Part of the lower jaw with dentary (d) plus dental lamina (arrowhead) and coronoid (co): note the absence of the dental lamina. **H:** Maxilla (m) and vomer (v) with monocuspid, undivided teeth. **I:** Part of the palatine (arrow); remnants of the dental lamina (arrowhead). Replacement teeth and tooth buds in C, E, and F are labelled with an asterisk.





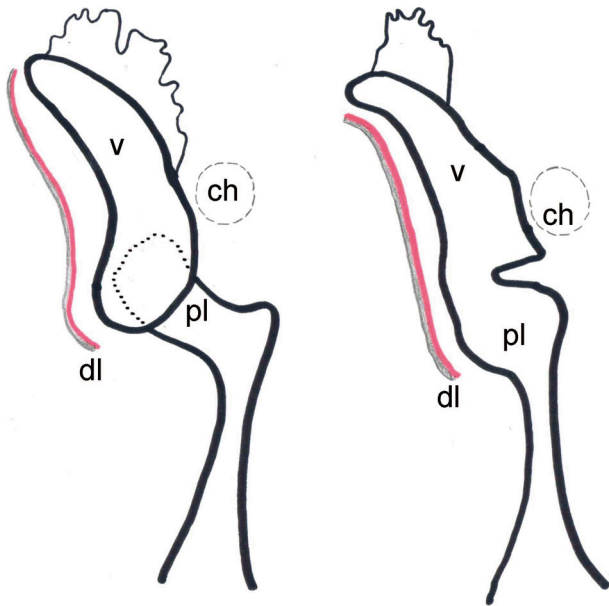


Fig. 3. Overlapping of vomer (v) and palatinum (pl) in *Salamandra salamandra* (left) and formation of a vomeropterygopalatinum in *Ichthyosaura alpestris* and *Lissotriton vulgaris* (right). dl = dental lamina (red) with the strong connective tissue (grey) on the unproductive side. For further explanation see text.

is stopped at the onset of metamorphosis. In overwintering larvae (see CLEMEN, 1978) as well as in the hypophysectomized larvae of these stages growth of the vomer leads to the enlargement of the posterior part of the tooth patch and to formation of many replacement teeth (Fig. 2 E, I). As a result, in larvae 3 and 4 the posterior part of the vomer overlaps the palatine, still connected with the pterygoid, on both sides, and in larva 5 on one side (Fig. 2 D, E, I). The boundary of the overlapping vomer was not easy to recognise; therefore the assignment of the most posterior teeth either to the vomer or the palatine was not always possible (compare Fig. 2 D and Fig. 2 I). Manipulation of these bones with a fine needle does not reveal a fusion of vomer and palatine.

At first glance this overlapping gives the impression of a vomeropterygopalatinum, *i. e.* a fusion of the palatine portion of the pterygopalatinum with the vomer, which occurs in the newts *Lissotriton vulgaris* and *Ichthyosaura alpestris* during delayed metamorphosis. However, in the newts the posterior edge of growing vomer meets the anterior edge of the palatines and both merge without a suture (Fig. 3; GREVEN *et al.*, 2015, 2017). Reasons for the different behaviour of the vomer and palatine in newts and salamanders during arrested (or delayed) metamor-

phosis are unknown, but may be influenced by the different structure and growth of the two bones. In *S. salamandra* the palatine has a shape that resembles a spoon used for feeding a toddler, *i. e.* bent at an angle of approx. 90°, whereas in newts vomer and palatine lay one behind the other in nearly a straight line (see Fig. 3).

Close proximity of these bones has been reported from the salamandrid *Notophthalmus viridescens*, where an interdigitating articulation of vomer and palatine occurred prior to metamorphosis (REILLY, 1986).

In very young TH treated *Ambystoma mexicanum* the vomer merged with the palatopterygoid forming a vomeropalatopterygoid (SMIRNOV & VASSILIEVA, 2005): For TH-treated *L. vulgaris* a fusion of the vomer with parts of the palatine was described and for TU treated specimens an expanded anterior part of the palatine sliding under the posterior portion of the vomer was reported (SMIRNOV & VASSILIEVA, 2001). This resembles the conditions described for *S. salamandra*.

Ad 2. Sixteen months after surgery the largely edentate palatines (very few degrading teeth in larva 3, no teeth in larva 4; a single tooth in larva 5) indicate that the degradation of the palatal dental lamina did not require high levels of TH, if any (see also larva 1 and 2).

Larva 6 (Stage IV a. o.)

This larva was the most developed at time of surgery, and was classified as stage IV (metamorphic), a stage at which the number of vomerine teeth is already considerably reduced (see Fig. 4 H for a comparable stage). After surgery, maxillae reached the midst of the elongate choana (Fig. 4 A, B). Again premaxillae showed a partial ‘bistichy’ (see above); the gap between premaxillae and maxillae was clearly visible and was bridged by a tooth; a single replacement tooth in this region was bicuspid; teeth were clearly divided (Fig. 4 A–F). The pedicellate condition of teeth also holds for the vomer, whose tooth patch was, however, highly reduced. On its posterior part some undivided teeth occurred (Fig. 4 E–G). Palatines were missing.

Noteworthy are (1) the considerable reduction of the larval vomer and the vomerine tooth patch, but absence of the vomerine bar; (2) the complete absence of the palatines and the bony bridge connecting the palatine with the pterygoid; and (3) presence of a bicuspid replacement tooth and some undivided vomerine teeth.

Ad 1. In *S. salamandra* the larval vomer appears not to be resorbed totally; rather parts of the larval vomer be-

← **Fig. 2.** Mouth roof, ventral view, and details of the dentition of larva 3 (A–E), larva 4 (F–I), and larva 5 (J, K) 16 months p.o. Schematic drawings are not in scale. **A:** Overview; note the reduced palatal tooth patches (small arrow), the vomers (v) posteriorly broadened and bent slightly inward, and bistichy on the maxillae (m). **B:** The gap between the bipartite dentate premaxillae (pm) is bridged by teeth (arrow); **C:** Bistichously arranged maxillary teeth (interference contrast). **D:** Overlapping of vomer and palatine. **E:** Boundary (arrow) between right vomer (v) and right palatine (pl). **F, G:** Overview; note broadened posterior parts of the vomers (v). **H:** Premaxilla (pm) and maxilla (m); note bistichy (arrow). **I:** Boundary (arrow) between right vomer (v) and right palatine (pl). **J:** Overview. **K:** Left vomer; note divided teeth; vomerine plate (vpl). Replacement teeth and tooth buds in B, H, and K are labelled with an asterisk.

came incorporated in the definite vomer (CLEMEN, 1979). Compared to larvae **3**, **4** and **5** vomers and vomerine tooth patches of larva **6** were not broadened posteriorly, but appeared considerably reduced suggesting that already before surgery the remodelling of the vomer had started. The same applies to the development of the vomerine bar that arises from an osteogenic field of connective tissue at the posterior edge of the larval vomer (see CLEMEN, 1979) and was seen as small elevation of the oral epithelium. The organisation of the vomer before surgery roughly corresponded to that shown in figure 4 H). Remodelling and the simultaneous development of the vomerine bar (inhibited by surgery) had prevented the posterior expansion of the vomer. Images of two stages of normogenesis may illustrate the progress of outgrowth (Fig. 4 H, I).

Ad 2. The thinning and the final break of the mid-part (bony bridge) of the palatopterygoid have been repeatedly considered as clearly visible morphological sign for the onset of metamorphosis (e. g. REILLY 1986). Its degradation, started already before surgery, and degradation of the palatine was completed after surgery suggesting that these processes, very probably stimulated by TH, were not halted by the withdrawal of TH (see HARTWIG, 1940). HARTWIG replanted TH treated larval skin fragments in the subcutis of larvae and could demonstrate transformation of larval skin into adult epidermis.

Ad 3. We did not exactly know the type of teeth in larva **6** before surgery. However, based on other studies (e. g. CLEMEN & GREVEN, 2013), they all must have been monocuspid and divided, i. e. they were in a late larval stage (see Fig. 4 H). Generally, this type of teeth persisted after surgery suggesting that the TH-level necessary for its transformation in bicuspid teeth had not yet reached. This is also indicated by the fact that all replacement teeth of the upper jaw and the vomer, so far examined, were monocuspid. Therefore, the presence of the single bicuspid replacement tooth between premaxilla and maxilla as well as the presence of undivided (early larval) teeth at the posterior end of the vomer seemed somewhat surprising. In a previous experiment hypophysectomized larvae (stage III–IV) developed mono- and bicuspid teeth on the upper jaw 103 days p.o. arranged in an alternating order. This suggested the presence of alternating functional and non-functional tooth loci or their counterparts in the dental lamina. The former may have temporarily inhibited the development of further teeth; whereas the

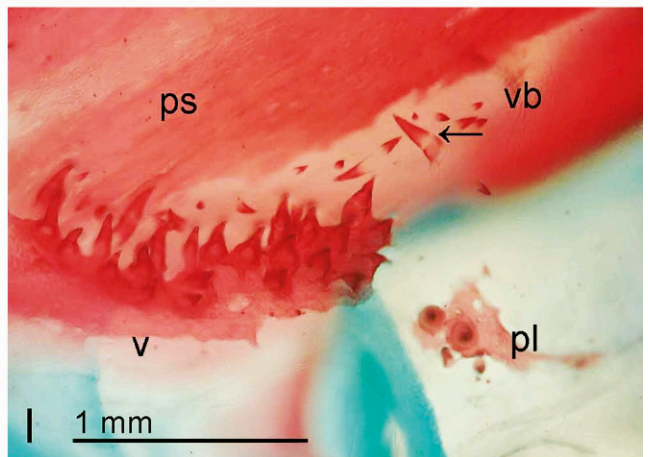
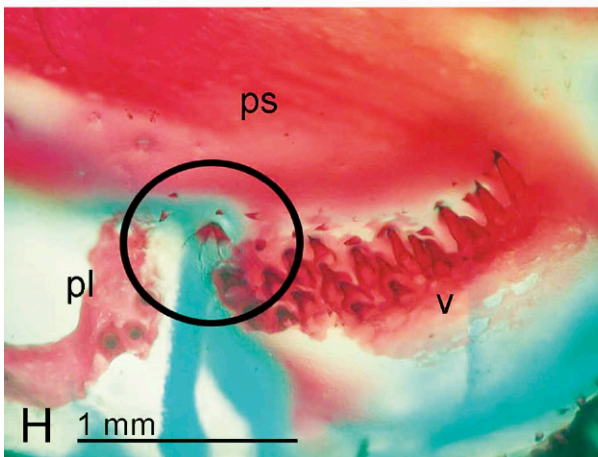
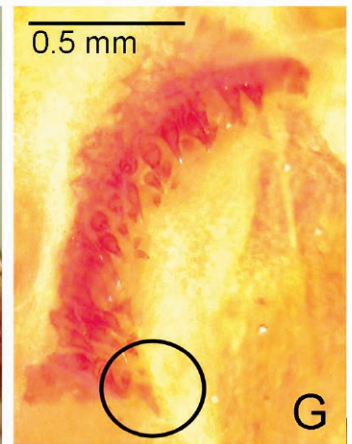
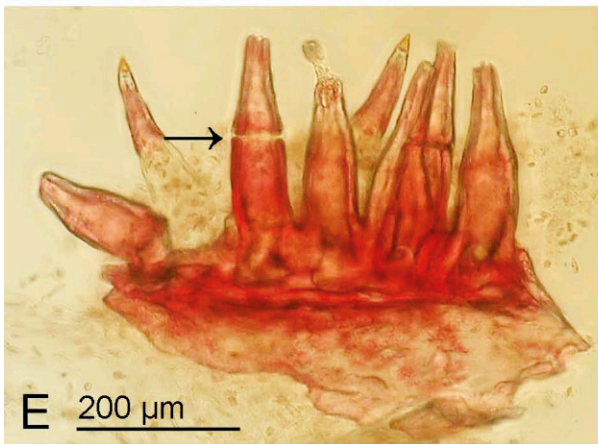
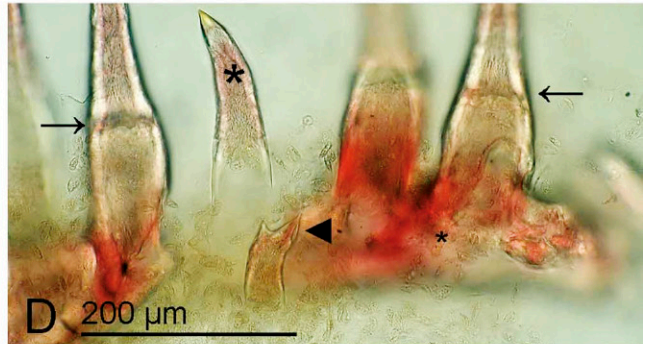
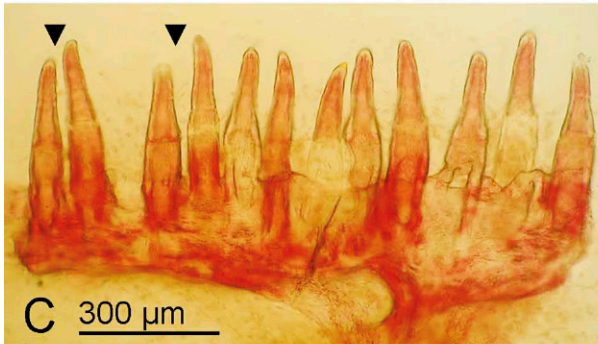
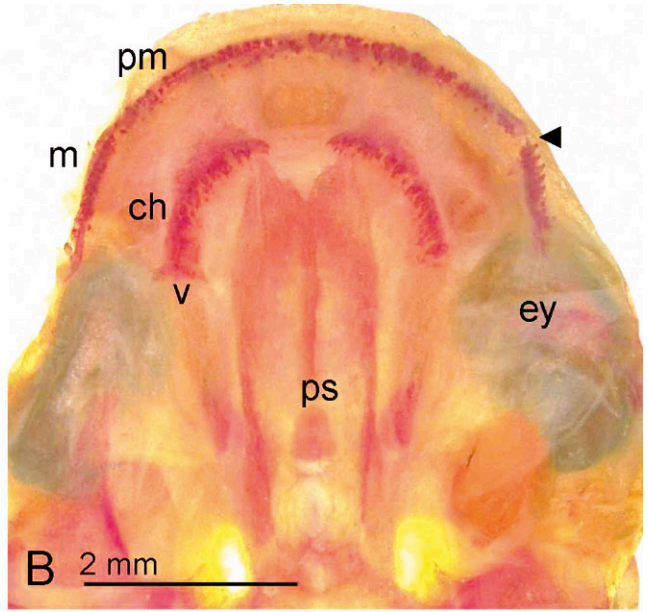
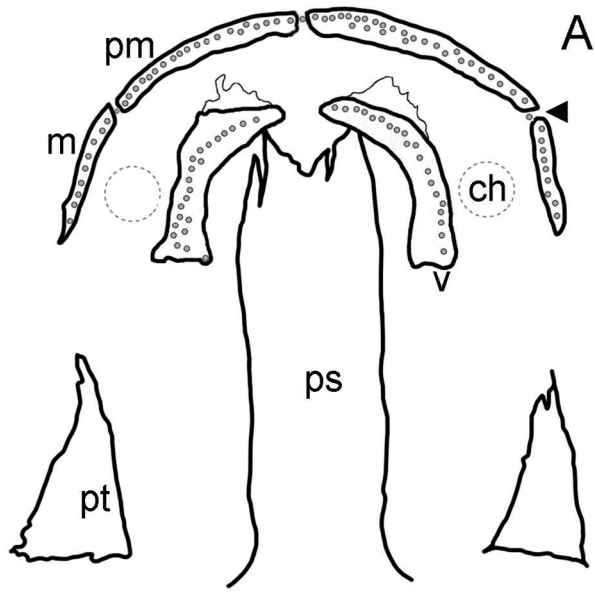
latter may have received stimuli to produce new teeth (see GREVEN & CLEMEN, 1990). In the present study the observed bicuspidity probably indicates a (premature?) local response of the tooth locus to some TH that may have been present before surgery (see below). Undivided teeth on the vomer, where bicuspid teeth are generally formed later than in the upper jaw, could have arisen from loci inactive before surgery.

Concluding remarks

The examination of the dental systems of six (early-, mid-larval, and metamorphic) hypophysectomized larvae of *Salamandra salamandra* confirmed a stage dependent response to TH deficiency and showed that some processes failed to appear, whereas others proceeded after surgery. Certainly, many of them respond to plasma concentrations of TH. TH levels have not been recorded in developing *S. salamandra*, but are considered to be generally low in transforming amphibians during embryonic and early larval development. TH levels increase towards metamorphosis, peak at metamorphic climax and decline thereafter (NORMAN et al., 1987; see also DODD & DODD, 1976; SHI, 2000).

TH-inducibility and TH-dependence of the remodelling of the palate, i. e. rebuilding of the vomer, loss of the palatine (and the coronoid in the lower jaw) during metamorphosis, is evident, as these processes are prevented by TH-deficiency (e. g. see ROSE, 1999, 2003; for more recent studies see SMIRNOV & VASSILIEVA, 2001, 2003, 2005; SMIRNOV et al., 2011). TH-induction, generally, occurs via the regulation of gene expression through TH-receptors and the reception of the message depends upon the presence and number of these receptors in the respective cells/tissues. Less sensitive cells require high TH concentrations to respond at correspondingly later stages. Such relations, demonstrated for several amphibian tissues are to our knowledge unexplored regarding the dental systems. That these systems react at different times during development, and therefore probably respond to different concentrations of TH can be deduced from the literature (summarized in ROSE, 1999, 2003; see also SMIRNOV & VASSILIEVA, 2001, 2003, 2005; SMIRNOV et al., 2011). However, to infer a direct TH-independency or TH-dependency only from the above results might be too simple due to the complex regulation of TH release

→ **Fig. 4.** Mouth roof, ventral view and details of dentition of larva **6** (11 months p.o.) (A–G) and cleared and stained specimens of larvae in normogenesis (H, I). **A, B:** Overview; note the absence of the palatine and the tooth (arrow) between premaxilla (pm) and maxillae (m). **C:** Premaxilla (labial aspect); note partial bistichy (arrowheads). **D:** Premaxilla (left) and left maxilla (right), lingual aspect. Note the divided teeth (arrows), the tooth between the two bones (asterisk) and a bicuspid replacement tooth (arrowhead). **E:** Fragment of the vomer (labial side) with divided teeth (arrow). **F:** Two fragments of the left vomer with undivided (asterisk) and divided (asterisks) teeth. **G:** Right vomer with largely monostichous dentition; note the position of the tooth at the posterior end (circle). **H, I:** Stage IV (H) and stage V (I) of normogenesis; note the posterior end (circle) of the vomer (v) in H, the divided non established tooth (arrow) on the prospective vomerine bar (vb) in I, and the different degradation of the palatines (pl).



and reception of TH, in which different tissues are involved (e. g., SHI, 2000; DENVER *et al.*, 2002).

It must also be considered that a complete developmental series and time scaling of the development of dental systems in *S. salamandra* are still missing and that the developmental stages of various urodeles on the basis of external appearance and even on developmental events during cranial ontogeny (see literature cited above) may be not readily comparable. Nevertheless, varying developmental sequences of the earliest cranial bones, which also include the dental systems except for the maxillae have been recorded (see tab. 3 in VASSILIEVA & SERBINOVA, 2013). The deviations reported herein, e. g. the relatively early formation of the maxillary dental system, the early regression of the coronoid dental system, and the early reduction of the palatal tooth patch may reflect taxon-specific differences, but might be also related to the mode of reproduction. GASCHÉ (1939) and SCHINDELMEISER (1985) demonstrated histologically and ultrastructurally activities of the thyroid gland in intrauterine larvae just before and at the beginning of hibernation. This activity was discussed in relation to the TH-dependent urea excretion and growth inhibition (possibly by keeping the prolactin concentration low) of intrauterine larvae (SCHINDELMEISER, 1985), but not in relation to other TH-dependent processes.

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