

Comparative Skull Osteology of *Karsenia koreana* (Amphibia, Caudata, Plethodontidae)

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ABSTRACT The recent discovery of a plethodontid salamander, *Karsenia koreana*, in Korea challenged our understanding of the biogeographic history of the family Plethodontidae, by far the largest family of salamanders, which otherwise is distributed in the New World with a few European species. Molecular studies suggest that *Karsenia* forms a clade with *Hydromantes* (*sensu lato*), which includes among its species the only other Old World plethodontids. We studied the skull of *K. koreana* and compared it with that of other plethodontid genera, especially members of the subfamily Plethodontinae, which it resembles most closely in general anatomy. The anatomy of its skull corresponds to the most generalized and apparently ancestral condition for plethodontids. No clearly autapomorphic states were detected, and no synapomorphies can be found that would link it to other genera. The *Karsenia* skull is cylindrical and well ossified, giving an impression of strength. In contrast, the skull of *Hydromantes* is highly derived; the skull is flattened and the bones are weakly ossified and articulated. *Hydromantes* and *Karsenia* share no unique anatomical features; differences between them are especially evident in the hyobranchial skeleton, which is generalized in *Karsenia* but highly modified in *Hydromantes*, which is well known for its highly projectile tongue. *Plethodon* and *Plethodon*-like species, including *Karsenia* and to a lesser degree *Ensatina*, represent the more generalized and apparently ancestral plethodontid morphology. Specialized morphologies have evolved along only a few morphological axes within the Plethodontidae, resulting in a pattern of rampant homoplasy. Our analysis of the anatomy of the new Asiatic lineage illuminates some potential mechanisms underlying adaptive morphological evolution within the Plethodontidae. *J. Morphol.* 271:533–558, 2010. © 2009 Wiley-Liss, Inc.

KEY WORDS: evolution; morphology; bones; lungless salamanders; hyobranchial apparatus

INTRODUCTION

The order Caudata comprises 583 species of salamanders (AmphibiaWeb, <http://amphibiaweb.org/index.html>, September 2009). The family Plethodontidae, with 394 species, is the most diverse family, both in number of species and in ecological and morphological variability. Plethodontid salamanders occupy a vast array of ecological niches, adapting to very different habitats (e.g., aquatic, fossorial, arboreal, stream, terrestrial or cave;

Wake, 1966). Most plethodontids lay eggs on land, which develop directly into miniatures of the adults without a larval stage. Species with the basal amphibian biphasic life cycle, as well as pedomorphic (permanently larval) forms, also occur, but at least in the Plethodontinae the larvae are secondarily derived (e.g., species of *Desmognathus*; Chippindale et al., 2004; Mueller et al., 2004). Despite morphological variability related to the ecological diversification of the group, synapomorphic traits characterize the family, such as the absence in adults of lungs, pterygoid and lacrimal bones, and the presence of a naso-labial groove and large posterior patches of vomerine teeth (Wake, 1966).

Several principal evolutionary lineages are recognized within the family, arrayed taxonomically in different groups depending on the authors (e.g., Wake, 1966; Lombard and Wake, 1986; Chippindale et al., 2004; Mueller et al., 2004; Frost et al., 2006; Vieites et al., 2007). The most recent revision of the group recognized two subfamilies within Plethodontidae: i) Hemidactyliinae, which includes the supergenus *Bolitoglossa* (12 genera; >250 species of tropical salamanders), the western North American genus *Batrachoseps* (20 species), the tribe Spelerpini (Eastern North American species, genera *Eurycea*, *Gyrinophilus*, *Pseudotriton*, *Stereochilus*, and *Ursperlerpes*), and the genus *Hemidactylium* (monotypic genus), the last a distinct lineage whose taxonomic position has been rather controversial; ii) Plethodontinae, which includes the supergenus *Desmognathus* (genera *Desmognathus* and *Phaeognathus*), the North American genera *Aneides*, *Ensatina*, and *Plethodon*, the

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super-genus *Hydromantes* (genera *Atylodes*, *Hydromantes*, and *Speleomantes*), and the recently described genus *Karsenia* (Vieites et al., 2007).

Karsenia is a monotypic genus that contains the only known Asiatic plethodontid, *Karsenia koreana*, recorded from only a few localities in South Korea, where individuals can be found under rocks on the humid slopes of montane woodlands with limestone soils (hardwoods and mixed hardwood/pine; Min et al., 2005). The species has the characteristic features of plethodontid salamanders (the absence of lungs, pterygoid and lacrimal bones in adults, and the presence of a naso-labial groove and large posterior patches of vomerine teeth). The first molecular analyses carried out confirmed the singularity of the new lineage (Min et al., 2005). This, together with some morphological particularities such as an attached protrusible tongue and a derived tarsus, as well as its geographic distribution, led to the description of a new genus for the species (Min et al., 2005). A thorough molecular study confirmed the inclusion of *K. koreana* within the plethodontine salamanders (Vieites et al., 2007). In that phylogenetic analysis, *Karsenia* forms a clade with the species from the super-genus *Hydromantes*, the clade being sister to the remaining plethodontines (see Fig. 1). Furthermore, *K. koreana* shares synapomorphic karyological characteristics with *Hydromantes* (Sessions et al., 2008).

Plethodontid salamanders are distributed primarily in the New World (99% of species). Most of the species are found in North and Middle America, with the exception of the eight that occur in the Mediterranean region. Several hypotheses have been proposed to explain the current geographic distribution and the patterns of diversification in Plethodontidae (e.g., Wilder and Dunn, 1920; Wake, 1966, 1987). However, these evolutionary and biogeographic scenarios were challenged by the discovery of the Asian species *Karsenia koreana* (Min et al., 2005; Vieites et al., 2007). This discovery has led to an acceptance of a postulated early connection across the Bering Strait between species in western North America and their relatives in Western Europe.

In addition to its relevance for recovering the biogeographic history of the family, *K. koreana* may also play an important role in understanding the patterns of morphological evolution and ecological diversification within the group. Plethodontid salamanders are the most speciose and diversified group of salamanders, but the diversity and disparity of forms are unevenly distributed within the family (Wake, 1966). Levels of specialization and speciation are not directly correlated, and highly specialized and generalist forms coexist within the different plethodontid lineages. The general morphology of the group is highly conserved, and the specialized forms have diversified

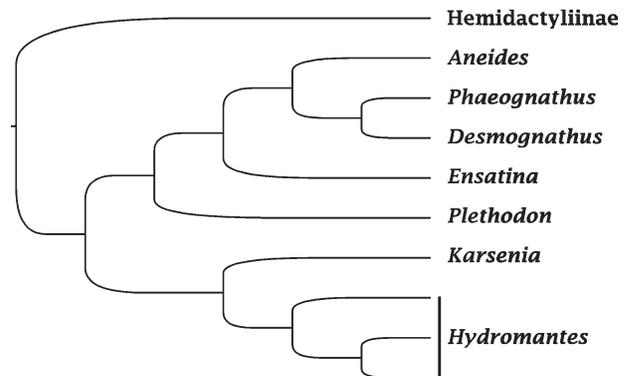


Fig. 1. Phylogenetic relationships within Plethodontinae. The phylogenetic tree has been simplified from Vieites et al. (2007) and is based on nuclear DNA markers. *Karsenia*, the only Asiatic plethodontid, is sister to the species of *Hydromantes*, which occur in North America and Europe. The remaining plethodontine and hemidactyliine species are distributed in the New World from southern Canada to Bolivia.

along a few similar morphological axes (Wake and Larson, 1987; Wake, 1991). Recurrent evolution of similar morphological traits across lineages, i.e. homoplasy, is a common theme among the Plethodontidae (Wake, 1991).

Given the singularity and importance of the new lineage, we describe the osteology of the skull of *K. koreana*. We compare the results with the characteristics of other members of the Plethodontinae (*Hydromantes*, *Aneides*, *Ensatina*, *Plethodon*, *Desmognathus*, and *Phaeognathus*). Superficially, *Karsenia* resembles members of *Plethodon*, as well as female *Aneides hardii*, and such members of *Desmognathus* as *D. ochrophaeus* and relatives, but we focus especially on *Hydromantes* because of its putative close phylogenetic relationship and its hypothetically shared biogeographic history. So far, little is known about the natural history, biology, morphological variability, and anatomical characteristics of *K. koreana*, so a detailed and comparative anatomical description may shed some light on the biology and evolution of the species. We also discuss the results in terms of the morphological variability, biology, and evolution of plethodontid salamanders in the framework of the molecule-based phylogenetic hypotheses recently proposed.

MATERIALS AND METHODS

Descriptions are based on high-resolution X-ray CT (HRXCT) scans of *Karsenia koreana* (DRV 5033, 50.0 snout-vent length (SVL) mm, female, Sanan-Ri, South Korea) and *Hydromantes platycephalus* (SMR 271, 67.2 mm SVL, male, Inyo Creek, Inyo County, CA) performed at the HRXCT Facility at The University of Texas (Austin). The specimen of *K. koreana* was scanned under the following conditions. A FeinFocus microfocal X-ray source operating at 180 kV, 0.13 mA, and with no filter was used. The source-to-object distance was 39 mm. For each slice, 1,400 views were taken, with four samples per view. The field of image reconstruction was 9 mm, with an offset of 6,500 and a reconstruction scale of 4,600. The final dataset consisted of

675 slices gathered at a resolution of 1024×1024 pixels, with an inplane resolution of $9 \mu\text{m}$ per pixel. For the *H. platycephalus* specimen, the scanning conditions were as follows. The FeinFocus microfocal X-ray source operated at 200 kV, 0.15 mA, and with no filter. The source-to-object distance was 55 mm. For each slice, 1,800 views were taken, with three samples per view. The field of image reconstruction was 16 mm, with an offset of 4,100 and a reconstruction scale of 4,500. The final dataset consisted of 559 slices gathered at a resolution of 1024×1024 pixels, with an inplane resolution of $15.625 \mu\text{m}$ per pixel. The descriptions of the specimens scanned were supplemented by the study of six cleared and bone-and-cartilage-stained *K. koreana* (DRV 5019, 5029, 5551, 5553, 5555, 5558, Jangtae San and Sanan-Ri, South Korea) and two *H. platycephalus* (MVZ 197502, 197506, Tuolumne County, CA). We compared the scanned specimens closely with the cleared individuals and, unless explicitly noted, the cleared specimens have the same morphology as reported for the scanned specimen. Data on ontogenetic or population variability of the structures described cannot yet be provided. All the specimens studied are or will be deposited in the Herpetological Collection of the Museum of Vertebrate Zoology (MVZ), U. C. Berkeley, and in the Herpetological Collection of Southern Illinois University (SIUC).

We present detailed descriptions and comparisons between *Karsenia* and *Hydromantes*, and we contrast the skulls of these genera with those of the remaining plethodontines. The taxonomy of the *Hydromantes* group is unstable, and *Hydromantes* has been considered by different authors as a supergenus containing the genera *Hydromantes*, *Speleomantes*, and *Atylodes* (Vieites et al., 2007) and as a genus containing three subgenera (e.g., Wake et al., 2005; van der Meijden et al., 2009), but an equally acceptable taxonomy is to recognize two genera, *Hydromantes* and *Speleomantes*, the latter containing two subgenera (*Speleomantes* and *Atylodes*) (Crochet, 2007). The results presented here are neutral with respect to taxonomy, and we find it most convenient to refer to *Hydromantes* (*sensu lato*) as a clade containing American species (*Hydromantes* in a restricted sense) and European taxa (*Speleomantes* and *Atylodes*). For comparison with other plethodontines (*Aneides*, *Desmognathus*, *Ensatina*, *Phaeognathus*, and *Plethodon*), we use the published comparative osteological descriptions by Wake (1963, 1966), in addition to studies of individual genera: *Aneides* (Wake et al., 1983b), *Hydromantes* (Wiedersheim, 1875; Adams, 1942; Hilton, 1945; Lanza et al., 1995), *Desmognathus* (Means, 1974). We refer to *Phaeognathus* and *Desmognathus* collectively as desmognathines. We made new observations of plethodontine taxa using the cleared and stained specimens from the collections of the Museum of Vertebrate Zoology, and we studied on-line CT scans of *Aneides lugubris* and *Phaeognathus hubrichti* (<http://digimorph.org/>). All comparative statements refer to the focal taxon in relation to *Karsenia*. For the description of the anatomy, we follow the anatomical nomenclature suggested on the Amphibian Anatomical Ontology web site (Leopold et al., 2007; Maglia et al., 2007), based largely on Francis (1934).

Segmentation of the individual elements of the skull was performed by tracing the sutures in the original scans, and then reconstructing the images in three dimensions using VGStudio MAX[®] 1.2 (Volume Graphics, Heidelberg, Germany). Right-side elements were segmented for paired structures. Measurements of structures and angles were taken with the software ImageJ 1.4.1 (Rasband, 2008). Contrast and brightness of the pictures were modified in Adobe Photoshop[®] 7.0 (San José, CA).

All reconstructions and labeled images will be available on the DigiMorph website (<http://digimorph.org/>). High resolution X-ray CT scans of *Aneides lugubris* and *Phaeognathus hubrichti* are also available on the website.

RESULTS

The skull of *Karsenia koreana* is oval in shape, robust, and well ossified. The skull is fully articulated and scantily ornamented (see Fig. 2). Longitu-

dinally, the skull measures 8.1 mm from the anteriormost tips of the premaxillae to the posterior ends of the occipital condyles. The maximum height is 3 mm (dorsomedial articulation of frontal and parietal to the transverse plane defined by the ventral surface of the dentaries), and the widest transverse expanse of the skull is 6 mm at the level of the anterior ends of the coronoid processes of the prearticulars.

The skull is divided into three areas for descriptive purposes. First, the olfactory capsules and investing bones, forming the facial area, palate and upper jaws, represent one fourth of the total length of the skull. The face is broadly rounded and it rises abruptly in the lateral plane, being slightly rounded anteriorly; the robust alary processes of the premaxillae, which rise almost perpendicularly, are responsible for the slight curvature. The curvature is more pronounced in the transverse plane, and is defined by the lateral surfaces of the nasals and the *pars facialis* of the maxillae, which are oriented ventrolaterally at a 60° angle (Fig. 2C,E). The second region, comprising large orbits and the anterior braincase with its investing bones, occupies one half of the skull and is defined by the frontals, the parietals, the parasphenoid, and the orbitosphenoids. The frontals and parietals cover the dorsal braincase and are more or less parallel with the ventral parasphenoid, and the lateral surfaces of the braincase are formed by the orbitosphenoids (which articulate dorsally with the frontals and parietals and ventrally with the parasphenoid; Fig. 2C). The otic capsules and the occipital complex represent the third area and the remaining one fourth of the skull. The otic capsules are rounded and not very prominent. They do not reach the level of the dorsal frontal-parietal surface in height. The squamosals project anteroventrally from the otic capsules toward the articulators, forming a 45° angle with the midline of the skull in the dorsal plane and a 33° angle with the midsagittal plane. Finally, the mandible is relatively stout and planar, incorporating dentaries and prearticulars.

In comparison with *Karsenia*, the skull of *Hydromantes platycephalus* is less well ossified and more loosely articulated. Proportionally, the skull is wider, flatter, and less robust (see Fig. 2); it is almost spherical in frontal section (Fig. 2B) and compressed dorsoventrally. Longitudinally, the skull measures 12 mm from the anteriormost tips of the premaxillae to the posterior ends of the occipital condyles. The maximum height is 4.5 mm (dorsomedial articulation of frontal and parietal to the transverse plane defined by the ventral surface of the dentaries), and the widest transverse expanse of the skull is 11 mm at the level of the posterior ends of the maxillae. The nasal capsules are poorly ossified and less robust. The alary processes of the premaxillae project vertically and

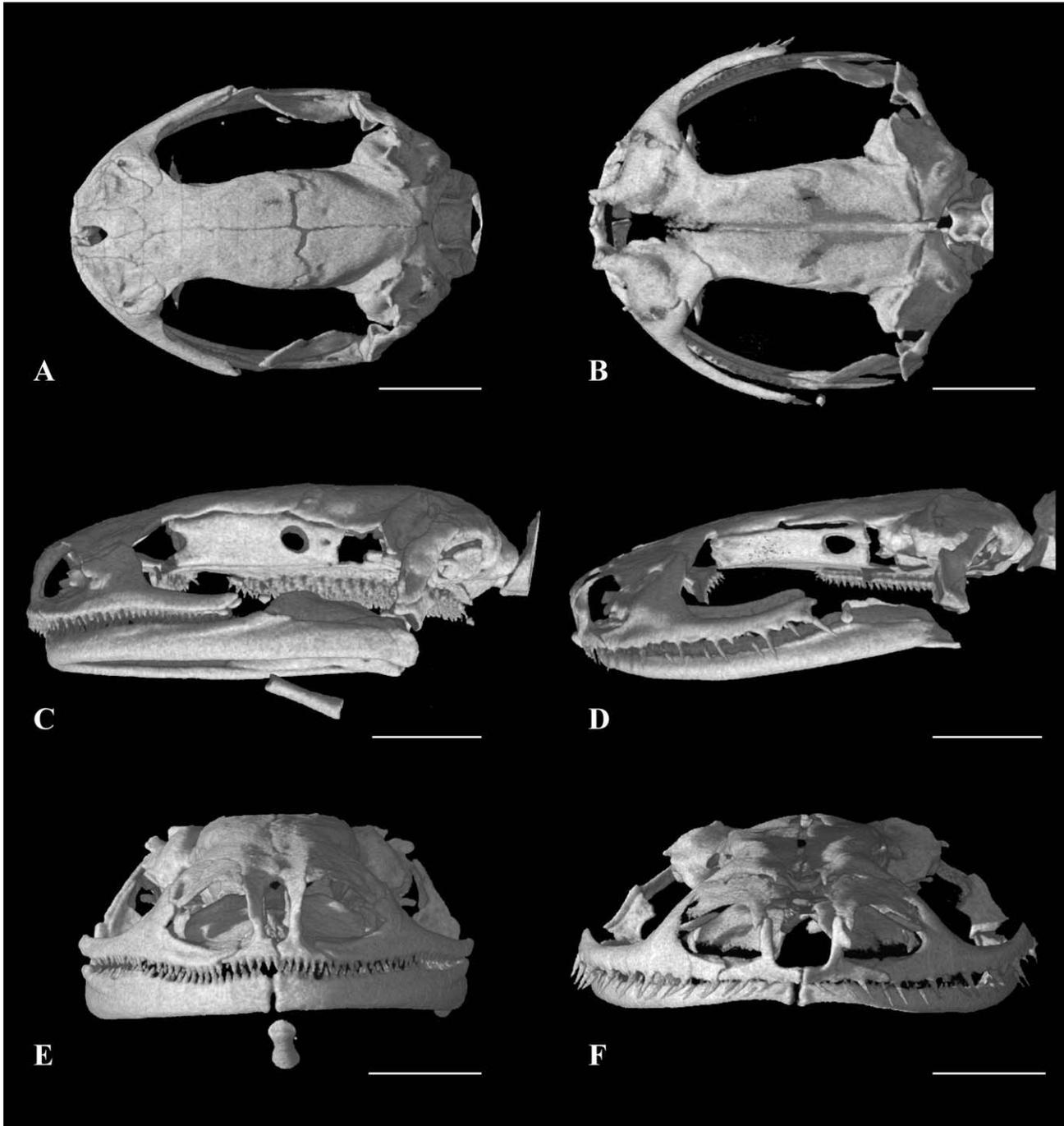


Fig. 2. General overview of the skull of *Karsenia koreana* (left panels: **A**, **C**, **E**) and *Hydromantes platycephalus* (right panels: **B**, **D**, **F**) in dorsal (**A**, **B**), lateral (**C**, **D**), and frontal (**E**, **F**) views, respectively (scale bars: **A**, **C**, **E** = 2 mm; **B**, **D**, **F** = 3 mm).

slightly anteriorly in the lateral plane, so the curvature is minimal. The anterior braincase region is more compressed, especially anteriorly, and the investing bones are less planar. The orbitosphenoids increase in height anteroposteriorly. The orbital vacuity is proportionally larger and wider. The otic-occipital area is the most robust part of

the skull. The otic capsules are proportionally higher, more prominent and better ossified. Given the wider mandibular arch, the squamosals project toward the articulators with a different inclination, forming a 61° angle with the midline of the skull in the dorsal plane and a 49° angle in the midsagittal plane. The squamosals are spread more lat-

erally in the dorsal plane and less vertically in the transverse plane. The mandibular arch is oriented ventrodorsally in the lateral plane (10° angle).

Premaxillae

Premaxillae are paired structures that are well articulated with each other at the anteriormost end of the skull (Fig. 3A,C). Premaxillae have three distinct regions. 1) The *pars dentalis* corresponds to the toothed area that forms the anterior end of the maxillary arch. In the specimen scanned, the premaxillary dentition consists of seven teeth on each bone (see "Dentition" section). The *pars dentalis* forms a rectangular trapezoid in the transverse plane. The longest edge of the trapezoid corresponds to the toothed area. Medially, the two premaxillae contact squarely, whereas a slender and toothless projection of the maxilla overlaps the flattened lateral ends. 2) The alary process (*pars facialis*) projects posterodorsally, first arising vertically as a robust, flattened column, then curving posteriorly and broadening into a flattened wing-like structure that overlaps the facial part of the frontal bone. The alary processes of the two premaxillae define the lateral borders of an oval fontanelle. The flattened portion of the alary process articulates medially with its counterpart, laterally with the nasal, and posteriorly with the frontal. The contact between the posterior ends of the premaxillae and the frontals forms an apparent concavity, in which the "nasal gland" is located. 3) The *pars palatina* consists of a small, flat surface that projects perpendicularly from the longest edge of the *pars dentalis* to the internal cavity of the cranium. The *pars palatina* articulates laterally with the lingual edge of the maxilla. The posterior edge of the *pars palatina* articulates with the anterior margin of the body of the vomer (Fig. 3A,C).

In *Hydromantes platycephalus*, the premaxillae are also paired structures that are not tightly articulated medially with each other (Fig. 3B,D). The *pars dentalis* is more rectangular and proportionally longer than in *K. koreana*, articulating squarely with its counterpart. The *pars palatina* is reduced, forming a small irregular shelf, more expanded at the posterolateral end. It does not contact the vomer. The lateral ends of the *pars dentalis* and the *pars palatina* merge and form a small tongue-and-groove articulation with the anterior end of the maxilla. The alary process is short and thin, perpendicular to the *pars dentalis*, and only slightly bent posteriorly. Along its posterolateral border, it has a reduced articulation with the medial border of the anterior end of the nasal. The alary processes of the premaxillae fall short of the frontal bones and do not contact each other posteriorly; they slightly overlap or lie immediately medial to anteromedial portions of the

nasals, and a large fontanelle occurs between them. In European *Hydromantes*, the alary process is considerably longer and is expanded and flattened posteriorly, where it typically slightly overlaps the anteromedial process of the frontal (except in *H. italicus*, where overlap is found in only 40% of specimens; Lanza et al., 1995).

Premaxillae of *Plethodon* and *Ensatina* generally resemble those of *Karsenia*; they are fused medially in *Aneides*, *Phaeognathus*, and *Desmognathus*. In some *Aneides* and desmognathines, the alary processes become massive and fuse to form a large flat plate that can become heavily co-ossified with the skin in both *Phaeognathus* and larger *Aneides* (Wake, 1966; Means, 1974). The European species of *Hydromantes* differ from the American species in having much larger alary processes that are often expanded into wing-like structures that reach the frontal bones in all species except *H. italicus*, in which 60% of specimens have alary processes that fall short of the frontals (Adams, 1942; Wake, 1966; Lanza et al., 1995). *Hydromantes* differs from all other genera in that the alary processes never contact each other behind the fontanelle.

Maxillae

These paired bones, together with the anteromedial premaxillae, form the upper jaw skeleton (Fig. 3A,E,G). Three regions are recognized. 1) The elongated *pars dentalis* extends approximately half the length of the *optic fenestra*. The entire extent is toothed. The anteriormost part overlaps the posterior area of the *pars dentalis* of the premaxilla. The posterior part connects to the palatopterygoid cartilage by means of the jugal ligament. 2) The *pars facialis*, an ascendant process from the *pars dentalis*, separates the nasal cavity and the *optic fenestra*. The *pars facialis* is trapezoidal in shape; its posterodorsal edge overlaps the prefrontal. It has a small extension directed anteriorly and pointing toward the dorsoventral edge of the nasal, but without contacting it. 3) The *pars palatina* is a flattened plate that extends from the *pars dentalis* into the oral cavity. It articulates with the *pars palatina* of the premaxilla and with the vomer, together forming the roof of the palate area.

Maxillae in *H. platycephalus* are less robust than in *K. koreana* (Fig. 3B,F,H). The *pars dentalis* is similar in length, extending to the middle of the *optic fenestra*, and is fully toothed. The *pars facialis* is narrowly triangular and concave, the posterior tip of the triangle overlapping the lateralmost edge of the nasals and the partly disconnected anterior part of the frontal (see later for description of the bipartite frontal in *Hydromantes*). The anteriorly directed process of the *pars facialis* is absent. The *pars palatina* is much reduced; it does not contact the vomer at any point. Accordingly,

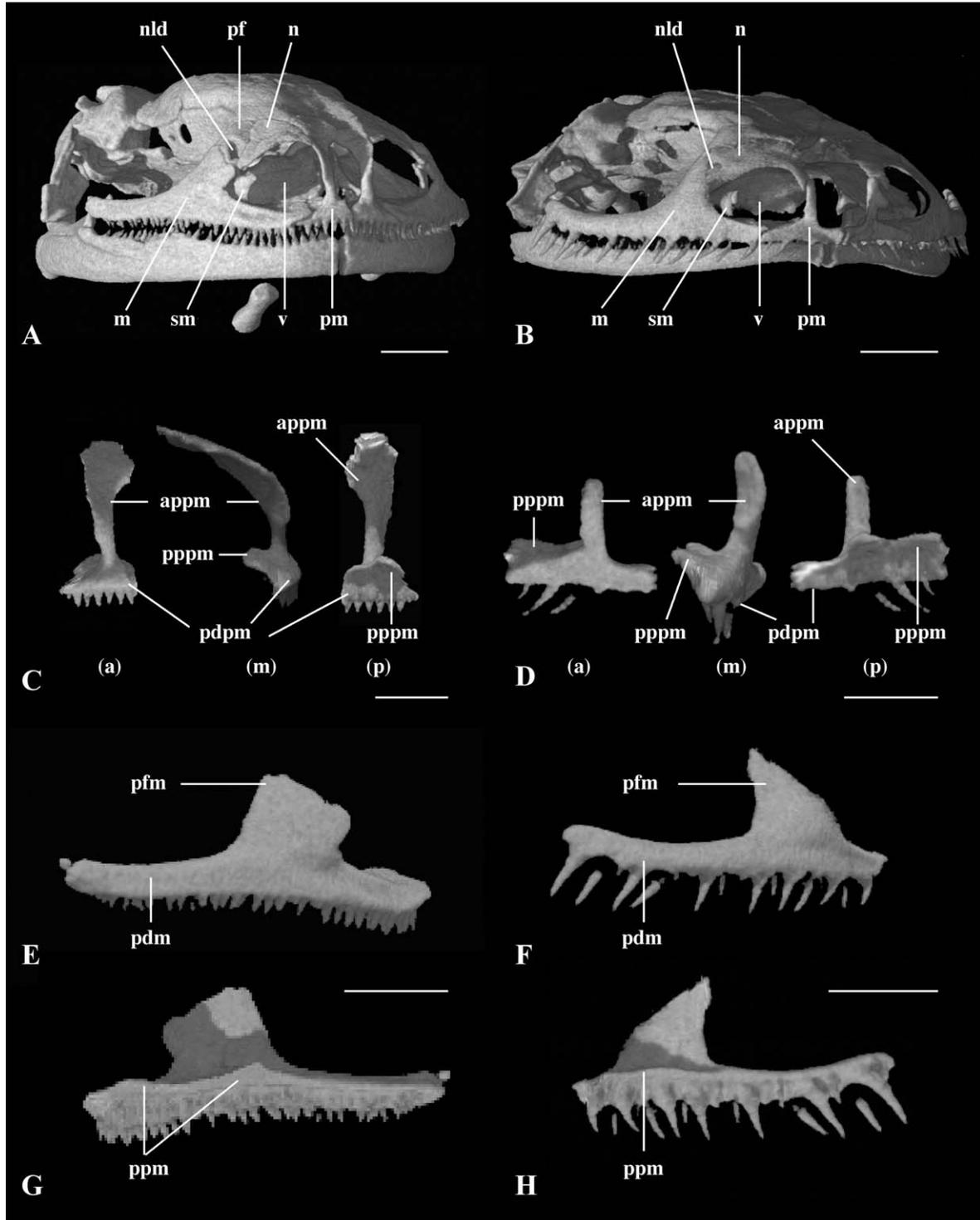


Fig. 3. Premaxillae, maxillae, and septomaxillae in *K. koreana* (left panels: A, C, E, G) and *H. platycephalus* (right panels: B, D, F, H). A, B: Frontolateral view of the skulls. Note the articulation in *K. koreana* between the septomaxilla and the prefrontal, and between the premaxilla, maxilla and vomer (A), articulations absent in *H. platycephalus* (B). C: Anterior (a), medial (m), and posterior (p) views of the premaxilla in *K. koreana*. D: Anterior (a), medial (m), and posterior (p) views of the premaxilla in *H. platycephalus*. E, G: Lateral and medial views of the maxilla in *K. koreana*, respectively. F, H: Lateral and medial views of the maxilla in *H. platycephalus*, respectively. appm, alary process of the premaxilla; m, maxilla; n, nasal; nld, nasolacrimal duct; pdpm, *pars dentalis* of the premaxilla; pdm, *pars dentalis* of the maxilla; pf, prefrontal; pfm, *pars facialis* of the maxilla; pppm, *pars palatina* of the premaxilla; ppm, *pars palatina* of the maxilla; pm, premaxilla; sm, septomaxilla; v, vomer (scale bars: A, C, E, G = 1 mm; B, D, F, H = 2 mm).

the anterior palate is incomplete and the body of the vomer is weakly connected, if at all, to lateral parts of the palate. In European *Hydromantes*, the *pars facialis* is rectangular rather than triangular and much shorter, barely contacting the nasal and well separated by a distinct gap from the frontal (Adams, 1942; Wake, 1966; Lanza et al., 1995).

Maxillaries of other plethodontines generally resemble those of *Karsenia*, especially those of *Plethodon* and *Ensatina*, although the *pars dentalis* of *Ensatina* is longer and more slender. The *pars facialis* of *Ensatina* is small and articulates only weakly, if at all, with the prefrontal. Maxillae of *Aneides* are much stouter and are more complexly articulated with the prefrontals and nasals; the *pars dentalis* is massive in the larger species and in males of smaller ones and bears enlarged teeth anteriorly, whereas posteriorly it is expanded dorsoventrally and is edentulous (Wake, 1963, 1966; Wake et al., 1983b). The maxillae of desmognathines are stout and well articulated via the *pars facialis* directly to the frontal, which it broadly overlaps. In *Phaeognathus* there is a very large, stout process that extends posteromedially, almost as far as the quadrate bone, into the ligament that connects the bone to the suspensorium (Wake, 1966).

Septomaxillae

These paired bones, embedded in the cartilages of the nasal capsules at the lateral edge of the nasal vacuity, are small and irregular in shape. The anteriormost part is somewhat triangular and projects anteriorly in the dorsolateral part of the nasal cavity (Fig. 3A). The small anterior extension of the *pars facialis* of the maxilla partially covers the septomaxilla. The septomaxilla articulates dorsally with the prefrontal at the anterior edge of the nasolacrimal duct, which passes through its posterior margin.

In *H. platycephalus*, the septomaxillae also are paired structures, irregular in shape and smaller than in *Karsenia* (Fig. 3B). The bones are weakly ossified and do not articulate with or otherwise contact any other bone.

Septomaxillae are typically present in all plethodontines, but usually they are smaller than in *Karsenia*. They are especially small in desmognathines. The bones are weakly developed in European *Hydromantes* and can be absent entirely (as many as 60% of *H. italicus*), absent on one side, or reduced in degree of ossification (Lanza et al., 1995).

Nasals

These paired bones are triangular in shape. The base of the triangle forms the dorsal part of the nasal capsule, and the vertex points posterodor-

sally (Fig. 4A). The nasal is slightly convex and presents an irregular surface. A foramen is present in the medial part of the bone, presumably for passage of the profundus branch of nerve V. The nasal articulates mesially with the premaxilla and frontal; laterally, it articulates with the prefrontal, which separates it from the maxilla.

In *H. platycephalus*, nasals are more irregular, somewhat rectangular, and less well ossified (Fig. 4B). The nasal has a medial projection that extends anteriorly, contacting the alary process of the premaxilla. Laterally, the nasal articulates with the *pars facialis* of the maxilla. Anteriorly, the lateral margin of the nasal is notched, forming a partial foramen for the nasolacrimal duct. Nasals are larger and more elongated, usually longer than broad, in European *Hydromantes*.

Nasals are roughly similar in all plethodontines but they are very small and packed between larger bones (e.g., well articulated to the *pars facialis* of the maxilla and the alary process of the premaxilla) in desmognathines. Shape varies (often specific to a given species) from triangular to quadrangular or pentagonal, and the degree of articulation with the *pars facialis* of the maxillary varies from close to none among species.

Prefrontals

Prefrontals are paired bones, oval and slightly convex, that are irregular in shape. They partially to completely fill the gaps between the orbit and the nasal vacuity, and between the frontal and the *pars facialis* of the maxilla (Fig. 4A). They are smaller than the nasals. The nasolacrimal duct passes through an elongate foramen in the anterior part of the prefrontal that is usually not enclosed in bone anteriorly. The prefrontal articulates anteriorly with the septomaxilla and lateroventrally with the *pars facialis* of the maxilla. The prefrontal is overlapped laterodorsally by the nasal; posteriorly, it broadly overlaps the frontals.

Prefrontals have been reported consistently as absent in *Hydromantes* (Adams, 1942; Wake, 1966; Lanza et al., 1995). In the specimen scanned, however, the anteriormost part of the frontal seems differentiated as a distinct element (Fig. 4B). This putative element occupies space that represents the anterior part of the frontal and at least part of the prefrontal (in the sense that it is overlapped by the *pars facialis* of the maxilla). It is not well ossified; the contact between anterior and posterior parts of the frontal is not clearly delimited. An incipient break in the bone extends from the midline, well in advance of the orbits, posterolaterally at about a 45° angle to the extreme anterolateral border of the orbit. Zones of weak ossification correspond only roughly to the prefrontal because the anterior element extends to the midline, which differs from prefrontals in all other salamanders. The

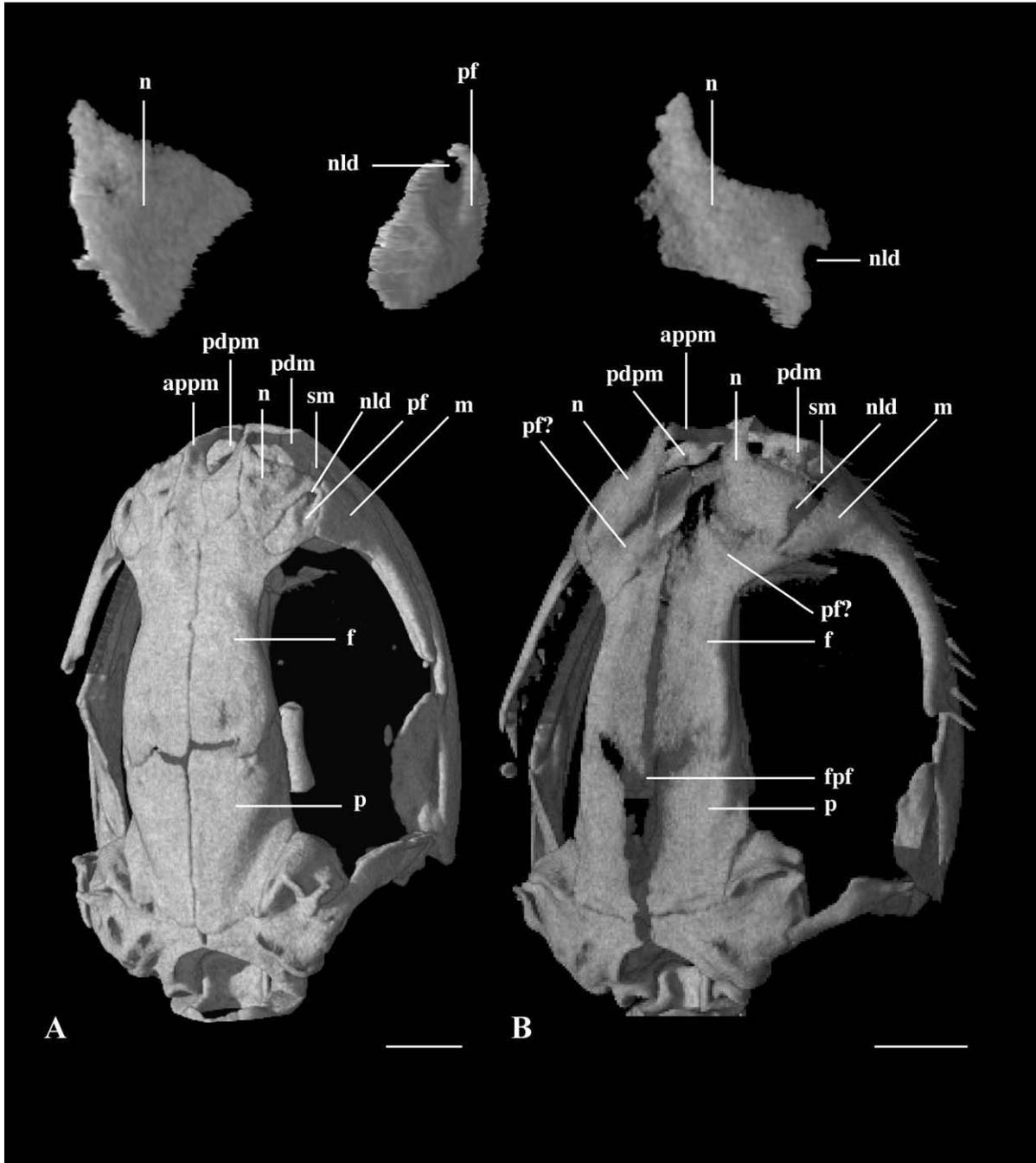


Fig. 4. Dorsolateral views of the skulls of *K. koreana* (A) and *H. platycephalus* (B), showing the articulations between, nasals, prefrontals, frontals, and parietals. An enlarged dorsal view of the nasal and prefrontal is provided. Prefrontals have been consistently reported absent in *Hydromantes*. In the specimen scanned, however, a differentiated element is found between frontals and nasal (pf in B). appm, alary process of the premaxilla; f, frontal; fpf, frontoparietal fontanelle; m, maxilla; n, nasal; nld, nasolacrimal duct; os, orbitosphenoid; p, parietal; pdm, *pars dentalis* of the maxilla; pdpm, *pars dentalis* of the premaxilla; pf, prefrontal; ps, parasphenoid; sm, septomaxilla (scale bars: A = 1 mm; B = 2 mm).

anterior element is posterior to the nasal and is irregular in shape (Fig. 4B). Long but very slender anterior projections are present medially and shorter ones laterally, but the latter does not separate the nasal from the *pars facialis* of the maxilla, as is typical of prefrontals in many taxa (Wake,

1966). The developmental origin of this putative element should be further explored to clarify the identity and homology of the structure.

Prefrontals are absent in desmognathines in which they are thought to be incorporated into expanded facial lobes of the frontal (Wake, 1966).

The bones are well developed in *Aneides*, and in the western species they are involved in a complex interlocking articulation with the *pars facialis* of the maxilla. The bones of *Ensatina* and *Plethodon* resemble those of *Karsenia*.

Frontals

These are long, paired bones, which, together with the parietals, form the roof of the cranial cavity (Fig. 4A). The frontal is rectangular in shape, although its anterior third is broader than the rest of the bone. It is slightly convex and has a smooth surface. Frontals articulate mesially with their counterparts but do not fuse. The premaxilla, nasal, and prefrontal overlap the enlarged anteriormost part of the frontal. The lateral edges of the frontal turn down laterally, articulating with the orbitosphenoid (Fig. 6A). Frontals articulate posteriorly with parietals, slightly overlapping them lateroventrally. The articulation is not square, but the contact between frontals and parietals does not leave a conspicuous frontoparietal fontanelle in adults.

Frontals in *H. platycephalus* are rectangular structures, enlarged anteriorly (Fig. 4B). They are poorly ossified, especially medially and posteriorly, and the anterior part is weakly connected to the interorbital part of the bone (see earlier, prefrontal). The frontals do not articulate either with their counterparts or posteromedially with the parietals, leaving a conspicuous frontoparietal fontanelle that has a shape reminiscent of a fleur-de-lis. Laterally, frontals articulate with the dorsal sides of the orbitosphenoids (Fig. 6C). This articulation extends through the anterior half of the orbitosphenoids. Posterolaterally, the frontals overlap the parietals. In American *Hydromantes*, a frontal lobe extends anterolaterally, overlapped extensively by the tip of the triangular *pars facialis* of the maxilla. The lobes are absent in European *Hydromantes*, but in some individuals the frontals extend as far as the reduced *pars facialis* of the maxillae.

The frontals are well-ossified, large bones in plethodontines. They are particularly large and stout with expanded anterior portions in desmognathines, which lack prefrontals. Desmognathines are unique in that the frontals have ventrolateral processes that invade the antorbital region, in the front of the orbit between the prefrontals and palatal parts of the maxillae. The frontals are relatively massive, especially in *Phaeognathus*, and they extend beyond the posterior margins of the orbitosphenoids. Usually small, diamond-shaped fontanelles separate the frontals from each other and the parietals except in desmognathines and larger *Aneides*. All of the anterior cranial elements of *Phaeognathus* and *Aneides lugubris* (and to a lesser extent *A. flavipunctatus*) are heavily rugose

with co-ossified skin. There is a discrete posterior boundary in the middle of the frontals that corresponds to the anterior extent of the mandibular adductor muscles.

Parietals

The parietals are large, rectangular, paired bones that cover the posterior braincase. The parietal is shorter than the frontal and its surface is also more irregular (Fig. 4A). The ventrolateral border is curved downward where it contacts the orbitosphenoid and covers the dorsal surface of the ascending process of the palatoquadrate cartilage at the posterior end of the orbitosphenoid (Fig. 6A). This lateral face of the parietal is more conspicuous than that of the frontal. The anterior half of the parietal contacts the orbitosphenoid from the *optic fenestra* to its posterior end. The broadly curved, posterior border has complex articulations. The ventrolateral border is thickened and becomes clawlike in transverse section, articulating within the claw first with the ascending process and more posteriorly with the inner margins of the otic capsule. The lateral edge becomes thinner posteriorly and conforms to the medial portions of the otic capsule. The anterior surface of the parietal forms a low dome, whereas the posterior surface, in front of the otic capsule, is depressed and forms a broad groove that accommodates the portion of the mandibular adductor musculature that extends from the atlas vertebra to the mandible. In the scanned specimen, there appears to be a significant gap between the two parietals, but in the five cleared-and-stained individuals there is no gap and the bones are sutured. In one instance, there is a clear overlap between the bilateral counterparts. The scanned specimen also displays a small gap between the frontals and the parietals, but there is no gap in the cleared specimens (the scanned and the cleared specimens are all similar in size). The frontals overlap the parietals, especially laterally.

In *H. platycephalus*, parietals are poorly ossified anteromedially and medially (Fig. 4A). The anterolateral edge of each parietal projects anteriorly and is overlapped by the frontal. Laterally, the parietal does not contact the orbitosphenoid except at its posterior end (Fig. 6C). Posteriorly, the parietal envelops the anterior half of the otic capsule. Compared with *Karsenia*, the bone is thinner, flatter, and less domed, and the depressed groove accommodating mandibular adductor musculature is less conspicuous.

The parietals of *Plethodon* and *Ensatina* are similar to *Karsenia*, whereas those of *Aneides* differ mainly in having a more conspicuous posterolateral depression. The paired bones in *Aneides* are tightly sutured and become fused in large specimens of the larger species. Parietals of des-

mognathines differ mainly in being more massive and in having even more conspicuous adductor depressions. This reaches an extreme in *Phaeognathus*, in which the anteromedial portions of the occipito-otic participate in the pronounced depression, with the articulation between parietal and occipito-otic lying at the midpoint of the depression. The parietal and the occipito-otic are drawn out into a large ventrolateral tab that extends the osseous depression for the atlantomandibular ligament, which appears to be unique to desmognathines. The two parietals are strongly sutured in desmognathines. In *Desmognathus*, but to a lesser extent in *Phaeognathus*, the two parts of the bone contrast strongly. The bones are relatively short and the anterior half is high, flat, and smooth, whereas the posterior half is strongly depressed. The bones are tightly articulated to the frontals and the occipito-otics.

Vomers

The vomers are paired bones that, together with the *pars palatina* of the maxillae and premaxillae, form the anterodorsal part of the roof of the oral cavity (Fig. 5A,C,E). Four areas are distinguished. 1) The anterior flattened body articulates squarely with the maxilla and the premaxilla and projects posterodorsally at a 45° angle. The anterior body does not articulate with its counterpart, leaving an internasal space between them. The mesial edge of the anterior body surrounding the internasal space is turned up, forming a septum-like process that increases in height posteriorly. 2) A short and broad posterior projection proceeds posteriorly from the vomerine body. Its ventrolateral edge surrounds the internal nares. The projection articulates posteromedially with its counterparts. The medial posterior edge of the projection overlaps the anterior edge of the parasphenoid. A topographically limited articulation occurs between the lateral posteroventral part of the vomer and the anteroventral end of the orbitosphenoid (Figs. 5A,E and 6B). 3) A conspicuous dentigerous ridge (dentigerous process, including a preorbital projection toward, but falls short of, the lateral margin of the vomerine body, posterior to the internal nares. 4) A posterior vomerine dental patch is constituted by two patches that meet at the midline, separated from the vomers proper. The patches are drop-shaped and constitute a dense extension of vomerine teeth (see "Dentition" section for a detailed description). The patches, attached (but not fused) to the ventral surface of the parasphenoid, are separated from the dentigerous process by a narrow gap.

The anterior part of the main body of the vomer in *H. platycephalus* is not as well ossified as that of *K. koreana* and does not contact the *pars pala-*

tina of the premaxilla and the maxilla (Fig. 5B,D,F). The vomerine body projects posterodorsally at a 45° angle. The curved, septum-like process on the medial border is absent, and the internasal space is proportionally larger than in *K. koreana*. The posterior projection from the main body is slightly curved ventrally. The vomer does not articulate with its counterpart in this male specimen. However, in females, there are articulations with the maxilla and premaxilla and also with its counterpart by means of portions of the posterior projections (Adams, 1942). The projection articulates posteriorly with the anteriormost end of the parasphenoid, but there is no contact between the vomer and the orbitosphenoid (Figs. 5B and 6D). The dentigerous process projects anteriorly toward the vomerine body; its length exceeds the width of the body. The posterior vomerine dental patches are more reduced than those of *K. koreana*. They are restricted to two narrow, widely separated strips of teeth, located posterolaterally on the ventral surface of the parasphenoid (Fig. 5F). The teeth occupy barely 20% of the total surface of the parasphenoid, and they are separated from the dentigerous process by a wide gap, three times as great as in *Karsenia*.

Vomers of *Plethodon*, *Ensatina*, and *Aneides* closely resemble those of *Karsenia* in most respects. In large *Aneides*, the septum-like dorso-medial margin of the body may articulate with the ventral surface of the alary process of the premaxilla. The bilateral counterparts articulate with each other for the posterior half of their extent. Preorbital processes of the dentigerous process typically fall short of the lateral extent of the vomer body, but in *Ensatina*, the process is especially long and exceeds the lateral extent of the body. Species of *Aneides* differ in structure of the preorbital process, which is absent entirely in western species. The gap between the dentigerous process and the posterior tooth patch is typically greater than in *Karsenia*, but less than in *Hydromantes*; however in *Ensatina*, the gap is less and may be completely closed. The posterior patches are not in contact and are especially widely separated in *Ensatina*. The vomers of desmognathines have features not found in other plethodontines and also display much variability related to size of organisms, sex, and habitat. The vomers are generally robust and are tightly articulated to each other. The internasal space is subject to much variation; in the most aquatic, largest species it is much reduced in size and the articulation of the bilateral counterparts is nearly complete. The preorbital process usually has few or no teeth and a bony plate extends posterolaterally from the dental arcade. The preorbital process usually falls far short of the lateral extent of the body. The process may articulate slightly with the peculiar antorbital ossification of desmognathines. The posterior tooth



Fig. 5. Vomers in *K. koreana* (left panels: A, C, E) and *H. platycephalus* (right panels: B, D, F). A, B: Frontal sections, showing the dorsal surfaces of the vomers and their articulation with premaxillae, maxillae, parasphenoid, and orbitosphenoids. C, D: Dorsal (d) and ventral (v) view of the isolated right vomers. E, F: Ventral view of the skulls, showing the ventral disposition of the vomers, and the two posterior vomerine dental patches, attached to the parasphenoid. appm, alary process of the premaxilla; c, choana; dr, dentigerous ridge; m, maxilla; n, nasal; nld, nasolacrimal duct; pdm, *pars dentalis* of the maxilla; pdpm, *pars dentalis* of the premaxilla; pf, prefrontal; v, vomer; vb, anterior vomerine body; vdp, vomerine dental patch (scale bars: A = 1 mm; B = 2 mm; C = 0.5 mm; D = 1 mm; E = 1 mm; F = 2 mm).

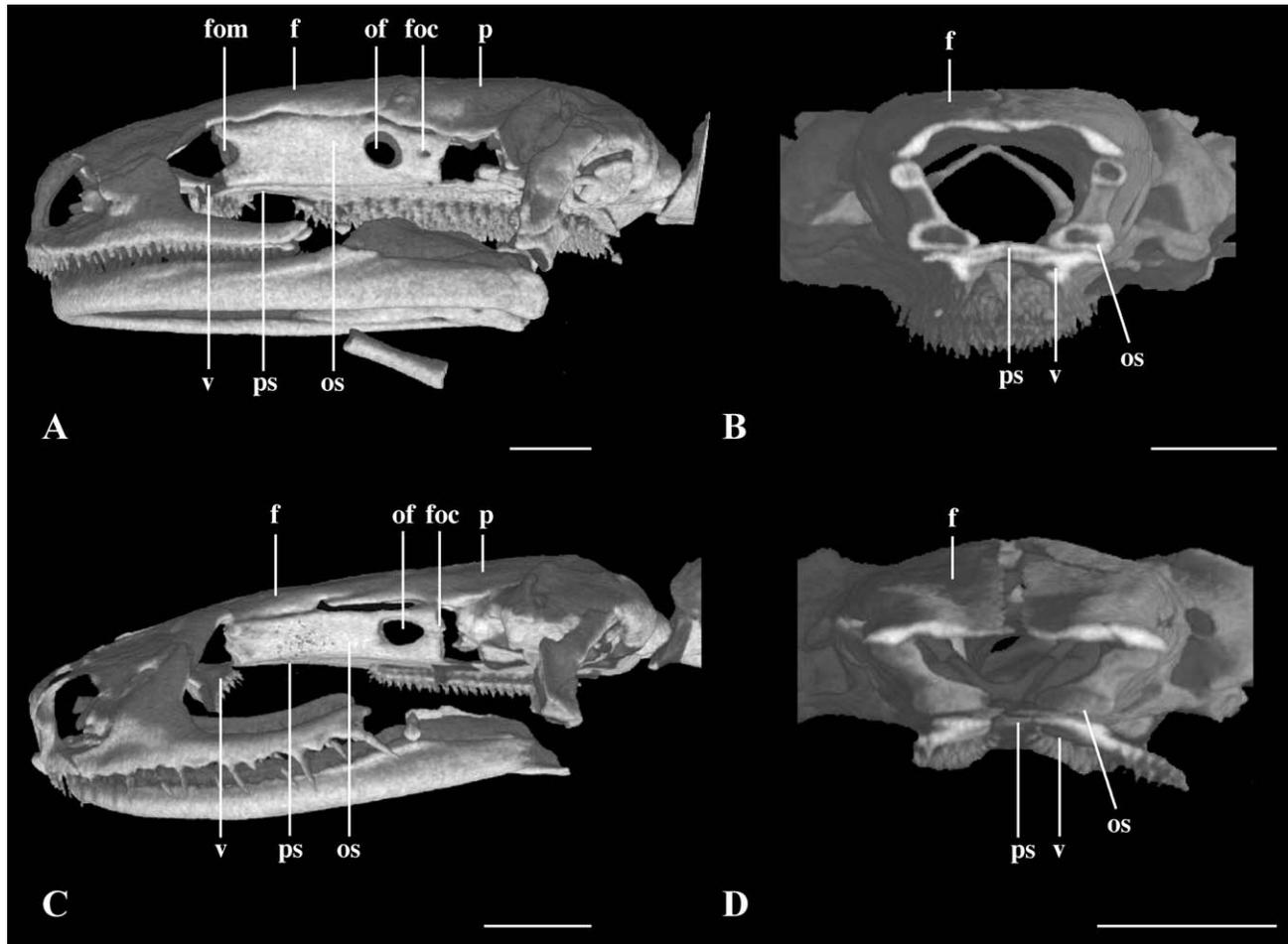


Fig. 6. Brainscase in *K. koreana* (A, B) and *H. platycephalus* (C, D). A, C: Lateral view of the skulls, showing the orbitosphenoids and their contact and articulation with vomers, parasphenoid, frontals, and parietals. B, D: Transverse section of the skulls, about the level of the anterior end of the orbitosphenoids. The brainscases in the two species are very different in shape. Note also the absence of contact between vomers and orbitosphenoids in *H. platycephalus*. f, frontal; foc, *foramen oculomotorium*; fom, *foramen orbitonasale mediale*; of, *optic fenestra*; os, orbitosphenoid; p, parietal; ps, parasphenoid; v, vomer (scale bars: A, B = 1 mm; C, D = 2 mm).

patches are relatively small and are widely separated from the denticerous portion of the vomer and from each other.

Orbitosphenoid

These paired rectangular bones are disposed vertically in an anterior–posterior direction (Fig. 6A,B). They form the lateral sides of the brainscase. They are widely separated dorsally and ventrally, and they do not contact each other at any point. Although mainly vertical, they are closer together ventrally than dorsally so that the brainscase is trapezoidal in shape anteriorly (Fig. 6B). The inner surface is slightly concave, and the structure is thicker at the anterior and posterior borders. The bone increases in height from the anterior edge to its medial part, decreasing toward its posterior margin. Three foramina for cranial nerves

are present (Fig. 6A). The anterior border consists of three projections (one dorsally and two ventrally) that partially encircle the opening of the *foramen orbitonasale mediale*. A conspicuous *optic fenestra* lies in the posterior third of the orbitosphenoid. Very large and completely surrounded by bone, it opens at about the level of the articulation of the frontals and parietals. The third foramen, a small *foramen oculomotorium*, opens posterior to the *optic fenestra* and close to the posterior margin of the orbitosphenoid. The anteriormost lateral and ventral margins of the orbitosphenoid contact the posterior edges of the vomers. With the exception of this anterior contact, the ventral surface of the orbitosphenoid contacts the dorsal surface of the parasphenoid (Fig. 6B). Dorsally, the orbitosphenoid articulates with the frontal and parietal.

The orbitosphenoids in *H. platycephalus* are more curved and inclined laterally, the cranial cav-

ity being more oval and flattened (Fig. 6C). The *foramen orbitonasale mediale* is not evident in the anterior part of the orbitosphenoid, and only a slight indication of the anterior part of the *foramen oculomotorium* is evident at the posterior end of the bone. A conspicuous *optic fenestra* occupies the posterior area of the orbitosphenoids. The orbitosphenoid articulates ventrally with the anterior half of the parasphenoid; it does not contact the vomer anteriorly (Fig. 6D). The anterior half of the dorsal surface of the orbitosphenoid articulates laterally with the frontal; the posterior half approximates the parietal, although it contacts only at the posterolateral end.

Orbitosphenoids are basically similar in all plethodontines, differing mainly in size and degree of robustness. In *Aneides lugubris*, the bilateral counterparts are in contact posteroventrally.

Parasphenoid

The parasphenoid is an unpaired, median, symmetrical bone, which forms the ventral surface of the braincase. Elongated and semirectangular, the bone is square and narrow at the anterior margin and wider and more rounded at its posterior end (Fig. 5E). Its anteriormost margin is thin and flat, but becomes dorsally concave toward the posterior margin. Several foramina perforate the lateral margin of the parasphenoid in the third quarter of its length. One of these may be the carotid canal, which is not well defined in salamanders; alternatively, all three might carry different branches of the internal carotid. Ventrally, the parasphenoid sustains the vomerine tooth patch, which occupies ~85% of its surface. The parasphenoid articulates laterally with the orbitosphenoids. The posterior edges curve dorsally, enveloping the anterior borders of the occipito-otic. The posterior end of the bone is expanded dorsoventrally and serves as the attachment for subvertebral muscles.

The parasphenoid in *H. platycephalus* is nearly triangular, with a narrow anterior end (Fig. 5F). It is flat anteriorly, but quickly becomes internally concave, especially at the level of the area occupied by the vomerine tooth patches. The posterodorsal ends articulate with much of the occipito-otic complex.

Plethodontines do not differ much with respect to the parasphenoid, but in desmognathines, the bone is shortened, thickened, and articulates squarely with the ends of the vomer, although a small, flattened process extends forward dorsally. The posterior part of the parasphenoid is greatly expanded and extends far laterally, finally contacting a dorsomedial process of the quadrate.

Occipito-Otic Complex

The posterior portion of the skull is a massive amalgamation of elements associated with the otic

capsules and the occipital complex (Fig. 7A,C). The bulbous otic capsules enclose the vestibular-auditory system; they have dorsal and ventral medial connections to each other and the exoccipital region. The otic region is irregular in shape and bears the impressions of the semicircular canals. These impressions form prominent crests, which bear additional projections. The capsule is connected to the braincase by two processes that extend to the base of the ascending process of the suspensorium, by another to the ventral surface of the lateral part of the parietal, and by processes extending to the parasphenoid; it also connects to the basal part of the suspensorium. In addition, there is a ridge on the outer surface of the capsule that articulates with the anterior edge of the squamosal, which also articulates with the capsule behind this ridge. A conspicuous crest, located mesially on the surface of the capsule above the anterior semicircular canal, is v-shaped, the vertex of the "v" pointing anteriorly. Posterolaterally is another prominent crest, directed outward toward the area above the middle of the squamosal. This crest is drawn into a stout process. The posterior part of the capsule is less well ossified. Many foramina pierce the capsule medially toward the cranial cavity, ventrally and posteriorly (the *foramina acustica*, *foramen endolymphaticum*, *foramen perilymphaticum*, and *foramen post-oticum*). The operculum covering the *fenestra ovalis*, the *pars media plectri*, is a circular, well-ossified plate (Fig. 7C). Associated with its lateral side is the columellar stylus of the *pars media plectri*. The stylus is roughly L-shaped, with an enlarged area in the *pars media plectri* that connects to the operculum and a relatively large, cylindrical, rod-like portion that proceeds anterolaterally toward the articulation between the squamosal and the quadrate. It does not directly contact these elements, apparently (based on study of cleared specimens) connecting to a long, slender process of the cartilaginous suspensorium and indirectly by a ligament to the squamosal. The exoccipitals form the posteriormost part of the occipito-otic complex, encircling the *foramen magnum* and articulating via the occipital condyles with the first vertebra. Well ossified and completely fused to the otic capsules, the exoccipitals are in contact dorsally by means of the fibrous *tectum synoticum*, but they are in direct contact at the posteriormost point. Conspicuous, rounded occipital condyles are concave posteriorly and articulate with the atlantal cotyles. The internal walls of the *foramen magnum* articulate by means of distinct facets with the large tuberculum interglenoideum of the atlas. The exoccipitals do not contact ventrally, but instead are connected by a rectangular, cartilaginous hypochordal commissure. The ventral part of each exoccipital is overlapped slightly by the posterior part of the parasphenoid.

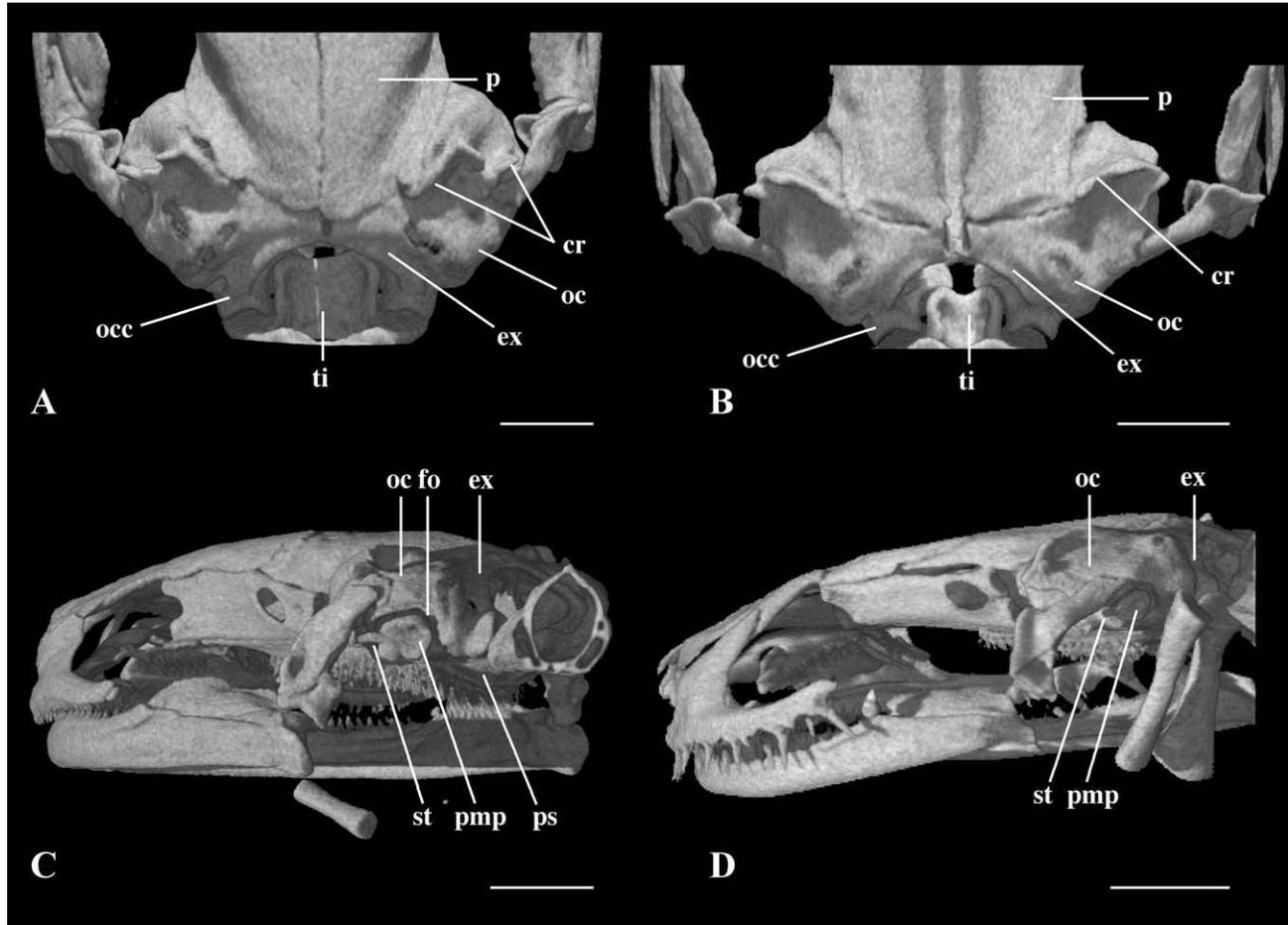


Fig. 7. Occipito-otic complex in *K. koreana* (A, C) and *H. platycephalus* (B, D). A, B: Dorsal view of the posterior part of the skull. Note the different articulations between exoccipitals and atlas vertebrae, which correspond to differences in the relative size of the tuberculum interglenoideum of the atlas in the two species. C, D: Posterolateral view of the skull. Note the different shape of the stylus of the *pars media plectri*. cr, crest; ex, exoccipital; fo, fenestra ovalis; oc, otic capsule; occ, occipital condyle; p, parietal; pmp, *pars media plectri*; st, stylus of the *pars media plectri*; ti, tuberculum interglenoideum (scale bars: A, C = 1 mm; B, D = 2 mm).

In *H. platycephalus*, the otic capsules are enlarged, flattened, and well ossified, but disposed similarly to those of *K. koreana* (Fig. 7B,D). The lateral walls of the capsules are swollen and heavily ossified anteromedially at the level of the connections to the orbitosphenoids. The otic capsules have conspicuous dorsal crests that end in small projections and align with the crests of the squamosals.

This complex differs little in all plethodontines except for the presence of crests of different sizes and shapes. These reach extremes in the case of high, wing-like crests in large species of *Aneides* and in males of all species of that genus (Wake, 1963). Crests of the desmognathines have unique configurations and shapes (Wake, 1966). The occipital condyles of desmognathines are massive and prominently stalked (Wake, 1966; Schwenk and Wake, 1993). The opercular-columellar system in plethodontines is relatively uniform. There is a rel-

atively large opercular footplate that appears to be fused (often so completely as to give the impression of a single element) to a more anterior unit comprised of a partial footplate and a distinct rod-like projection. These vary in shape and length among taxa (Monath, 1965; Wake, 1966).

Squamosal

The paired squamosals extend from the otic capsules to the quadrates, covering the suspensorium (Fig. 8A). The relatively robust, flattened bone arises just behind the prominent crests on the capsule and descends laterally and slightly anteriorly. Along its path, it undergoes a twist of about 45° or more. Two-thirds of the internal-proximal surface of the squamosal is also in contact with the enlarged ascending process of the quadrate.

The squamosal of *Hydromantes platycephalus* is dog-legged in shape and it is smaller and more

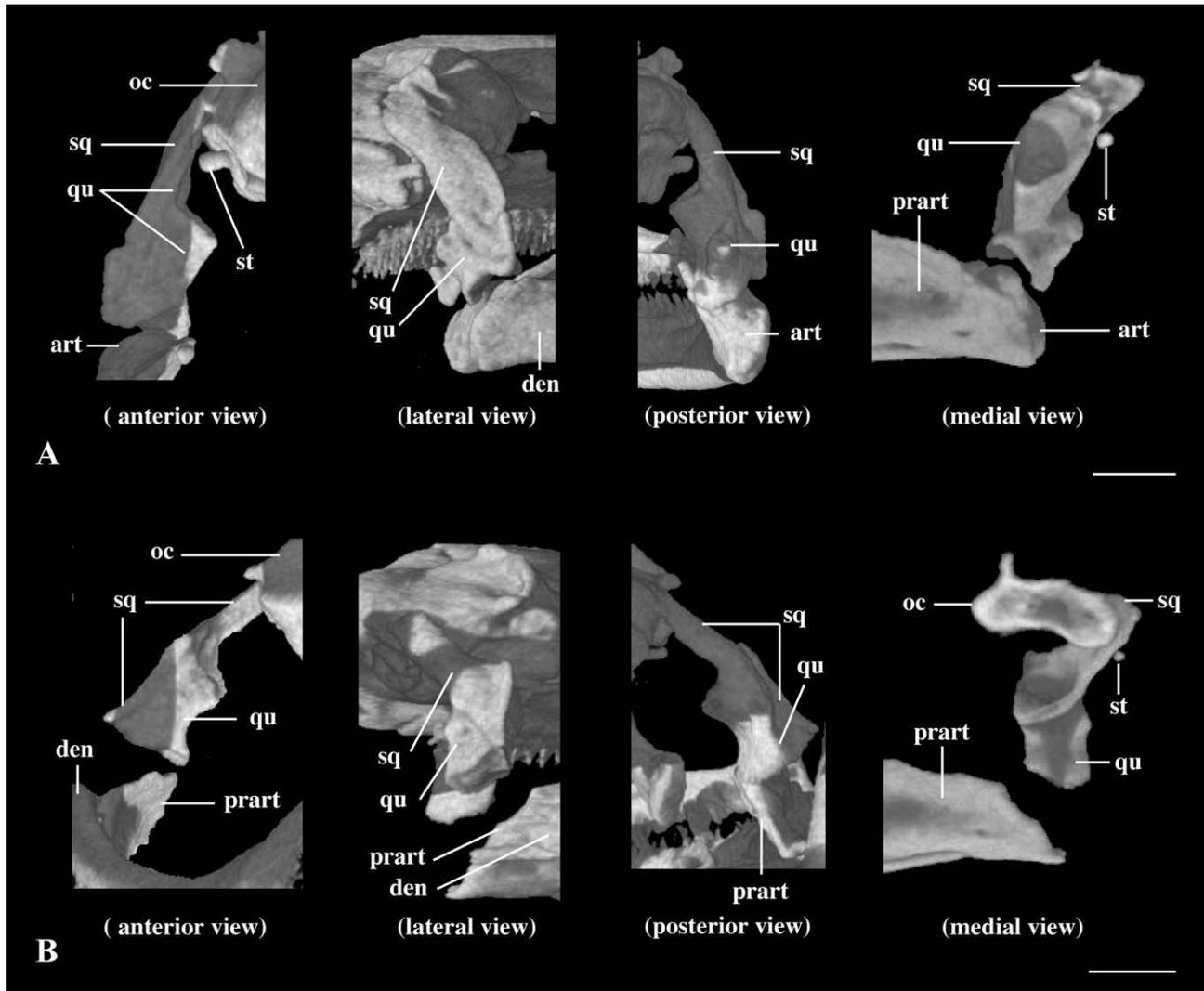


Fig. 8. Anterior, lateral, posterior, and medial views of the right squamosal and the quadrate, the only ossified element in the suspensorium, in *K. koreana* (A) and *H. platycephalus* (B). The medial view (m) corresponds to a sagittal section at the level of the articulation between the otic capsule and the squamosal. Note the different articulation between quadrate and articular in the two species. art, articular; den, dentary; prart, prearticular; oc, otic capsules; qu, quadrate; sq, squamosal; st, stylus of the *pars media plectri* (scale bars: A = 0.5 mm; B = 1 mm).

slender (Fig. 8B). It is attached to the otic capsule posterior to a prominent crest. It is directed anterolaterally and at about its midpoint it bends sharply laterally and ventrally to articulate with the quadrate.

The squamosals of plethodontines are generally similar, differing mainly in the degree of robustness, which is largely related to the overall strength of jaws and size of the mandibular adductor muscles and associated otic and squamosal crests. The bones reach a maximal size and complexity in large *Aneides* (Wake, 1963), in which they participate in extended otic crests. The bones are relatively robust in desmognathines, in which the relatively straight bones are tucked behind an

otic crest and wedged up against another more horizontally oriented crest of the otic capsule.

Suspensorium

The suspensorium of *Karsenia* is largely cartilaginous and we have been able to visualize it well with doubly cleared and stained specimens. The only ossified portion is the quadrate and its ascending process, whereas the basal, otic, ascending, and pterygoid portions are cartilaginous. The least well-visualized and smallest portion is the slender, elongate process that connects to the columellar stylus. The quadrate is conspicuous and well ossified, and is a blocky bone (Fig. 8A). It has

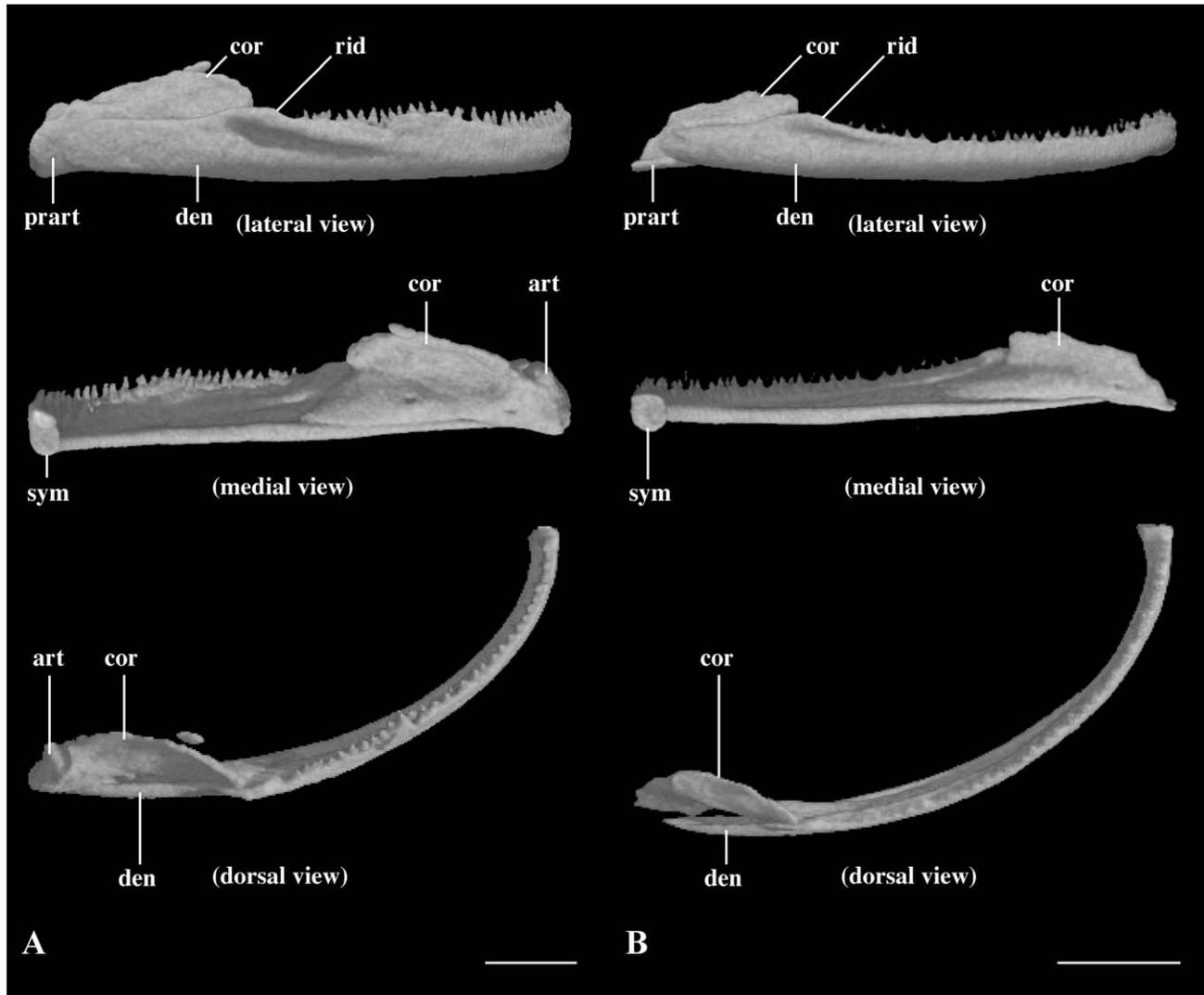


Fig. 9. Lateral, medial, and dorsal views of the right lower jaw in *K. koreana* (A) and *H. platycephalus* (B). The posterior end of the mandible is not so robust in *H. platycephalus*. The coronoid process of the prearticular is less developed and the articular does not ossify. art, articular; cor, coronoid process of the prearticular; den, dentary; prart, prearticular; rid, ridge; sym, mandibular symphysis (scale bars: A = 1 mm; B = 2 mm).

a relatively long and very thin ascending process, which is completely covered by the squamosal. The ventral end of the quadrate is capped by a thick cartilage that forms a concave surface, which articulates with the articular portion of the mandible. The posterior end of the ventral surface forms a small bony projection, the insertion point for the hyoquadrate ligament. Proximally (interior surface), the quadrate is also enlarged, forming an attachment surface for the jugal ligament.

In *H. platycephalus*, the quadrates are smaller and less robust, although they retain the same generally blocky form (Fig. 8B). The ascending process is shorter.

Quadrates of all plethodontids are generally similar, differing mainly in size and degree of development, which is correlated with the overall size of

the taxon studied. In desmognathines, the quadrates have extended dorsal and medial processes that articulate with the otic capsule and the cartilaginous parasphenoid.

Lower Jaw

The paired mandibulae form the lower jaw, which is relatively stout and well developed in *Karsenia* (Fig. 9A). Each ramus contains a well-ossified dentary surrounding a persistent Meckel's cartilage. Viewed laterally each mandible is planar on its ventral surface. The anterodorsal ends of the two dentaries contact at the mandibular symphysis, and they are a bit spread apart ventrally. In transverse section, the dentary is O-shaped anteriorly, enclosing Meckel's cartilage. It opens

and increases in height posteriorly, forming an elongated V-shaped region exposing the cartilage at the lingual margin. A well-formed ridge arises along the dorsolateral surface of the dentary at about its midpoint, before the end of the maxilla; this ridge and adjacent parts of the dentary serve as the insertion of more anterior mandibular adductors. The ridge is extended for a short distance posteriorly as a small projection. The dentary rises slightly as the ridge forms but there is no triangular process as in some other plethodontids. Teeth are borne on the dentary from the symphysis region to the origin of the ridge, at the level of the posterior tip of the maxilla, or for about three-quarters of the length of the dentary. The posterior end of the dentary is flattened, narrow, and blade-like, and is closely applied to the prearticular. Each of the paired well-developed prearticulars, located medially to the dentaries, has a long, tapering process that enters the dentary canal so that the bone is inserted into the elongate triangular opening described earlier. A moderately high shelf-like coronoid process arises just past the posterior end of the maxilla, immediately adjacent to the dentary ridge, and is inflected dorsomedially. The extensive surface of this shelf is the insertion of more posterior mandibular adductor muscles. The coronoid process extends posteriorly and then rapidly slopes ventrally and flattens, ending just before the posterior end of the prearticular. The posterior portion of Meckel's cartilage, the articular, is enlarged and mineralized or ossified, with a cartilaginous articular surface. The articular is co-ossified with the lateral and posterior parts of the prearticular and is also tightly connected to the dentary.

In *H. platycephalus*, the mandible is weaker and more slender (Fig. 9B). In lateral view, it is somewhat concave, with the symphyseal region being lower than the articular region. The dentaries are not in direct contact at the mandibular symphysis. The dentary is more slender, but a subdued dorsolateral ridge is present. The ridge is the high point of the dentary viewed laterally, and there is no triangular process. Teeth are present as in *Karsenia*. The prearticular is smaller and shorter in antero-posterior length, and the coronoid process is sigmoid-shaped in cross-section. The process is relatively low and it slopes rapidly toward the end of the prearticular. The posterior end of the prearticular is drawn into a short rod-like projection. The articular appears to be entirely unossified.

Mandibles of plethodontines vary greatly in size but not in composition. *Plethodon* and *Ensatina* and the smaller species of *Aneides* closely resemble *Karsenia*. *Aneides* has an edentulous triangular extension of the dentary, anterior to the coronoid process of the prearticular and associated with the muscular dorsolateral ridge. It becomes very large in *A. lubugris* and *A. flavipunctatus*. The extension

is variable but generally low in *Plethodon*, and only slightly evident in *Ensatina*. The coronoid process of the prearticular is generally similar to *Karsenia*, except in *Ensatina*, in which it is especially low and small, and *Aneides*, in which it is enormously enlarged in the larger species. Teeth are borne along about three-quarters of the dentary in *Ensatina*, between one-half and two-thirds the length in *Plethodon*, and the sometimes very large teeth of *Aneides* are restricted to less than one-half to less than one-quarter the length of the dentary (see Dentition section). Although the dentaries of the largest *Aneides* are massive, they are not as massive, in a relative sense, as those of desmognathines. In species such as *Phaeognathus hubrichti*, the dentaries are very high in lateral view and densely ossified, bearing teeth along less than two-thirds of the length of the bone. A very large dorsolateral ridge provides a wide shelf for insertion of adductor muscles. The desmognathine prearticular has a unique shape. The coronoid process is restricted to an anterior pointed but stout projection; this forms the posterior border of the unique atlantomandibular ligament, which attaches to the dentary just in front of it (Means, 1974). All plethodontids lack a significant retroarticular process, although there is some extension of the prearticular slightly posterior to the articulation. In desmognathines and larger species of *Aneides*, a small ventrally oriented process of the dentary is present near the symphysis, presumably to serve as the point of insertion of hyoid muscles.

Hyobranchial Apparatus

The hyobranchial apparatus in adults consists of paired ceratohyals, basibranchial, paired radials, paired ceratobranchials I and II, paired epibranchials I, and a urohyal (see Fig. 10). All parts of the apparatus are cartilaginous, except the central portion of the basibranchial. The latter is a solid rod of mineralized cartilage viewed laterally in the scanned specimen as floating below the skull, orientated posteroventrally relative to the snout and forming a 45° angle with the mandibular plane. The basibranchial is enlarged and has three distinct areas. 1) A central, rod-shaped and mainly mineralized body changes to cartilage both anteriorly and posteriorly. The mineralized portion, which constitutes about 45% of the total length of the basibranchial, increases in diameter toward the ends. 2) A well-developed, knob-shaped cartilage forms an anterior process. 3) A posterior cartilaginous enlarged area, pointed at the posterior-most end, connects to the two pairs of ceratobranchials. The paired radials articulate at the base of the anterior process of the basibranchial. They are rod-shaped proximally but more flattened and irregular in outline distally. The radials are ori-

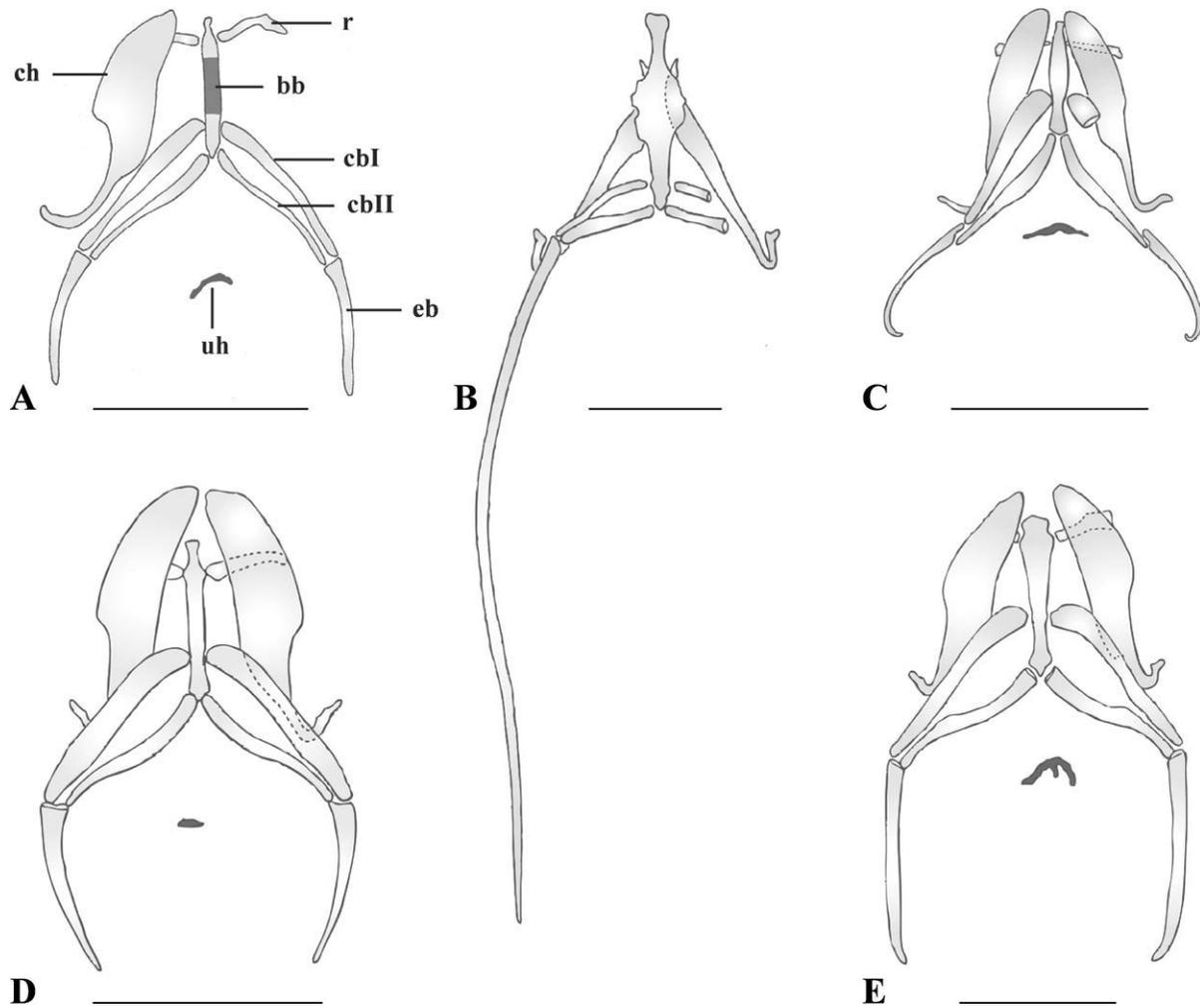


Fig. 10. Ventral views of the hyobranchial apparatus in *Karsenia koreana* (A), *Hydromantes platycephalus* (B), *Desmognathus monticola* (C), *Plethodon neomexicanus* (D), and *Ensatina eschscholtzii* (E). Hyobranchial parts are largely cartilaginous, with the exception of the central portion of the basibranchial in *K. koreana* and the urohyal in all species except *H. platycephalus*, which lacks it. Ossified segments are shown in dark gray. (B–E) have been modified from Wake and Deban (2000). bb, basibranchial; cbI, ceratobranchial I; cbII, ceratobranchial II; ch, ceratohyal; eb, epibranchial; r, radial; uh, urohyal (scale bars = 5 mm).

ented almost perpendicular to the basibranchial, pointing slightly anteriorly. The first ceratobranchial is the longest articulated structure of the hyobranchial apparatus. Ceratobranchial I is nearly straight, with a relatively flat and broad anterior end, the structure becoming more cylindrical and narrower posteriorly. The anterior end articulates with the basibranchial at the level of the posterior cartilaginous area and the posterior end articulates with epibranchials. Ceratobranchial II is shorter, more slender, slightly sigmoidal, and cylindrical. The anterior end of the ceratobranchial articulates with the basibranchial on two sides of the pointed posterior end. Posterolaterally, the ceratobranchial articulates with the epibranchial. Ceratobranchials I and II do not overlap or contact each other. The epibranchial is a cylindrical cone that decreases in size and curves ante-

roposteriorly; it is directed posterodorsally, in the direction of the shoulder. The posterior end nearly reaches the level of the pectoral girdle. The bowed, ossified urohyal lies medially, slightly posterior to the level of the epibranchial-ceratobranchial articulation. The ceratohyal is a large element that is cylindrical proximally, where it connects by ligaments to the quadrate. It becomes flattened and rather abruptly greatly expanded distally, with a broadly rounded anterolateral margin and nearly straight anteromedial margins parallel to the medial axis.

The ceratohyal is the longest element of the hyobranchial apparatus (1.7 times the length of the basibranchial), and the first ceratobranchial is the longest of the articulated elements (1.25 times the basibranchial). The second ceratobranchial is 1.2 times the basibranchial, and the epibranchial is

about 0.8 times the first ceratobranchial and essentially the same length as the basibranchial. The radials are 0.5 times the basibranchial and the urohyal is 0.3 times the basibranchial. The knob-like anterior extension of the basibranchial (from the midpoint of the attachment of the radials) is about 0.16 times the length of the element. The proportions are similar in all the specimens studied.

The hyobranchial apparatus of *Hydromantes* (all members of the group) differs greatly from that of all other salamanders (Wiedersheim, 1875; Lombard and Wake, 1977; Wake and Deban, 2000), and the contrast with *Karsenia* is especially great. It has the form of an extremely long projectile. The basibranchial is longer relative to body size than in any other plethodontid and has an anterior extension that is flattened at the end and flexible near the tip (Lombard and Wake, 1977). The basibranchial is expanded near its midpoint with thin lateral flanges. The epibranchials are extremely long, reaching lengths 2.7–3.2 times the basibranchial length. In contrast, the ceratobranchials are short and slender, with the second being slightly shorter and slightly stouter than the first. The first ceratobranchial is only 0.52–0.54 times basibranchial length, whereas the epibranchial is nearly six times the length of the first ceratobranchial. Radials and urohyal are absent. The ceratohyals are relatively short, less than 0.9 times basibranchial length, and have only a narrowly expanded blade. The epibranchials are about three times longer than the ceratohyal. No parts are mineralized.

The hyobranchial apparatus of *Aneides* and *Plethodon* closely resembles that of *Karsenia* in shape and proportions, and the basibranchial is mineralized in its midsection in several species. *Desmognathus* and *Phaeognathus* also have similar hyobranchial structure, although the tip of the basibranchial is not knobbed in the latter, instead tapering to a point. The radials are somewhat longer and substantially more slender in desmognathines than in the other plethodontines. The urohyal is variable; it can be as long as two-thirds the length of the basibranchial in *Desmognathus*, but is much shorter and more slender in *Phaeognathus*. *Ensatina* differs from other plethodontines in having longer epibranchials (about 1.8 times basibranchial length) and in lacking the slender, knobbed anterior extension of the basibranchial. Instead, that part of the basibranchial is short and trapezoidal-to-triangular in shape. It is partly disconnected from the main body and hence is flexible.

Dentition

Toothed areas include the premaxillae, maxillae, dentaries, vomers, and two posterior vomerine

patches associated with the parasphenoid. All teeth are pedicellate, small, and barely bicuspid in adults, and nearly homogeneous in size and shape in all the toothed areas. The lingual cusp is the largest. There is scant evidence of sexual dimorphism. Premaxillary teeth are slightly enlarged and reduced in numbers (mean of 7.9 ± 1.3 total for both bones, range of 6–10 in 12 females, 6.4 ± 1.1 ; range: 4–7 in 8 males) in comparison with such relatives as *Plethodon* and *Ensatina*. Maxillary teeth are slightly more numerous in females (mean 49.5 ± 4.5 ; range: 40–56) than in males (45.6 ± 3.3 ; range: 40–50). Vomerine teeth are small and extend only a short distance onto the otherwise toothless preorbital process. They number 16.8 ± 2.7 ; range: 12–20 in females and 15.5 ± 2.6 ; range: 13–21 in males. The posterior vomerine patch occupies most of the ventral surface of the parasphenoid, extending forward to the midpoint between the anterior rim of the *optic fenestra* and the anterior border of the orbitosphenoid; it is densely populated with small bicuspid teeth. Teeth are organized in rows that are oriented anteriorly and form a 45° angle with the medial sagittal plane of the parasphenoid. The patch narrows to a single tooth in width anteriorly and consists of as many as four roughly parallel rows at the widest site posteriorly. Teeth are difficult to count accurately because there are many unankylosed teeth. Numbers of ankylosed teeth range between ~60 and 70 per patch. Replacement teeth are found in the lingual areas of the premaxillae, maxillae, and dentaries, and along the posterolateral margins of the vomerine tooth row.

In *Hydromantes playcephalus* (and other species of the genus) teeth are distributed as in *Karsenia*. Dentition has been described by Greven and Clemen (1976) and Lanza et al. (1995). Teeth of females and nonreproductive males are similar to those of *Karsenia* in being obscurely bicuspid and small. The vomers bear teeth nearly to the lateral end of the preorbital process. Posterior vomerine patches are narrower and more widely separated than in *Karsenia*, but tooth numbers vary from 45–105 (mean 65.4) in *H. italicus* (Lanza et al., 1995). There are usually between 40 and 50 in American species of *Hydromantes*. Significant differences in dental morphology are found between European and American species. In the European species, males have fewer premaxillary teeth (1–2) than females (3–5), and the teeth are enlarged and monocuspid with sharp, conical, recurved tips that protrude from the mouth and function to scarify the skin of courted females, thus delivering secretions of the mental glands. In the American species, females have more premaxillary teeth that are of normal form, whereas males have fewer but mainly bicuspid teeth (but not so few as in the European species), although the more posterior teeth are transitional to the shape of the maxillary

teeth. Females have maxillary teeth similar to their premaxillary teeth and they number between about 20 and 30 in European species, but they are more numerous in American species (25–40). Males have typical small bicuspid teeth in European species, with about the same numbers as females, but in the American species males have greatly enlarged, monocuspid teeth that protrude outward from the bone and the skin forming the upper lip (Noble and Brady, 1930; Noble, 1931; Adams, 1942). These narrowly conical, sharp maxillary teeth apparently scarify the skin of females during courtship to deliver secretions of mental glands. The maxillary teeth of American males number from 8 to 12. Dentary teeth of all species are small and bicuspid, numbering between 30 and 50.

Dentition in *Desmognathus* and *Phaeognathus* was described by Means (1974), and in general resembles that of *Karsenia*. However, there is substantial interspecific variation in the shape and size of tooth crowns. Typical bicuspid teeth are present, but lingual cusps of some species are enlarged and recurved, and in a few species, the labial cusp is greatly reduced in size and is nearly imperceptible. Some species display sexual dimorphism in cusp morphology, with females having typical small bicuspid teeth and males developing monocuspid conical premaxillary teeth that project forward, and enlarged, forward-projecting teeth at the anterior end of the dentary that have hypertrophied lingual cusps (Noble and Pope, 1929). In two species, teeth of the jaws have short flattened cusps that have been described as fungiform (Means, 1974). The dentaries of some species become concave and may even lose most teeth at sexual maturity. *Ensatina* resembles *Karsenia* in dentition but has higher numbers of generally smaller but clearly bicuspid teeth. No sexual dimorphism has been noted. The vomerine tooth series is especially long, and the posterior vomerine tooth patches are large, narrowly separated, and bear high numbers of teeth. *Plethodon* resembles *Karsenia* in most respects, but has more diversity of crown shapes. In general larger species have more teeth, with numbers of maxillary teeth varying between 30 and 80 for different species (Coss, 1974). Most teeth are simple and bicuspid, but in males of some species (eastern small group) premaxillary and anterior maxillary teeth are elongated and have enlarged labial cusps. In most of the western species premaxillary teeth are monocuspid and spike-like, and in some species these teeth are also found on the maxilla, especially anteriorly; in *P. elongatus* and *P. stormi* these anterior maxillary teeth are less elongated but enlarged and flattened, even in females (Coss, 1974). The dentition of *Aneides* is similar to that of *Karsenia* in respect to vomerine teeth, which are small and bicuspid. However, western species of

Aneides have especially small vomerine teeth and the row is short because the preorbital process of the vomer is absent (Wake, 1963). Teeth of the jaws differ substantially from those of *Karsenia*. The species that most closely resembles *Karsenia* is *A. hardii*, the females of which have relatively small, bicuspid premaxillary, maxillary, and dentary teeth (Wake, 1963). Male *A. hardii* have premaxillary teeth that are monocuspid, elongated and recurved; anterior maxillary and dentary teeth are small and bicuspid, but posteriorly on the maxilla the teeth become longer and are laterally compressed with an obscure or absent labial cusp (Coss, 1974). Adult *A. aeneus* have monocuspid and “spike-like” teeth (Coss, 1974) and bicuspid teeth are observed only in juveniles. The western species, *A. ferreus*, *A. flavipunctatus*, *A. lugubris*, and *A. vagrans*, all have specialized dentition, with small bicuspid premaxillary teeth in females but monocuspid premaxillary teeth in males (“needle-like” in *A. flavipunctatus*; Coss, 1974), and enlarged, recurved and laterally compressed maxillary and dentary teeth, although anterior maxillary teeth may be small and bicuspid. The posterior part of the maxilla is toothless and greatly expanded dorsoventrally, and the monocuspid teeth immediately in front of this expanded portion are large and blade-like (Wake, 1963). In *A. lugubris*, maxillary and dentary teeth both increase in size and degree of compression and decrease in number with increasing body size, with as few as five per bone in large adults (Wake et al., 1983b). Numbers of maxillary and dentary teeth are reduced to as few as three per bone in *A. flavipunctatus* (Wake, 1963).

DISCUSSION

Modern salamanders constitute an old clade that originated by the Triassic; diversification of the extant families traces from the Jurassic to the mid-Cretaceous (Vieites et al., 2009). Salamanders have invaded heterogeneous geographic regions, inhabiting a vast array of terrestrial and aquatic habitats. Furthermore, they have experienced very different cycles of climatic and environmental conditions since their origin. However, the characteristic general morphology of salamanders has persisted for more than 160 million years (Gao and Shubin, 2003). This morphological persistence despite profound environmental changes has been related to the characteristic biology of these organisms (Wake et al., 1983a; Larson, 1984; Wake and Larson, 1987).

Variations, needless to say, occur. Morphological diversity among salamanders reflects adaptations to specific habitats, as well as the evolution of highly specialized life histories, such as direct development and pedomorphosis. These variations characterize the distinct families of salamanders,

and, occasionally, within-family clades (Wake, 1966; Larson, 1984). Nonetheless, the morphological “solutions” that have evolved are, in several cases, rather similar among clades, leading to a pattern of rampant homoplasy within the group (Wake, 1991).

Homoplasy, the independent evolution of similar features without a common ancestry, is ubiquitous in Caudata. Moreover, it is not restricted to any taxonomic-phylogenetic level, but it can be detected at diverse phylogenetic depths. The extensive homoplasy has made phylogenetic reconstruction problematic (e.g., Wake, 1966; Mueller et al., 2004; Wiens et al., 2005). The monophyly of the 10 families of salamanders has been confirmed with morphological, molecular, and paleontological data. The phylogenetic relationships among families, however, have been more difficult to establish, especially when using only morphological characters, because of the high levels of homoplasy. For example, pedomorphic forms, in which individuals are sexually mature but retain a larval morphology, have independently evolved in several families of salamanders. The retention of larval characters in “adult” individuals and the absence of diagnostic adult characters in these individuals have obscured the attempts to reconstruct the phylogenetic relationships between families by means of standard morphological characters (Good and Wake, 1992; Larson and Dimmick, 1993; Gao and Shubin, 2001; Wiens et al., 2005). Furthermore, the evolution of homoplastic features is also substantial *within* families, in particular, the Plethodontidae (Larson, 1984; Wake, 1991; Parra-Olea and Wake, 2001).

The family Plethodontidae comprises more than two thirds of all the species of salamanders, and is the most speciose clade among salamanders. The family originated around 125 mya (divergence between Amphiumidae and Plethodontidae; Vieites et al., 2009). The evolutionary history of plethodontids has been characterized by several successful radiations. In some cases, the radiations entailed the geographic expansion and diversification of the group, but with no substantial change in the morphology and ecology of the lineage (non-adaptive radiation). The genus *Plethodon* includes more than 50 species distributed in western and eastern North America, all occupying similar habitats and niches (Kozak et al., 2006). Morphology is generally conserved among species of *Plethodon*, with no significant differences except in coloration, relative sizes and proportions (Wake et al., 1983a; Kozak et al., 2006). This scenario contrasts with the adaptive radiation that permitted the expansion southward and the colonization of the New World tropics by the supergenus *Bolitoglossa*, which includes more than 250 species of plethodontid salamanders (Wake, 1987). The bolitoglossine radiation entailed the invasion of new niches

and habitats and the evolution of new behavioral and morphological specializations. Highly specialized and derived forms also occur in smaller clades such as the genus *Aneides*, which includes species with the strongest and most specialized jaws of the family, and *Phaeognathus*, which has a highly derived and heavily ossified skull related to its burrowing behavior. Diversity and disparity are thus not correlated within the family, and generalists and specialists are unevenly distributed across the phylogeny (Larson, 1984). The diversity of forms and specializations has evolved along only a few morphological axes, constrained by a persistent morphological design and leading to the homoplastic pattern observed within the family (Wake, 1991).

The discovery of *Karsenia koreana*, the only Asiatic plethodontid, not only has challenged our understanding of the biogeographic history of plethodontid salamanders, but also is of relevance in comprehending the evolution of morphological patterns and trends within the family. We discuss the anatomical description of *K. koreana* with two goals in mind. First, we approach the biology of the species through its morphological description, profiting from the detailed comparative data set available for most plethodontid species. The general, global links among anatomical parts, functional morphology, and ecological characteristics of the different species are well understood (Wake, 1966; Larson, 1984). Then, we analyze the morphological trends observed in *K. koreana* and its closest relatives to examine the potential factors that have driven the evolution of morphology in plethodontids.

Habitat in Relation to the General Morphology of the Skull

There is a broad correlation between the general morphology of the skull and the habitat of plethodontine salamanders. For example, species within *Plethodon* are generally ground-dwellers, and are normally found under rocks and logs in forest habitats. The morphology of their skulls represents the most generalized and likely ancestral form (Wake, 1966). The *Plethodon* radiation contrasts with other functionally specialized types such as occur in *Desmognathus*, which has flattened and streamlined skull morphologies adapted for living in mountain streams, and the robust and bullet-shaped skull in the burrower *Phaeognathus* (Wake, 1966; Larson, 1984; Schwenk and Wake, 1993).

The skull of *Karsenia* is compact and well articulated. Jaws are robust although teeth are not enlarged. In general, the skull resembles that of members of the genus *Plethodon* more than that of other plethodontine genera. It is apparently stronger than any *Plethodon* skull that we have

observed, resembling that of members of the *P. elongatus* group more than others in this respect. It lacks, however, the crests on the occipito-otic that receive a peg-like projection of the squamosal, which is unique to members of that latter group (Wake, 1963). Like *Plethodon*, *Karsenia* is thought to be fully terrestrial, living at or near the surface, but unlike most *Plethodon*, it is associated with rock crevices as its vernacular name, Korean crevice salamander, implies. The species of *Plethodon* that share this habit to some degree include *P. larselli* (*P. vandykei* group) and *P. asupak* (*P. elongatus* group; Mead et al., 2005). Although behavior of *Karsenia koreana* in relation to habitat use is known only from the observations of collectors, we suspect that the species uses its solid skull, in part, in relation to seeking refuge in tight spaces between rocks.

The skull of *Hydromantes* is similar to that of *Karsenia* in general composition, but in overall shape and degree of ossification it is the plethodontine genus that is most distinct. In contrast to the rounded, strong skull of *Karsenia*, that of *Hydromantes platycephalus*, as revealed in the CT scans and confirmed in the cleared and stained specimens, is dorsoventrally compressed and weakly articulated. The contrast is greatest in sexually mature males: The skull is especially flattened with maxillae that are oriented ventrolaterally and bear elongated, spine-like teeth that extend laterally well beyond the margins of the head (first noted in the original description; Camp, 1916). *Hydromantes* and especially *H. platycephalus* wedge themselves into very tight spaces, but generally in cracks in massive outcrops of granite. Rather than having evolved a heavily ossified skull, they appear to have followed an alternative adaptive route in which the skull gives way to pressure and conforms, to a degree, to its incompressible surroundings.

Feeding in Relation to Skull Form and the Anatomy of the Hyobranchial Apparatus

Feeding systems include the jaws, the dentition, the tongue, and the tongue skeleton, i.e., the hyobranchial apparatus. Feeding systems and behaviors are highly diverse in Plethodontidae, related to the different habitats and life styles of the species. The diversification of feeding mechanisms, in fact, has played a substantial role in the evolution of the family (Lombard and Wake, 1976, 1977, 1986; Roth and Wake, 1985).

The hyobranchial apparatus in salamanders is involved in respiration and feeding during the larval period, and in pulmonary respiration in terrestrial adults. The evolutionary loss of lungs in plethodontid salamanders, coupled with the evolution of direct development in most of the species, permitted the use of the hyobranchial apparatus

in highly specialized methods of feeding, such as tongue projection. The hyobranchial apparatus, released from its functional constraints, has repeatedly evolved highly derived feeding mechanisms within the family (Wake, 1991). Feeding mechanisms are grouped in three main categories: attached protrusible tongues (ancestral condition; e.g., *Plethodon*, *Aneides*), attached projectile tongues (e.g., *Ensatina*, *Batrachoseps*, *Hemidactylium*), and free projectile tongues (most derived condition, e.g., *Hydromantes*, *Eurycea*, *Bolitoglossa*; Lombard and Wake, 1986). The structure, extent of ossification, and proportions among elements of the hyobranchial apparatus are highly modified in each of the three categories. These changes are accompanied by concomitant changes in the associated jaw musculature and articulation (Hinderstein, 1971; Lombard and Wake, 1976; Schwenk and Wake, 1993).

Karsenia koreana represents the ancestral condition, with a protrusible attached tongue and a hyobranchial apparatus similar in shape and proportions to that of *Plethodon* and *Aneides*. We know nothing concerning the feeding behavior of *Karsenia*, but based on the close resemblance of the tongue and hyobranchial apparatus to that of *Plethodon* and on the absence of the highly modified jaws of desmognathines, we assume that it feeds by contacting the prey with its protrusible tongue and then rapidly delivering the prey to the buccal cavity, where it is held by means of the posterior vomerine tooth patches and the tongue (Maglia and Pyles, 1995; Wake and Deban, 2000). All plethodontines feed mainly on arthropods, usually of small size in relation to the size of the mouth. Teeth and jaws play a minor to negligible role in prey capture and processing in other plethodontines, except when prey is large. While *Hydromantes* also uses tongue-feeding, it is specialized to an extreme degree and has the longest and at the same time the most powerful tongue of any salamander yet studied (Deban et al., 1997, 2007). Its relatively weak jaws do not appear to play a role in processing of food.

Hydromantes has been well studied with respect to visual behavior and feeding (Roth, 1976, 1987). The eyes are large and protuberant and the head is mobile. Associated with head mobility is a small and gracile tuberculum interglenoideum on the atlas vertebra, which extends into the foramen magnum and has small articular facets, oriented ventrolaterally. We know little concerning head mobility in *Karsenia*, but its large and robust tuberculum interglenoideum suggests that it is associated with less craniovertebral mobility and increased resistance to bending than in *Hydromantes* and possibly other plethodontines as well. The extreme in reduction of the tuberculum interglenoideum is in desmognathines, in which the occipital condyles are enlarged and stalked, thus

moving the head away from the vertebral column and facilitating up-and-down movements of the head (Schwenk and Wake, 1993).

Whereas the skull of *Karsenia* is rather solidly built and cylindrical, with a high, well-supported braincase and relatively small orbits, that of *Hydromantes* is relatively light and flat with weak articulations of bones, and its jaws are light and lack strong musculature and bear teeth that are directed outside the mouth. The braincase of *Hydromantes* is weakly supported, the jaws are weak and the orbits are especially large. Together with the flattening of the entire skull, the impression is of a skull that is not involved in food processing, as is appropriate for an organism with an elongate, highly specialized tongue that delivers the prey toward the back of the buccal cavity where it is immobilized by the posteriorly directed vomerine tooth patches.

Secondary Sexual Traits of the Skull in Relation to Courtship and Mating Behavior

Courtship behavior is uniform among plethodontids (summaries in Arnold, 1977; Wells, 2007). During an elaborate tail-straddling walk, the male delivers one or a few spermatophores that are taken into the female's cloaca. The tail-straddling behavior is usually accompanied by the delivery of pheromones by the male. Two distinct modes of delivery have been described. In the first mode, which is assumed to be ancestral and more generalized among plethodontid salamanders, males deliver pheromones from their mental glands by means of scratching the dorsal skin of females with their seasonally hypertrophied premaxillary teeth. Species showing this "vaccination" behavior have a strong seasonal sexual dimorphism in dentition. A second derived mode of pheromone transfer has evolved in a clade of the large species of *Plethodon*. In this case, males do not develop the protruding premaxillary teeth and the delivery of pheromones is conducted by repeatedly touching the mental gland to a female's nares. Courtship and mating behavior in most of the plethodontid species studied to date involve the delivery of pheromones, although a few species have secondarily lost the mental glands and the delivery behavior (e.g., *Ensatina*) (Arnold, 1977; Wells, 2007).

There is scant evidence of sexual dimorphism in our material of *Karsenia*, whereas most species of plethodontines show at least seasonal sexual dimorphism in premaxillary teeth, males having fewer and larger teeth (Coss, 1974). In our seasonally limited samples of *Karsenia*, although the premaxillary teeth of males are only slightly enlarged and, while fewer in number in males than in females, the minimum number of four per bone is not especially low. However, larger series of males

and females must be studied to assess the degree of seasonality and sexual dimorphism in the premaxillary dentition of *Karsenia*. Nothing is known concerning courtship and mating in *Karsenia* but, given the absence of protruding premaxillary teeth and the presence of mental glands in males, it is reasonable to assume that they are similar to the derived mode observed in *Plethodon*. The situation is similar in the European species of *Hydromantes*, but in the American species, sexual dimorphism is expressed in a unique way. The maxillary teeth of males are at least seasonally enlarged and spine-like. Although their precise role in courtship has not been specifically studied, based on behavior in other plethodontids, it is likely that the teeth are used to vaccinate females with pheromonal secretions from the mental glands.

Morphology of the Skull with Respect to Social and Aggressive Behavior

Patterns of social and aggressive behaviors are extremely diverse among plethodontids, involving visual and chemical cues in species, sexual, and individual recognition. Agonistic behaviors are normally related to intraspecific territorial combat, but also to interspecific spacing strategies and parental care (Jaeger and Forester, 1993). Plethodontines are known to be aggressive, both toward sympatric congeners and to conspecifics (reviewed in Wells, 2007). In some cases, the morphological correlates of aggressive behavior have been studied in detail (e.g., Staub, 1993; Wiltenmuth, 1996). The relatively strong jaws of *Karsenia*, which might be related either to locomotor use of the head or to feeding, suggest that these forms may be aggressive. However, scarring has not been observed in the specimens collected to date, so no further conclusions can be extrapolated. Aggression has not been observed in *Hydromantes* and it is not expected, given the weakness of the jaws. One must be cautious in inferring function from these salamanders, however, because the relatively weak-jawed *Ensatina* can be very aggressive toward conspecifics (Wiltenmuth, 1996; Wiltenmuth and Nishikawa, 1998).

Phylogenetic Implications of Comparative Skull Morphology in Plethodontine Salamanders

The skulls of the genera of plethodontine salamanders are distinctive, within a framework of conservative composition (see later). Furthermore, even within genera such as *Aneides* (Wake, 1963), *Desmognathus* (Means, 1974), and *Hydromantes* (Wake, 1966; Lanza et al., 1995; this work) important differences characterize either individual species or small clades (such as the western species of *Aneides*, and the European as compared with the

American *Hydromantes*). Some multispecies genera, e.g., *Aneides*, *Hydromantes*, and *Desmognathus*, are characterized by numerous synapomorphies (Wake, 1966), but *Plethodon* is not (Wake, 1963). *Plethodon* is the second largest genus of salamanders (after *Bolitoglossa*), currently with 55 recognized species (AmphibiaWeb, <http://amphibia-web.org/index.html>, September, 2009). These species are remarkably uniform, with relatively minor differences among them; only the *P. elongatus* group has significant synapomorphies associated with the jaws, squamosal, and cranial crests (Wake, 1963). Furthermore, *Plethodon* displays apparently plesiomorphic states for most skull and hyobranchial characters, the only exceptions being the somewhat strengthened jaws and slightly reduced numbers of teeth, which distinguish it from *Ensatina* and from distant outgroups such as *Rhyacotriton*. *Karsenia* also is plesiomorphic with respect to nearly all traits of the skull and hyobranchial apparatus, the exceptions being the strong jaws and the ossified first basibranchial. It shares the latter and its plesiomorphic traits with at least some species of *Plethodon*; with respect to skull and hyobranchial morphology *Karsenia* would fit well within *Plethodon*. The major features separating the genera relate to tarsal anatomy (Min et al., 2005) and molecular characters (Min et al., 2005; Vieites et al., 2007).

We attempted a phylogenetic analysis of data from the skull and hyobranchial apparatus, with characters used by Wake (1963, 1966), Good and Wake (1992), Larson and Dimmick (1993), and Wiens et al. (2005), but to little effect (data not shown). Larson and Dimmick (1993) and Wiens et al. (2005) attempted to reconstruct the phylogenetic relationships among salamander families, but even at that phylogenetic level many of the morphological characters employed were not informative due to the great amount of homoplasy among and within lineages. The majority of the characters used for the phylogenetic analysis, as expected, were not informative at the phylogenetic depth of this study. There are very few synapomorphies in the dataset, the most numerous being those that support the *Desmognathus*, *Aneides*, and *Hydromantes* clades. The sole multigeneric clade supported with several synapomorphies is *Phaeognathus* + *Desmognathus*. This clade, long recognized taxonomically as the subfamily Desmognathinae (Wake, 1966), was recently and unexpectedly found to be deeply nested within the Plethodontinae in all relevant molecular analyses (e.g., Chippindale et al., 2004; Mueller et al., 2004; Vieites et al., 2007). No single morphological synapomorphy defines a *Karsenia-Hydromantes* clade.

Composition of the skull of plethodontine salamanders is remarkably conservative. The divergence of the two major clades of plethodontid salamanders, Plethodontinae and Hemidactyliinae, is

~94 mya (Vieites et al., 2007; Zhang and Wake, 2009). During that vast period of time, in which the range of the plethodontines expanded from North America to East Asia and Mediterranean Europe, very little compositional change in skull configuration has taken place. Septomaxillary bones are variably absent from some populations of European *Hydromantes*, prefrontals are absent from desmognathines and *Hydromantes*, and urohials and radii of basibranchials are absent from *Hydromantes*. Premaxillary bones are fused in *Aneides*, *Desmognathus*, and *Phaeognathus*. Otherwise all species share a common composition. There are differences in degree of ossification, general strength of the skull and in desmognathines a major change in the jaw-opening and feeding and locomotory mechanisms associated with the skull. The putative sister-taxon relationship of *Karsenia* and *Hydromantes* associates the apparently conserved, likely ancestral condition of the skull and hyobranchial apparatus in *Karsenia* with the extreme of specialization for tongue feeding and the weakest and most compositionally deficient plethodontine skull of *Hydromantes*.

The pattern of generalist-specialized forms within clades is repeated along the phylogeny of plethodontids, and the clade comprising *Karsenia* and *Hydromantes* provides a new example to analyze the processes driving morphological evolution and specialization in Plethodontidae. As discussed, homoplasy, frequent within and between salamander families, makes attempts at phylogenetic reconstruction problematic at all phylogenetic levels. Homoplastic patterns, which are nonrandomly distributed both among characters and among taxa, reflect real biological processes that underlie the recurrent evolution of morphological similarities among groups (Hufford, 1996; McShea, 1996). In salamanders, these processes include the modification of otherwise conserved developmental programs (e.g., ontogenetic repatterning, differential metamorphosis; Roth and Wake, 1985; Wake and Roth, 1989). In Plethodontidae, these developmental modifications, coupled with extreme life history strategies such as pedomorphosis and especially direct development, and morphological peculiarities such as the loss of lungs, created an unusual evolutionary arena that permitted the diversification of the group but within a framework of homoplasy and morphological constraint (Wake et al., 1983a; Wake, 1991; Wake and Hanken, 1996). Descriptions of new lineages, such as *K. koreana*, are important because they test our understanding of the limits of morphological diversity, and provide insight into the dimensions of evolutionary patterns within bounds, or constraints, on change. Our anatomical description of the skull of the only Asiatic plethodontid is conducted within this conceptual framework. Obtaining relevant biological information for the species, such as habitat use,

feeding habits, and life history traits, will enable us to extend analysis of plethodontid evolution in a multidimensional approach (Wake and Larson, 1987), enhancing our understanding of the relative importance of the processes that have driven the evolution and diversification of plethodontid salamanders. A more general outcome of our study will be progress toward an understanding of how lineage differentiation proceeds in the context of limits to morphological expression and apparent phylogenetic and developmental constraints.

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