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

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1 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 to 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.

Key words: evolution, morphology, bones, lungless salamanders, hyobranchial apparatus.

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INTRODUCTION

The order Caudata comprises 583 species of salamanders (AmphibiaWeb, September 2009. <http://amphibiaweb.org/index.html>). 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 an intervening larval stage. Species with the basal amphibian biphasic life cycle, as well as paedomorphic (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 *Urspelerpes*), and the genus *Hemidactylum* (monotypic genus), the last a distinct lineage whose taxonomic position has

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4 70 been rather controversial; (ii) Plethodontinae, which includes the supergenus
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6 71 *Desmognathus* (genera *Desmognathus* and *Phaeognathus*), the North American genera
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8 72 *Aneides*, *Ensatina*, and *Plethodon*, the supergenus *Hydromantes* (genera *Atylodes*,
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10 73 *Hydromantes*, and *Speleomantes*), and the recently described genus *Karsenia* (Vieites et al.,
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12 74 2007).

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15 75 *Karsenia* is a monotypic genus that contains the only known Asiatic plethodontid,
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17 76 *Karsenia koreana*, recorded from only a few localities in South Korea, where individuals
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19 77 can be found under rocks on the humid slopes of montane woodlands with limestone soils
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21 78 (hardwoods and mixed hardwood/pine; Min et al., 2005). The species has the characteristic
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23 79 features of plethodontid salamanders (the absence of lungs, pterygoid and lacrimal bones in
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25 80 adults, and the presence of a naso-labial groove and large posterior patches of vomerine
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27 81 teeth). The first molecular analyses carried out confirmed the singularity of the new lineage
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29 82 (Min et al., 2005). This, together with some morphological particularities such as an
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31 83 attached protrusible tongue and a derived tarsus, as well as its geographic distribution, led
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33 84 to the description of a new genus for the species (Min et al., 2005). A thorough molecular
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35 85 study confirmed the inclusion of *K. koreana* within the plethodontine salamanders (Vieites
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37 86 et al., 2007). In that phylogenetic analysis, *Karsenia* forms a clade with the species from
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39 87 the supergenus *Hydromantes*, the clade being sister to the remaining plethodontines (Fig.
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41 88 1). Furthermore, *K. koreana* shares synapomorphic karyological characteristics with
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43 89 *Hydromantes* (Sessions et al., 2008).

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46 90 Plethodontid salamanders are distributed primarily in the New World (99% of species).
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48 91 Most of the species are found in North and Middle America, with the exception of the eight
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50 92 that occur in the Mediterranean region. Several hypotheses have been proposed to explain
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the current geographic distribution and the patterns of diversification in Plethodontidae (e.g., Wilder and Dunn, 1920; Wake, 1966; Wake, 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 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

116 phylogenetic relationship and its hypothetically shared biogeographic history. So far, little
117 is known about the natural history, biology, morphological variability and anatomical
118 characteristics of *K. koreana*, so a detailed and comparative anatomical description may
119 shed some light on the biology and evolution of the species. We also discuss the results in
120 terms of the morphological variability, biology, and evolution of plethodontid salamanders
121 in the framework of the molecule-based phylogenetic hypotheses recently proposed.

123 MATERIAL AND METHODS

124 Descriptions are based on High Resolution X-ray CT (HRXCT) scans of *Karsenia*
125 *koreana* (DRV 5033, 50.0 snout-vent length [SVL] mm, female, Sanan-Ri, South Korea)
126 and *Hydromantes platycephalus* (SMR 271, 67.2 mm SVL, male, Inyo creek, Inyo County,
127 California, USA) performed at the High-Resolution X-ray CT Facility at The University of
128 Texas (Austin). The specimen of *K. koreana* was scanned under the following conditions.
129 A FeinFocus microfocal X-ray source operating at 180kV, 0.13mA, and with no filter was
130 used. The source-to-object distance was 39 mm. For each slice, 1400 views were taken,
131 with 4 samples per view. The field of image reconstruction was 9 mm, with an offset of
132 6500 and a reconstruction scale of 4600. The final dataset consisted of 675 slices gathered
133 at a resolution of 1024x1024 pixels, with an inplane resolution of 9 μ m per pixel. For the
134 *H. platycephalus* specimen, the scanning conditions were as follows. The FeinFocus
135 microfocal X-ray source operated at 200kV, 0.15mA, and with no filter. The source-to-
136 object distance was 55 mm. For each slice, 1800 views were taken, with 3 samples per
137 view. The field of image reconstruction was 16 mm, with an offset of 4100 and a
138 reconstruction scale of 4500. The final dataset consisted of 559 slices gathered at a

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139 resolution of 1024x1024 pixels, with an inplane resolution of 15.625 μm per pixel. The
140 descriptions of the specimens scanned were supplemented by the study of 6 cleared and
141 bone-and-cartilage-stained *K. koreana* (DRV 5019, 5029, 5551, 5553, 5555, 5558, Jangtae
142 San and Sanan-Ri, South Korea) and two *H. platycephalus* (MVZ 197502, 197506,
143 Tuolumne County, California, USA). We compared the scanned specimens closely with the
144 cleared individuals and, unless explicitly noted, the cleared specimens have the same
145 morphology as reported for the scanned specimen. Data on ontogenetic or population
146 variability of the structures described cannot yet be provided. All the specimens studied are
147 or will be deposited in the Herpetological Collection of the Museum of Vertebrate Zoology
148 (MVZ), U. C. Berkeley, and in the Herpetological Collection of Southern Illinois
149 University (SIUC).

150 We present detailed descriptions and comparisons between *Karsenia* and
151 *Hydromantes*, and we contrast the skulls of these genera with those of the remaining
152 plethodontines. The taxonomy of the *Hydromantes* group is unstable, and *Hydromantes* has
153 been considered by different authors as a supergenus containing the genera *Hydromantes*,
154 *Speleomantes* and *Atylodes* (Vieites et al., 2007) and as a genus containing three subgenera
155 (e.g., Wake et al., 2005; van der Meijden et al., 2009), but an equally acceptable taxonomy
156 is to recognize two genera, *Hydromantes* and *Speleomantes*, the latter containing two
157 subgenera (*Speleomantes* and *Atylodes*) (Crochet, 2007). The results presented here are
158 neutral with respect to taxonomy, and we find it most convenient to refer to *Hydromantes*
159 (*sensu lato*) as a clade containing American species (*Hydromantes* in a restricted sense) and
160 European taxa (*Speleomantes* and *Atylodes*). For comparison with other plethodontines
161 (*Aneides*, *Desmognathus*, *Ensatina*, *Phaeognathus*, and *Plethodon*), we use the published

162 comparative osteological descriptions by Wake (1963, 1966), in addition to studies of
163 individual genera: *Aneides* (Wake et al., 1983), *Hydromantes* (Wiedersheim, 1875; Adams,
164 1942; Hilton, 1945; Lanza et al., 1995), *Desmognathus* (Means, 1974). We refer to
165 *Phaeognathus* and *Desmognathus* collectively as desmognathines. We made new
166 observations of plethodontine taxa using the cleared and stained specimens from the
167 collections of the Museum of Vertebrate Zoology, and we studied on-line CT scans of
168 *Aneides lugubris* and *Phaeognathus hubrichti* (<http://digimorph.org/>). All comparative
169 statements refer to the focal taxon in relation to *Karsenia*. For the description of the
170 anatomy, we follow the anatomical nomenclature suggested on the Amphibian Anatomical
171 Ontology web site (www.amphibanat.org, June 2009; Leopold et al., 2007; Maglia et al.,
172 2007), based largely on Francis (1934).

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

179 All reconstructions and labeled images will be available on the DigiMorph website
180 (<http://digimorph.org/>). HRXCT scans of *Aneides lugubris* and *Phaeognathus hubrichti* are
181 also available on the website.

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183 RESULTS

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The skull of *Karsenia koreana* is oval in shape, robust, and well ossified. The skull is fully articulated and scantily ornamented (Fig. 2). Longitudinally, 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 surfaces of the dentary), 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

207 otic capsules toward the articulators, forming a 45° angle with the midline of the skull in the
208 dorsal plane and a 33° angle with the mid-sagittal plane. Finally, the mandible is relatively
209 stout and planar, incorporating dentaries and prearticulars.

210 In comparison to *Karsenia*, the skull of *Hydromantes platycephalus* is less well
211 ossified and more loosely articulated. Proportionally, the skull is wider, flatter and less
212 robust (Fig. 2); it is almost spherical in frontal section (Fig. 2B) and compressed
213 dorsoventrally. Longitudinally, the skull measures 12 mm from the anteriormost tips of the
214 premaxillae to the posterior ends of the occipital condyles. The maximum height is 4.5 mm
215 (dorsomedial articulation of frontal and parietal to the transverse plane defined by the
216 ventral surfaces of the dentary), and the widest transverse expanse of the skull is 11 mm at
217 the level of the posterior ends of the maxillae. The nasal capsules are poorly ossified and
218 less robust. The alary processes of the premaxillae project vertically and slightly anteriorly
219 in the lateral plane, so the curvature is minimal. The anterior braincase region is more
220 compressed, especially anteriorly, and the investing bones are less planar. The
221 orbitosphenoids increase in height anteroposteriorly. The orbital vacuity is proportionally
222 larger and wider. The otic-occipital area is the most robust part of the skull. The otic
223 capsules are proportionally higher, more prominent and better ossified. Given the wider
224 mandibular arch, the squamosals project toward the articulators with a different inclination,
225 forming a 61° angle with the midline of the skull in the dorsal plane and a 49° angle in the
226 mid-sagittal plane. The squamosals are spread more laterally in the dorsal plane and less
227 vertically in the transverse plane. The mandibular arch is oriented ventrodorsally in the
228 lateral plane (10° angle).

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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, while 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 form 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 also are 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

252 at the posterolateral end. It does not contact the vomer. The lateral ends of the *pars*
253 *dentalis* and the *pars palatina* merge and form a small tongue-and-groove articulation with
254 the anterior end of the maxilla. The alary process is short and thin, perpendicular to the *pars*
255 *dentalis*, and only slightly bent posteriorly. Along its posterolateral border it has a reduced
256 articulation with the medial border of the anterior end of the nasal. The alary processes of
257 the premaxillae fall short of the frontal bones and do not contact each other posteriorly;
258 they slightly overlap or lie immediately medial to anteromedial portions of the nasals, and a
259 large fontanelle occurs between them. In European *Hydromantes* the alary process is
260 considerably longer and is expanded and flattened posteriorly, where it typically slightly
261 overlaps the anteromedial process of the frontal (except in *H. italicus*, where overlap is
262 found in only 40% of specimens [Lanza et al., 1995]).

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

273 **Maxillae:** These paired bones, together with the anteromedial premaxillae, form the upper
274 jaw skeleton (Fig. 3A,E,G). Three regions are recognized. 1) The elongated *pars dentalis*

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275 extends approximately half the length of the *optic fenestra*. The entire extent is toothed.

276 The anteriormost part overlaps the posterior area of the *pars dentalis* of the premaxilla. The

277 posterior part connects to the palatopterygoid cartilage by means of the jugal ligament. 2)

278 The *pars facialis*, an ascendant process from the *pars dentalis*, separates the nasal cavity

279 and the *optic fenestra*. The *pars facialis* is trapezoidal in shape; its posterodorsal edge

280 overlaps the prefrontal. It has a small extension directed anteriorly and pointing towards the

281 dorsoventral edge of the nasal, but without contacting it. 3) The *pars palatina* is a flattened

282 plate that extends from the *pars dentalis* into the oral cavity. It articulates with the *pars*

283 *palatina* of the premaxilla and with the vomer, together forming the roof of the palate area.

284 Maxillae in *H. platycephalus* are less robust than in *K. koreana* (Fig. 3 B,F,H). The

285 *pars dentalis* is similar in length, extending to the middle of the *optic fenestra*, and is fully

286 toothed. The *pars facialis* is narrowly triangular and concave, the posterior tip of the

287 triangle overlapping the lateralmost edge of the nasals and the partly disconnected anterior

288 part of the frontal (see below for description of the bipartite frontal in *Hydromantes*). The

289 anteriorly directed process of the *pars facialis* is absent. The *pars palatina* is very reduced;

290 it does not contact the vomer at any point. Accordingly, the anterior palate is incomplete

291 and the body of the vomer is weakly connected, if at all, to lateral parts of the palate. In

292 European *Hydromantes*, the *pars facialis* is rectangular rather than triangular and much

293 shorter, barely contacting the nasal and well separated by a distinct gap from the frontal

294 (Adams, 1942; Wake, 1966; Lanza et al., 1995).

295 Maxillaries of other plethodontines generally resemble those of *Karsenia*, especially

296 those of *Plethodon* and *Ensatina*, although the *pars dentalis* of *Ensatina* is longer and more

297 slender. The *pars facialis* of *Ensatina* is small and articulates only weakly, if at all, with the

298 prefrontal. Maxillae of *Aneides* are much stouter and are more complexly articulated with
299 the prefrontals and nasals; the *pars dentalis* is massive in the larger species and in males of
300 smaller ones and bears enlarged teeth anteriorly, while posteriorly it is expanded
301 dorsoventrally and is edentulous (Wake, 1963, 1966; Wake et al., 1983). The maxillae of
302 desmognathines are stout and well articulated via the *pars facialis* directly to the frontal,
303 which it broadly overlaps. In *Phaeognathus* there is a very large, stout process that extends
304 posteromedially, almost as far as the quadrate bone, into the ligament that connects the
305 bone to the suspensorium (Wake, 1966).

306 **Septomaxillae:** These paired bones, embedded in the cartilages of the nasal capsules at the
307 lateral edge of the nasal vacuity, are small and irregular in shape. The anteriormost part is
308 somewhat triangular and projects anteriorly in the dorsolateral part of the nasal cavity (Fig.
309 3A). The small anterior extension of the *pars facialis* of the maxilla partially covers the
310 septomaxilla. The septomaxilla articulates dorsally with the prefrontal at the anterior edge
311 of the nasolacrimal duct, which passes through its posterior margin.

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

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

319 **Nasals:** These paired bones are triangular in shape. The base of the triangle forms the
320 dorsal part of the nasal capsule, and the vertex points posterodorsally (Fig. 4A). The nasal

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

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

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

336 **Prefrontals:** Prefrontals are paired bones, oval and slightly convex, that are irregular in
337 shape. They partially to completely fill the gaps between the orbit and the nasal vacuity,
338 and between the frontal and the *pars facialis* of the maxilla (Fig. 4A). They are smaller than
339 the nasals. The nasolacrimal duct passes through an elongate foramen in the anterior part of
340 the prefrontal that is usually not enclosed in bone anteriorly. The prefrontal articulates
341 anteriorly with the septomaxilla and lateroventrally with the *pars facialis* of the maxilla.
342 The prefrontal is overlapped laterodorsally by the nasal; posteriorly, it broadly overlaps the
343 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 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 of is broader than the rest of the bone. It is slightly convex and has a smooth surface. Frontals

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367 articulate mesially with their counterparts but do not fuse. The premaxilla, nasal, and
368 prefrontal overlap the enlarged anteriormost part of the frontal. The lateral edges of the
369 frontal turn down laterally, articulating with the orbitosphenoid (Fig. 6A). Frontals
370 articulate posteriorly with parietals, slightly overlapping them lateroventrally. The
371 articulation is not square, but the contact between frontals and parietals does not leave a
372 conspicuous frontoparietal fontanelle in adults.

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

384 The frontals are well-ossified, large bones in plethodontines. They are particularly
385 large and stout with expanded anterior portions in desmognathines, which lack prefrontals.
386 Desmognathines are unique in that the frontals have ventrolateral processes that invade the
387 antorbital region, in the front of the orbit between the prefrontals and palatal parts of the
388 maxillae. The frontals are relatively massive, especially in *Phaeognathus*, and they extend
389 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 coossified 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, while 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

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413 cleared specimens (the scanned and the cleared specimens are all similar in size). The
414 frontals overlap the parietals, especially laterally.

415 In *H. platycephalus*, parietals are poorly ossified anteromedially and medially (Fig.
416 4A). The anterolateral edge of each parietal projects anteriorly and is overlapped by the
417 frontal. Laterally, the parietal does not contact the orbitosphenoid except at its posterior end
418 (Fig. 6C). Posteriorly, the parietal envelops the anterior half of the otic capsule. Compared
419 with *Karsenia* the bone is thinner, flatter and less domed, and the depressed groove
420 accommodating mandibular adductor musculature is less conspicuous.

421 The parietals of *Plethodon* and *Ensatina* are similar to *Karsenia*, while those of
422 *Aneides* differ mainly in having a more conspicuous posterolateral depression. The paired
423 bones in *Aneides* are tightly sutured and become fused in large specimens of the larger
424 species. Parietals of desmognathines differ mainly in being more massive and in having
425 even more conspicuous adductor depressions. This reaches an extreme in *Phaeognathus*, in
426 which the anteromedial portions of the occipito-otic participate in the pronounced
427 depression, with the articulation between parietal and occipito-otic lying at the midpoint of
428 the depression. The parietal and the occipito-otic are drawn out into a large ventrolateral tab
429 that extends the osseous depression for the atlantomandibular ligament, which appears to be
430 unique to desmognathines. The two parietals are strongly sutured in desmognathines. In
431 *Desmognathus*, but to a lesser extent in *Phaeognathus*, the two parts of the bone contrast
432 strongly. The bones are relatively short and the anterior half is high, flat and smooth,
433 whereas the posterior half is strongly depressed. The bones are tightly articulated to the
434 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. 5 A,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 (Fig. 5A,E, 6B). 3) A conspicuous dentigerous ridge (dentigerous process, including a preorbital process) extends laterally from the posterior 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 palatina* 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

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458 proportionally larger than in *K. koreana*. The posterior projection from the main body is
459 slightly curved ventrally. The vomer does not articulate with its counterpart in this male
460 specimen. However, in females there are articulations with the maxilla and premaxilla and
461 also with its counterpart by means of portions of the posterior projections (Adams, 1942).
462 The projection articulates posteriorly with the anteriormost end of the parasphenoid, but
463 there is no contact between the vomer and the orbitosphenoid (Fig 5B, 6D). The
464 dentigerous process projects anteriorly toward the vomerine body; its length exceeds the
465 width of the body. The posterior vomerine dental patches are more reduced than those of *K.*
466 *koreana*. They are restricted to two narrow, widely separated strips of teeth, located
467 posterolaterally on the ventral surface of the parasphenoid (Fig. 5F). The teeth occupy
468 barely 20% of the total surface of the parasphenoid, and they are separated from the
469 dentigerous process by a wide gap, three times as great as in *Karsenia*.

470 Vomers of *Plethodon*, *Ensatina* and *Aneides* closely resemble those of *Karsenia* in
471 most respects. In large *Aneides* the septum-like dorsomedial margin of the body may
472 articulate with the ventral surface of the alary process of the premaxilla. The bilateral
473 counterparts articulate with each other for the posterior half of their extent. Preorbital
474 processes of the dentigerous process typically fall short of the lateral extent of the vomer
475 body, but in *Ensatina* the process is especially long and exceeds the lateral extent of the
476 body. Species of *Aneides* differ in structure of the preorbital process, which is absent
477 entirely in western species. The gap between the dentigerous process and the posterior tooth
478 patch is typically greater than in *Karsenia* but less than in *Hydromantes*, but in *Ensatina* the
479 gap is less and may be completely closed. The posterior patches are not in contact and are
480 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 patches are relatively small and are widely separated from the dentigerous 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 braincase. 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 braincase 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 towards 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

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504 contact the posterior edges of the vomers. With the exception of this anterior contact, the
505 ventral surface of the orbitosphenoid contacts the dorsal surface of the parasphenoid (Fig.
506 6B). Dorsally, the orbitosphenoid articulates with the frontal and parietal.

507 The orbitosphenoids in *H. platycephalus* are more curved and inclined laterally, the
508 cranial cavity being more oval and flattened (Fig. 6C). The *foramen orbitonasale mediale* is
509 not evident in the anterior part of the orbitosphenoid, and only a slight indication of the
510 anterior part of the *foramen oculomotorium* is evident at the posterior end of the bone. A
511 conspicuous *optic fenestra* occupies the posterior area of the orbitosphenoids. The
512 orbitosphenoid articulates ventrally with the anterior half of the parasphenoid; it does not
513 contact the vomer anteriorly (Fig. 6D). The anterior half of the dorsal surface of the
514 orbitosphenoid articulates laterally with the frontal; the posterior half approximates the
515 parietal, although it contacts it only at the posterolateral end.

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

519 **Parasphenoid:** The parasphenoid is an unpaired, median, symmetrical bone, which forms
520 the ventral surface of the braincase. Elongated and semi-rectangular, the bone is square and
521 narrow at the anterior margin and wider and more rounded at its posterior end (Fig. 5E). Its
522 anteriormost margin is thin and flat but becomes dorsally concave toward the posterior
523 margin. Several foramina perforate the lateral margin of the parasphenoid in the third
524 quarter of its length. One of these may be the carotid canal, which is not well defined in
525 salamanders; alternatively, all three might carry different branches of the internal carotid.
526 Ventrally, the parasphenoid sustains the vomerine tooth patch, which occupies

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3 527 approximately 85% of its surface. The parasphenoid articulates laterally with the
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5 528 orbitosphenoids. The posterior edges curve dorsally, enveloping the anterior borders of the
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7 529 occipito-otic. The posterior end of the bone is expanded dorsoventrally and serves as the
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9 530 attachment for subvertebral muscles.
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12 531 The parasphenoid in *H. platycephalus* is nearly triangular, with a narrow anterior end
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14 532 (Fig. 5F). It is flat anteriorly, but quickly becomes internally concave, especially at the level
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16 533 of the area occupied by the vomerine tooth patches. The posterodorsal ends articulate with
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18 534 much of the occipito-otic complex.
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21 535 Plethodontines do not differ much with respect to the parasphenoid, but in
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23 536 desmognathines the bone is shortened, thickened and articulates squarely with the ends of
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25 537 the vomer, although a small, flattened process extends forward dorsally. The posterior part
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27 538 of the parasphenoid is greatly expanded and extends far laterally, finally contacting a
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29 539 dorsomedial process of the quadrate.
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34 540 **Occipito-otic complex:** The posterior portion of the skull is a massive amalgamation of
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36 541 elements associated with the otic capsules and the occipital complex (Fig. 7A,C). The
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38 542 bulbous otic capsules enclose the vestibular-auditory system; they have dorsal and ventral
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40 543 medial connections to each other and the exoccipital region. The otic region is irregular in
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42 544 shape and bears the impressions of the semicircular canals. These impressions form
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44 545 prominent crests, which bear additional projections. The capsule is connected to the
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46 546 braincase by two processes that extend to the base of the ascending process of the
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48 547 suspensorium, by another to the ventral surface of the lateral part of the parietal, and by
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50 548 processes extending to the parasphenoid; it also connects to the basal part of the
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52 549 suspensorium. In addition there is a ridge on the outer surface of the capsule that articulates
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550 with the anterior edge of the squamosal, which also articulates with the capsule behind this
551 ridge. A conspicuous crest, located mesially on the surface of the capsule above the anterior
552 semicircular canal, is v-shaped, the vertex of the ‘v’ pointing anteriorly. Posterolaterally is
553 another prominent crest, directed outward toward the area above the middle of the
554 squamosal. This crest is drawn into a stout process. The posterior part of the capsule is less
555 well ossified. Many foramina pierce the capsule medially toward the cranial cavity,
556 ventrally and posteriorly (the *foramina acustica*, *foramen endolymphaticum*, *foramen*
557 *perilymphaticum*, and *foramen post-oticum*). The operculum covering the *fenestra ovalis*,
558 the *pars media plectri*, is a circular, well-ossified plate (Fig. 7C). Associated with its lateral
559 side is the columellar stylus of the *pars media plectri*. The stylus is roughly L-shaped, with
560 an enlarged area in the *pars media plectri* that connects to the operculum and a relatively
561 large, cylindrical, rod-like portion that proceeds anterolaterally toward the articulation
562 between the squamosal and the quadrate. It does not directly contact these elements,
563 apparently (based on study of cleared specimens) connecting to a long, slender process of
564 the cartilaginous suspensorium and indirectly by a ligament to the squamosal. The
565 exoccipitals form the posteriormost part of the occipito-otic complex, encircling the
566 *foramen magnum* and articulating via the occipital condyles with the first vertebra. Well
567 ossified and completely fused to the otic capsules, the exoccipitals are in contact dorsally
568 by means of the fibrous *tectum synoticum*, but they are in direct contact at the posteriormost
569 point. Conspicuous, rounded occipital condyles are concave posteriorly and articulate with
570 the atlantal cotyles. The internal walls of the *foramen magnum* articulate by means of
571 distinct facets with the large tuberculum interglenoideum of the atlas. The exoccipitals do
572 not contact ventrally, but instead are connected by a rectangular, cartilaginous hypochordal

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6 574 of the parasphenoid.

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8 575 In *H. platycephalus*, the otic capsules are enlarged, flattened and well ossified, but
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10 576 disposed similarly to those of *K. koreana* (Fig. 7B,D). The lateral walls of the capsules are
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12 577 swollen and heavily ossified anteromedially at the level of the connections to the
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14 578 orbitosphenoids. The otic capsules have conspicuous dorsal crests that end in small
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17 579 projections that align with the crests of the squamosals.

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20 580 This complex differs little in all plethodontines except for the presence of crests of
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22 581 different sizes and shapes. These reach extremes in the case of high, wing-like crests in
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24 582 large species of *Aneides* and in males of all species of that genus (Wake, 1963). Crests of
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26 583 the desmognathines have unique configurations and shapes (Wake, 1966). The occipital
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28 584 condyles of desmognathines are massive and prominently stalked (Wake, 1966; Schwenk
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30 585 and Wake, 1993). The opercular-columellar system in plethodontines is relatively uniform.
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32 586 There is a relatively large opercular footplate that appears to be fused (often so completely
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34 587 as to give the impression of a single element) to a more anterior unit comprised of a partial
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36 588 footplate and a distinct rod-like projection. These vary in shape and length among taxa
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39 589 (Monath, 1965; Wake, 1966).

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43 590 **Squamosal:** The paired squamosals extend from the otic capsules to the quadrates,
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45 591 covering the suspensorium (Fig. 8A). The relatively robust, relatively flattened bone arises
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47 592 just behind the prominent crests on the capsule and descends laterally and slightly
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49 593 anteriorly. Along its path, it undergoes a twist of about 45° or more. Two-thirds of the
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51 594 internal-proximal surface of the squamosal is also in contact with the enlarged ascending
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55 595 process of the quadrate.
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596 The squamosal of *Hydromantes platycephalus* is dog-legged in shape and it is smaller
597 and more slender (Fig. 8B). It is attached to the otic capsule posterior to a prominent crest.
598 It is directed anterolaterally and at about its midpoint it bends sharp laterally and ventrally
599 to articulate with the quadrate.

600 The squamosals of plethodontines are generally similar, differing mainly in the degree
601 of robustness, which is largely related to the overall strength of jaws and size of the
602 mandibular adductor muscles and associated otic and squamosal crests. The bones reach a
603 maximal size and complexity in large *Aneides* (Wake, 1963), in which they participate in
604 extended otic crests. The bones are relatively robust in desmognathines, in which the
605 relatively straight bones are tucked behind an otic crest and wedged up against another
606 more horizontally oriented crest of the otic capsule.

607 **Suspensorium:** The suspensorium of *Karsenia* is largely cartilaginous and we have been
608 able to visualize it well with doubly cleared and stained specimens. The only ossified
609 portion is the quadrate and its ascending process, whereas the basal, otic, ascending, and
610 pterygoid portions are cartilaginous. The least well-visualized and smallest portion is the
611 slender, elongate process that connects to the columellar stylus. The quadrate is
612 conspicuous and well ossified, and is a blocky bone (Fig. 8A). It has a relatively long and
613 very thin ascending process that is completely covered by the squamosal. The ventral end
614 of the quadrate is capped in a thick cartilage that forms a concave surface, which articulates
615 with the articular portion of the mandible. The posterior end of the ventral surface forms a
616 small bony projection, the insertion point for the hyoquadrate ligament. Proximally (interior
617 surface), the quadrate is also enlarged, forming an attachment surface for the jugal
618 ligament.

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3 619 In *H. platycephalus* the quadrates are smaller and less robust, although they retain the
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6 620 same generally blocky form (Fig. 8B). The ascending process is shorter.
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8 621 Quadrates of all plethodontids are generally similar, differing mainly in size and degree
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10 622 of development, which is correlated with the overall size of the taxon studied. In
11
12 623 desmognathines the quadrates have extended dorsal and medial processes that articulate
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14 624 with the otic capsule and the cartilaginous parasphenoid.
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17 625 **Lower jaw:** The paired mandibulae form the lower jaw, which is relatively stout and well
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19 626 developed in *Karsenia* (Fig. 9A). Each ramus contains a well-ossified dentary surrounding
20
21 627 a persistent Meckel's cartilage. Viewed laterally each mandible is planar on its ventral
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23 628 surface. The anterodorsal ends of the two dentaries contact at the mandibular symphysis,
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25 629 and they are a bit spread apart ventrally. In transverse section, the dentary is O-shaped
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27 630 anteriorly, enclosing Meckel's cartilage. It opens and increases in height posteriorly,
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29 631 forming an elongated V-shaped region exposing the cartilage at the lingual margin. A well-
30
31 632 formed ridge arises along the dorsolateral surface of the dentary at about its midpoint,
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33 633 before the end of the maxilla; this ridge and adjacent parts of the dentary serve as the
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35 634 insertion of more anterior mandibular adductors. The ridge is extended for a short distance
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37 635 posteriorly as a small projection. The dentary rises slightly as the ridge forms but there is
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39 636 no triangular process as in some other plethodontids. Teeth are borne on the dentary from
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41 637 the symphysis region to the origin of the ridge, at the level of the posterior tip of the
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43 638 maxilla, or for about three-quarters of the length of the dentary. The posterior end of the
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45 639 dentary is flattened, narrow, and blade-like, and is closely applied to the prearticular. Each
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47 640 of the paired well-developed prearticulars, located medially to the dentaries, has a long,
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49 641 tapering process that enters the dentary canal so that the bone is inserted into the elongate
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642 triangular opening described above. A moderately high shelf-like coronoid process arises
643 just past the posterior end of the maxilla, immediately adjacent to the dentary ridge, and is
644 inflected dorsomedially. The extensive surface of this shelf is the insertion of more
645 posterior mandibular adductor muscles. The coronoid process extends posteriorly and then
646 rapidly slopes ventrally and flattens, ending just before the posterior end of the prearticular.
647 The posterior portion of Meckel's cartilage, the articular, is enlarged and mineralized or
648 ossified, with a cartilaginous articular surface. The articular is co-ossified with the lateral
649 and posterior parts of the prearticular and is also tightly connected to the dentary.

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

659 Mandibles of plethodontines vary greatly in size but not in composition. *Plethodon* and
660 *Ensatina* and the smaller species of *Aneides* closely resemble *Karsenia*. *Aneides* has an
661 edentulous triangular extension of the dentary, anterior to the coronoid process of the
662 prearticular and associated with the muscular dorsolateral ridge. It becomes very large in *A.*
663 *lubugris* and *A. flavipunctatus*. The extension is variable but generally low in *Plethodon*,
664 and only slightly evident in *Ensatina*. The coronoid process of the prearticular is generally

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3 665 similar to *Karsenia*, except in *Ensatina*, in which it is especially low and small, and
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5 666 *Aneides*, in which it is enormously enlarged in the larger species. Teeth are borne along
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8 667 about three-quarters of the dentary in *Ensatina*, between one-half and two-thirds the length
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10 668 in *Plethodon*, and the sometimes very large teeth of *Aneides* are restricted to less than one-
11
12 669 half to less than one-quarter the length of the dentary (see Dentition section). While the
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14 670 dentaries of the largest *Aneides* are massive, they are not as massive, in a relative sense, as
15
16 671 those of desmognathines. In species such as *Phaeognathus hubrichti*, the dentaries are very
17
18 672 high in lateral view and densely ossified, bearing teeth along less than two-thirds of the
19
20 673 length of the bone. A very large dorso-lateral ridge provides a wide shelf for insertion of
21
22 674 adductor muscles. The desmognathine prearticular has a unique shape. The coronoid
23
24 675 process is restricted to an anterior pointed but stout projection; these form the posterior
25
26 676 border of the unique atlantomandibular ligament, which attaches to the dentary just in front
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28 677 of them (Means, 1974). All plethodontids lack a significant retroarticular process, although
29
30 678 there is some extension of the prearticular slightly posterior to the articulation. In
31
32 679 desmognathines and larger species of *Aneides* a small ventrally oriented process of the
33
34 680 dentary is present near the symphysis, presumable to serve as the point of insertion of hyoid
35
36 681 muscles.

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38 682 **Hyobranchial apparatus:** The hyobranchial apparatus in adults consists of paired
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40 683 ceratohyals, basibranchial, paired radials, paired ceratobranchials I and II, paired
41
42 684 epibranchials I, and a urohyal (Fig. 10). All parts of the apparatus are cartilaginous, except
43
44 685 the central portion of the basibranchial. The latter is a solid rod of mineralized cartilage
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46 686 viewed laterally in the scanned specimen as floating below the skull, orientated
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48 687 posteroventrally relative to the snout and forming a 45° angle with the mandibular plane.
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688 The basibranchial is enlarged and has three distinct areas. 1) A central, rod-shaped and
689 mainly mineralized body changes to cartilage both anteriorly and posteriorly. The
690 mineralized portion, which constitutes about 45% of the total length of the basibranchial,
691 increases in diameter towards the ends. 2) A well-developed, knob-shaped cartilage forms
692 an anterior process. 3) A posterior cartilaginous enlarged area, pointed at the posteriormost
693 end, connects to the two pairs of ceratobranchials. The paired radials articulate at the base
694 of the anterior process of the basibranchial. They are rod-shaped proximally but more
695 flattened and irregular in outline distally. The radials are oriented almost perpendicular to
696 the basibranchial, pointing slightly anteriorly. The first ceratobranchial is the longest
697 articulated structure of the hyobranchial apparatus. Ceratobranchial I is nearly straight, with
698 a relatively flat and broad anterior end, the structure becoming more cylindrical and
699 narrower posteriorly. The anterior end articulates with the basibranchial at the level of the
700 posterior cartilaginous area and the posterior end articulates with epibranchials.
701 Ceratobranchial II is shorter, more slender, slightly sigmoidal, and cylindrical. The anterior
702 end of the ceratobranchial articulates with the basibranchial on two sides of the pointed
703 posterior end. Posterolaterally, the ceratobranchial articulates with the epibranchial.
704 Ceratobranchials I and II do not overlap or contact each other. The epibranchial is a
705 cylindrical cone that decreases in size and curves anteroposteriorly; it is directed
706 posterodorsally, in the direction of the shoulder. The posterior end nearly reaches the level
707 of the pectoral girdle. The bowed, ossified urohyal lies medially, slightly posterior to the
708 level of the epibranchial-ceratobranchial articulation. The ceratohyal is a large element that
709 is cylindrical proximally, where it connects by ligaments to the quadrate. It becomes

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3 710 flattened and rather abruptly greatly expanded distally, with a broadly rounded anterolateral
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5 711 margin and nearly straight anteromedial margins parallel to the medial axis.
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8 712 The ceratohyal is the longest element of the hyobranchial apparatus (1.7 times the
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10 713 length of the basibranchial), and the first ceratobranchial is the longest of the articulated
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12 714 elements (1.25 time the basibranchial). The second ceratobranchial is 1.2 times the
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14 715 basibranchial, and the epibranchial is about 0.8 times the first ceratobranchial and
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16 716 essentially the same length as the basibranchial. The radials are 0.5 times the basibranchial
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18 717 and the urohyal is 0.3 times the basibranchial. The knob-like anterior extension of the
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20 718 basibranchial (from the midpoint of the attachment of the radials) is about 0.16 times the
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22 719 length of the element. The proportions are similar in all the specimens studied.
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27 720 The hyobranchial apparatus of *Hydromantes* (all members of the group) differs greatly
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29 721 from that of all other salamanders (Wiedersheim, 1875; Lombard and Wake, 1977; Wake
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31 722 and Deban, 2000), and the contrast with *Karsenia* is especially great. It has the form of an
32
33 723 extremely long projectile. The basibranchial is longer relative to body size than in any other
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35 724 plethodontid and has an anterior extension that is flattened at the end and flexible near the
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37 725 tip (Lombard and Wake, 1977). The basibranchial is expanded near its midpoint with thin
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39 726 lateral flanges. The epibranchials are extremely long, reaching lengths 2.7-3.2 times the
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41 727 basibranchial length. In contrast, the ceratobranchials are short and slender, with the second
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43 728 being slightly shorter and slightly stouter than the first. The first ceratobranchial is only
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45 729 0.52-0.54 times basibranchial length, whereas the epibranchial is nearly 6 times the length
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47 730 of the first ceratobranchial. Radials and urohyal are absent. The ceratohyals are relatively
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49 731 short, less than 0.9 times basibranchial length, and have only a narrowly expanded blade.
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732 The epibranchials are about three times longer than the ceratohyal. No parts are
733 mineralized.

734 The hyobranchial apparatus of *Aneides* and *Plethodon* closely resembles that of
735 *Karsenia* in shape and proportions, and the basibranchial is mineralized in its midsection in
736 several species. *Desmognathus* and *Phaeognathus* also have similar hyobranchial structure,
737 although the tip of the basibranchial is not knobbed in the latter, instead tapering to a point.

738 The radials are somewhat longer and substantially more slender in desmognathines than in
739 the other plethodontines. The urohyal is variable; it can be as long as two-thirds the length
740 of the basibranchial in *Desmognathus*, but is much shorter and more slender in
741 *Phaeognathus*. *Ensatina* differs from other plethodontines in having longer epibranchials
742 (about 1.8 times basibranchial length) and in lacking the slender, knobbed anterior
743 extension of the basibranchial. Instead, that part of the basibranchial is short and
744 trapezoidal-to-triangular in shape. It is partly disconnected from the main body and hence is
745 flexible.

746 **Dentition:** Toothed areas include the premaxillae, maxillae, dentaries, vomers, and two
747 posterior vomerine patches associated with the parasphenoid. All teeth are pedicellate,
748 small, and barely bicuspid in adults, and nearly homogeneous in size and shape in all the
749 toothed areas. The lingual cusp is the largest. There is scant evidence of sexual
750 dimorphism. Premaxillary teeth are slightly enlarged and reduced in numbers (mean of 7.9
751 +/-1.3 total for both bones, range of 6-10 in 12 females, 6.4 +/-1.1, 4-7 in 8 males) in
752 comparison to such relatives as *Plethodon* and *Ensatina*. Maxillary teeth are slightly more
753 numerous in females (mean 49.5 +/- 4.5, 40-56) than in males (45.6 +/- 3.3, 40-50).

754 Vomerine teeth are small and extend only a short distance onto the otherwise toothless

preorbital process. They number 16.8 ± 2.7 , 12-20 in females and 15.5 ± 2.6 , 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 approximately 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 non-reproductive 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

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778 teeth that are of normal form, while males have fewer but mainly bicuspid teeth (but not so
779 few as in the European species), although the more posterior teeth are transitional to the
780 shape of the maxillary teeth. Females have maxillary teeth similar to their premaxillary
781 teeth and they number between about 20 and 30 in European species, but they are more
782 numerous in American species (25-40). Males have typical small bicuspid teeth in
783 European species, with about the same numbers as females, but in the American species
784 males have greatly enlarged, monocuspid teeth that protrude outward from the bone and the
785 skin forming the upper lip (Noble and Brady, 1930; Noble, 1931; Adams, 1942). These
786 narrowly conical, sharp maxillary teeth apparently scarify the skin of females during
787 courtship to deliver secretions of mental glands. The maxillary teeth of American males
788 number from 8 to 12. Dentary teeth of all species are small and bicuspid, numbering
789 between 30 and 50.

790 Dentition in *Desmognathus* and *Phaeognathus* was described by Means (1974) and
791 in general resembles that of *Karsenia*. However, there is substantial interspecific variation
792 in the shape and size of tooth crowns. Typical bicuspid teeth are present, but lingual cusps
793 of some species are enlarged and recurved, and in a few species the labial cusp is greatly
794 reduced in size and is nearly imperceptible. Some species display sexual dimorphism in
795 cusp morphology, with females having typical small bicuspid teeth and males developing
796 monocuspid conical premaxillary teeth that project forward, and enlarged, forward
797 projecting teeth at the anterior end of the dentary that have hypertrophied lingual cusps
798 (Noble and Pope, 1929). In two species, teeth of the jaws have short flattened cusps that
799 have been described as fungiform (Means, 1974). The dentaries of some species become
800 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* has 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

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824 monocuspid premaxillary teeth in males ("needle-like" in *A. flavipunctatus*; Coss, 1974),
825 and enlarged, recurved and laterally compressed maxillary and dentary teeth, although
826 anterior maxillary teeth may be small and bicuspid. The posterior part of the maxilla is
827 toothless and greatly expanded dorsoventrally, and the monocuspid teeth immediately in
828 front of this expanded portion are large and blade-like (Wake, 1963). In *A. lugubris*,
829 maxillary and dentary teeth both increase in size and degree of compression and decrease in
830 number with increasing body size, with as few as five per bone in large adults (Wake et al.,
831 1983). Numbers of maxillary and dentary teeth are reduced to as few as three per bone in *A.*
832 *flavipunctatus* (Wake, 1963).

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834 **DISCUSSION**

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

844 Variations, needless to say, occur. Morphological diversity among salamanders
845 reflects adaptations to specific habitats, as well as the evolution of highly specialized life
846 histories, such as direct development and paedomorphosis. 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 ten 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, paedomorphic 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.,

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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.,
1983; 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

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3 893 within the family. We discuss the anatomical description of *K. koreana* with two goals in
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6 894 mind. First, we approach the biology of the species through its morphological description,
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8 895 profiting from the detailed comparative data set available for most plethodontid species.
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10 896 The general, global links among anatomical parts, functional morphology, and ecological
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12 897 characteristics of the different species are well understood (Wake, 1966; Larson, 1984).
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14 898 Then, we analyze the morphological trends observed in *K. koreana* and its closest relatives
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16 899 to examine the potential factors that have driven the evolution of morphology in
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18 900 plethodontids.
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23 24 902 **Habitat in Relation to the General Morphology of the Skull**

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27 903 There is a broad correlation between the general morphology of the skull and the habitat of
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29 904 plethodontine salamanders. For example, species within *Plethodon* are generally ground-
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31 905 dwellers, and are normally found under rocks and logs in forest habitats. The morphology
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33 906 of their skulls represents the most generalized and likely ancestral form (Wake, 1966). The
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35 907 *Plethodon* radiation contrasts with other functionally specialized types such as occur in
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37 908 *Desmognathus*, which has flattened and streamlined skull morphologies adapted for living
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39 909 in mountain streams, and the robust and bullet-shaped skull in the burrower *Phaeognathus*
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41 910 (Wake, 1966; Larson, 1984; Schwenk and Wake, 1993).
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46 911 The skull of *Karsenia* is compact and well articulated. Jaws are robust although teeth are
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48 912 not enlarged. In general the skull resembles that of members of the genus *Plethodon* more
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50 913 than that of other plethodontine genera. It is apparently stronger than any *Plethodon* skull
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52 914 that we have observed, resembling that of members of the *P. elongatus* group more than
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55 915 others in this respect. It lacks, however, the crests on the occipito-otic that receive a peg-
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916 like projection of the squamosal, which is unique to members of that latter group (Wake,
917 1963). Like *Plethodon*, *Karsenia* is thought to be fully terrestrial, living at or near the
918 surface, but unlike most *Plethodon* it is associated with rock crevices as its vernacular
919 name, Korean crevice salamander, implies. The species of *Plethodon* that share this habit to
920 some degree include *P. larselli* (*P. vandykei* group) and *P. asupak* (*P. elongatus* group;
921 Mead et al., 2005). Although behavior of *Karsenia koreana* in relation to habitat use is
922 known only from the observations of collectors, we suspect that the species uses its solid
923 skull in part in relation to seeking refuge in tight spaces between rocks.

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

936
937 **Feeding in Relation to Skull Form and the Anatomy of the Hyobranchial Apparatus**

938 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*, *Hemidactylum*), 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 (Lombard and Wake, 1976; Hinderstein, 1971; 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

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962 contacting the prey with its protrusible tongue and then rapidly delivering the prey to the
963 buccal cavity, where it is held by means of the posterior vomerine tooth patches and the
964 tongue (Maglia and Pyles, 1995; Wake and Deban, 2000). All plethodontines feed mainly
965 on arthropods, usually of small size in relation to the size of the mouth. Teeth and jaws play
966 a minor to negligible role in prey capture and processing in other plethodontines, except
967 when prey are large. While *Hydromantes* also uses tongue-feeding, it is specialized to an
968 extreme degree and has the longest and at the same time the most powerful tongue of any
969 salamander yet studied (Deban et al., 1997; Deban et al., 2007). Its relatively weak jaws do
970 not appear to play a role in processing of food.

971 *Hydromantes* has been well studied with respect to visual behavior and feeding (Roth,
972 1976, 1987). The eyes are large and protuberant and the head is mobile. Associated with
973 head mobility is a small and gracile tuberculum interglenoideum on the atlas vertebra,
974 which extends into the foramen magnum and has small articular facets, oriented
975 ventrolaterally. We know little concerning head mobility in *Karsenia*, but its large and
976 robust tuberculum interglenoideum suggests that it is associated with less craniovertebral
977 mobility and increased resistance to bending than in *Hydromantes* and possibly other
978 plethodontines as well. The extreme in reduction of the tuberculum interglenoideum is in
979 desmognathines, in which the occipital condyles are enlarged and stalked, thus moving the
980 head away from the vertebral column and facilitating up-and-down movements of the head
981 (Schwenk and Wake, 1993).

982 Whereas the skull of *Karsenia* is rather solidly built and cylindrical, with a high, well-
983 supported braincase and relatively small orbits, that of *Hydromantes* is relatively light and
984 flat with weak articulations of bones, and its jaws are light and lack strong musculature and

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3 985 bear teeth that are directed outside the mouth. The braincase of *Hydromantes* is weakly
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5 986 supported, the jaws are weak and the orbits are especially large. Together with the
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8 987 flattening of the entire skull, the impression is of a skull that is not involved in food
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10 988 processing, as is appropriate for an organism with an elongate, highly specialized tongue
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12 989 that delivers the prey toward the back of the buccal cavity where it is immobilized by the
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14 990 posteriorly directed vomerine tooth patches.
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992 **Secondary Sexual Traits of the Skull in Relation to Courtship and Mating Behavior**

993 Courtship behavior is uniform among plethodontids (summaries in Arnold, 1977; Wells,
994 2007). During an elaborate tail-straddling walk, the male delivers one or a few
995 spermatophores that are taken into the female's cloaca. The tail-straddling behavior is
996 usually accompanied by the delivery of pheromones by the male. Two distinct modes of
997 delivery have been described. In the first mode, which is assumed to be ancestral and more
998 generalized among plethodontid salamanders, males deliver pheromones from their mental
999 glands by means of scratching the dorsal skin of females with their seasonally
1000 hypertrophied premaxillary teeth. Species showing this 'vaccination' behavior have a strong
1001 seasonal sexual dimorphism in dentition. A second derived mode of pheromone transfer has
1002 evolved in a clade of the large species of *Plethodon*. In this case, males do not develop the
1003 protruding premaxillary teeth and the delivery of pheromones is conducted by repeatedly
1004 touching the mental gland to a female's nares. Courtship and mating behavior in most of the
1005 plethodontid species studied to date involve the delivery of pheromones, although a few
1006 species have secondarily lost the mental glands and the delivery behavior (e.g., *Ensatina*)
1007 (Arnold, 1977; Wells, 2007).

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1008 There is scant evidence of sexual dimorphism in our material of *Karsenia*, whereas most
1009 species of plethodontines show at least seasonal sexual dimorphism in premaxillary teeth,
1010 males having fewer and larger teeth (Coss, 1974). In our seasonally limited samples of
1011 *Karsenia*, although the premaxillary teeth of males are only slightly enlarged and, while
1012 fewer in number in males than in females, the minimum number of 4 per bone is not
1013 especially low. However, larger series of males and females must be studied to assess the
1014 degree of seasonality and sexual dimorphism in the premaxillary dentition of *Karsenia*.
1015 Nothing is known concerning courtship and mating in *Karsenia* but, given the absence of
1016 protruding premaxillary teeth and the presence of mental glands in males, it is reasonable to
1017 assume that they are similar to the derived mode observed in *Plethodon*. The situation is
1018 similar in the European species of *Hydromantes*, but in the American species sexual
1019 dimorphism is expressed in a unique way. The maxillary teeth of males are at least
1020 seasonally enlarged and spine-like. While their precise role in courtship has not been
1021 specifically studied, based on behavior in other plethodontids it is likely that the teeth are
1022 used to vaccinate females with pheromonal secretions from the mental glands.
1023
Morphology of the Skull with Respect to Social and Aggressive Behavior
1025 Patterns of social and aggressive behaviors are extremely diverse among plethodontids,
1026 involving visual and chemical cues in species, sexual, and individual recognition. Agonistic
1027 behaviors are normally related to intraspecific territorial combat, but also to interspecific
1028 spacing strategies and parental care (Jaeger and Forester, 1993). Plethodontines are known
1029 to be aggressive, both toward sympatric congeners and to conspecifics (reviewed in Wells,
1030 2007). In some cases, the morphological correlates of aggressive behavior have been

1031 studied in detail (e.g., Staub, 1993; Wiltenmuth, 1996). The relatively strong jaws of
1032 *Karsenia*, which might be related either to locomotor use of the head or to feeding, suggest
1033 that these forms may be aggressive. However, scarring has not been observed in the
1034 specimens collected to date, so no further conclusions can be extrapolated. Aggression has
1035 not been observed in *Hydromantes* and it is not expected, given the weakness of the jaws.
1036 One must be cautious in inferring function from form in these salamanders, however,
1037 because the relatively weak-jawed *Ensatina* can be very aggressive toward conspecifics
1038 (Wiltenmuth, 1996; Wiltenmuth and Nishikawa, 1998).

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1040 **Phylogenetic Implications of Comparative Skull Morphology in Plethodontine**

1041 **Salamanders**

1042 The skulls of the genera of plethodontine salamanders are distinctive, within a framework
1043 of conservative composition (see below). Furthermore, even within genera such as *Aneides*
1044 (Wake, 1963), *Desmognathus* (Means, 1974), and *Hydromantes* (Wake, 1966; Lanza et al.,
1045 1995; this work) important differences characterize either individual species or small clades
1046 (such as the western species of *Aneides*, and the European as compared with the American
1047 *Hydromantes*). Some multispecies genera, e.g. *Aneides*, *Hydromantes*, and *Desmognathus*,
1048 are characterized by numerous synapomorphies (Wake, 1966), but *Plethodon* is not (Wake,
1049 1963). *Plethodon* is the second largest genus of salamanders (after *Bolitoglossa*), currently
1050 with 55 recognized species (AmphibiaWeb, September, 2009.
1051 <http://amphibiaweb.org/index.html>). These species are remarkably uniform, with relatively
1052 minor differences among them; only the *P. elongatus* group has significant synapomorphies
1053 associated with the jaws, squamosal, and cranial crests (Wake, 1963, and unpublished

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1054 data). Furthermore, *Plethodon* displays apparently plesiomorphic states for most skull and
1055 hyobranchial characters, the only exceptions being the somewhat strengthened jaws and
1056 slightly reduced numbers of teeth, which distinguish it from *Ensatina* and from distant
1057 outgroups such as *Rhyacotriton*. *Karsenia* also is plesiomorphic with respect to nearly all
1058 traits of the skull and hyobranchial apparatus, the exceptions being the strong jaws and the
1059 ossified first basibranchial. It shares the latter and its plesiomorphic traits with at least some
1060 species of *Plethodon*; with respect to skull and hyobranchial morphology *Karsenia* would
1061 fit well within *Plethodon*. The major features separating the genera relate to tarsal anatomy
1062 (Min et al., 2005) and molecular characters (Min et al., 2005; Vieites et al. 2007).

1063 We attempted a phylogenetic analysis of data from the skull and hyobranchial
1064 apparatus, with characters used by Wake (1963, 1966), Good and Wake (1992), Larson and
1065 Dimmick (1993), and Wiens et al. (2005), but to little effect (data not shown). Larson and
1066 Dimmick and Wiens et al. attempted to reconstruct the phylogenetic relationships among
1067 salamander families, but even at that phylogenetic level many of the morphological
1068 characters employed were not informative due to the great amount of homoplasy among
1069 and within lineages. The majority of the characters used for the phylogentic analysis, as
1070 expected, were not informative at the phylogenetic depth of this study. There are very few
1071 synapomorphies in the dataset, the most numerous being those that support the
1072 *Desmognathus*, *Aneides*, and *Hydromantes* clades. The sole multigeneric clade supported
1073 with several synapomorphies is *Phaeognathus* + *Desmognathus*. This clade, long
1074 recognized taxonomically as the subfamily Desmognathinae (Wake, 1966), was recently
1075 and unexpectedly found to be deeply nested within the Plethodontinae in all relevant

1076 molecular analyses (e.g., Mueller et al., 2004; Chippindale et al., 2004; Vieites et al., 2007).

1077 No single morphological synapomorphy defines a *Karsenia-Hydromantes* clade.

1078 Composition of the skull of plethodontine salamanders is remarkably conservative.

1079 The divergence of the two major clades of plethodontid salamanders, Plethodontinae and

1080 Hemidactyliinae, is approximately 94 mya (Vieites et al., 2007; Zhang and Wake, 2009).

1081 During that vast period of time, in which the range of the plethodontines expanded from

1082 North America to east Asia and Mediterranean Europe, very little compositional change in

1083 skull configuration has taken place. Septomaxillary bones are variably absent from some

1084 populations of European *Hydromantes*, prefrontals are absent from desmognathines and

1085 *Hydromantes*, and urohyals and radii of basibranchials are absent from *Hydromantes*.

1086 Premaxillary bones are fused in *Aneides*, *Desmognathus* and *Phaeognathus*. Otherwise all

1087 species share a common composition. There are differences in degree of ossification,

1088 general strength of the skull and in desmognathines a major change in the jaw-opening and

1089 feeding and locomotory mechanisms associated with the skull. The putative sister-taxon

1090 relationship of *Karsenia* and *Hydromantes* associates the apparently conserved, likely

1091 ancestral condition of the skull and hyobranchial apparatus in *Karsenia* with the extreme of

1092 specialization for tongue feeding and the weakest and most compositionally deficient

1093 plethodontine skull of *Hydromantes*.

1094 The pattern of generalist-specialized forms within clades is repeated along the phylogeny

1095 of plethodontids, and the clade comprising *Karsenia* and *Hydromantes* provides a new

1096 example with which to analyze the processes driving morphological evolution and

1097 specialization in Plethodontidae. As discussed, homoplasy, frequent within and between

1098 salamander families, makes attempts at phylogenetic reconstruction problematic at all

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1099 phylogenetic levels. Homoplastic patterns, which are non-randomly distributed both among
1100 characters and among taxa, reflect real biological processes that underlie the recurrent
1101 evolution of morphological similarities among groups (Hufford, 1996; McShea, 1996). In
1102 salamanders, these processes include the modification of otherwise conserved
1103 developmental programs (e.g., ontogenetic repatterning, differential metamorphosis [Roth
1104 and Wake, 1985; Wake and Roth, 1989]). In Plethodontidae, these developmental
1105 modifications, coupled with extreme life history strategies such as paedomorphosis and
1106 especially direct development, and morphological peculiarities such as the loss of lungs,
1107 created an unusual evolutionary arena that permitted the diversification of the group but
1108 within a framework of homoplasy and morphological constraint (Wake et al., 1983; Wake,
1109 1991; Wake and Hanken, 1996). Descriptions of new lineages, such as *K. koreana*, are
1110 important because they test our understanding of the limits of morphological diversity, and
1111 provide insight into the dimensions of evolutionary patterns within bounds, or constraints,
1112 on change. Our anatomical description of the skull of the only Asiatic plethodontid is
1113 conducted within this conceptual framework. Obtaining relevant biological information for
1114 the species, such as habitat use, feeding habits, and life history traits, will enable us to
1115 extend analysis of plethodontid evolution in a multidimensional approach (Wake and
1116 Larson, 1987), enhancing our understanding of the relative importance of the processes that
1117 have driven the evolution and diversification of plethodontid salamanders. A more general
1118 outcome of our study will be progress toward an understanding of how lineage
1119 differentiation proceeds in the context of limits to morphological expression and apparent
1120 phylogenetic and developmental constraints.
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1132 REFERENCES

- 1133 Adams L. 1942. The natural history and classification of the Mount Lyell salamander,
1134 *Hydromantes platycephalus*. Univ Calif Pub Zool 46:179–204.
- 1135 Arnold SJ. 1977. The evolution of courtship behavior in New World salamanders with
1136 some comments on Old World salamanders. In: Taylor DH, Guttman SI, editors. The
1137 Reproductive Biology of Amphibians. New York: Plenum Press. p 141–183.
- 1138 Camp CL. 1916. *Spelerpes platycephalus*, a new alpine salamander from the Yosemite
1139 National Park, California. Univ Calif Pub Zool 17:11–14.
- 1140 Chippindale PT, Bonett RM, Baldwin AS, Wiens JJ. 2004. Phylogenetic evidence for a
1141 major reversal of life-history evolution in plethodontid salamanders. Evolution
1142 58:2809–2822.

- 1143 Coss HT. 1974. Maxillary and premaxillary dentition of salamanders of the tribe
1144 Plethodontini (Family Plethodontidae) [Ph.D. Dissertation]. Clemson (SC): Clemson
1145 University. ix + 70 p.
- 1146 Crochet PA. 2007. Nomenclature of European plethodontid salamanders: *Speleomantes*
1147 Dubois, 1984 has precedence over *Atylodes* Gistel, 1868. *Amphibia-Reptilia* 28:170-
1148 172.
- 1149 Deban SM, O'Reilly JC, Dicke U, van Leeuwen JL. 2007. Extremely high-power tongue
1150 projection in plethodontid salamanders. *J Exp Biol* 210:655–667.
- 1151 Deban SM, Wake DB, Roth G. 1997. Salamander with a ballistic tongue. *Nature* 389:27–
1152 28.
- 1153 Francis ETB. 1934. *The Anatomy of the Salamander*. Oxford: Clarendon Press. p 381, 25
1154 pls.
- 1155 Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CF, de Sá RO, Channing A,
1156 Wilkinson M, Donnellan S, Raxworthy CJ, Campbell JA, Blotto BL, Moler P,
1157 Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC. 2006. The
1158 Amphibian Tree of Life. *Bull Am Mus Nat Hist* 297:1–371.
- 1159 Gao KQ, Shubin NH. 2001. Late Jurassic salamanders from China. *Nature* 410:574–577.
- 1160 Gao KQ, Shubin NH. 2003. Earliest known crown-group salamanders. *Nature* 422:424–
1161 428.
- 1162 Good DA, Wake DB. 1992. Geographic variation and speciation in the Torrent
1163 Salamanders of the genus *Rhyacotriton* (Caudata: Rhyacotritonidae). *Univ Calif Pub*
1164 *Zool* 126:1–91.

- 1165 Greven H, Clemen G. 1976. Morphologische Untersuchungen an der Mundhöhle von
1166 Urodelen. II. Die Gaumenzahnfelder von *Hydromantes italicus* Cunn
1167 (Plethodontidae: Amphibia). Zool Beitr 22:489–506.
- 1168 Hilton WA. 1945. The skeleton of *Hydromantes*. J Ent Zool 37:98–99.
- 1169 Hinderstein B. 1971. The desmognathine jaw mechanism (Amphibia: Caudata:
1170 Plethodontidae). Herpetologica 27:467–476.
- 1171 Hufford L. 1996. Ontogenetic evolution, clade diversification, and homoplasy. In:
1172 Sanderson MJ, Hufford L, editors. Homoplasy: The Recurrence of Similarity in
1173 Evolution. San Diego: Academic Press. p 271–301.
- 1174 Jaeger RG, Forester DC. 1993. Social behavior of plethodontid salamanders. Herpetologica
1175 49:163–175.
- 1176 Kozak KH, Weisrock DW, Larson A. 2006. Rapid lineage accumulation in a non-adaptive
1177 radiation: phylogenetic analysis of diversification rates in eastern North American
1178 woodland salamanders (Plethodontidae: *Plethodon*). Proc R Soc Lond B 275:539–
1179 546.
- 1180 Lanza B, Caputo V, Nascetti G, Bullini L. 1995. Morphological and genetic studies of the
1181 European plethodontid salamanders: taxonomic inferences (genus *Hydromantes*).
1182 Monogr Mus Reg Sci Nat 16:1–366.
- 1183 Larson A. 1984. Neontological inferences of evolutionary patterns and processes in the
1184 salamander family Plethodontidae. Evol Biol 17:119–217.
- 1185 Larson A, Dimmick WW. 1993. Phylogenetic relationships of the salamander families: an
1186 analysis of congruence among morphological and molecular characters. Herp Monogr
1187 7:77–93.

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1188 Leopold JL, Maglia AM, Pugener LA, Gauch S. 2007. The Amphibian Anatomical
1189 Ontology web project. <http://www.amphibanat.org>
1190 Lombard RE, Wake DB. 1976. Tongue evolution in the lungless salamanders, family
1191 Plethodontidae. I. Introduction, theory and a general model of dynamics. J Morphol
1192 148:265–286.
1193 Lombard RE, Wake DB. 1977. Tongue evolution in the lungless salamanders, family
1194 Plethodontidae. II. Function and evolutionary diversity. J Morphol 153:39–80.
1195 Lombard RE, Wake DB. 1986. Tongue evolution in the lungless salamanders, family
1196 Plethodontidae. IV. Phylogeny of plethodontid salamanders and the evolution of
1197 feeding dynamics. Syst Zool 35:532–551.
1198 Maglia AM, Leopold JL, Pugener LA, Gauch S. 2007. An anatomical ontology for
1199 amphibians. Proc Pac Symp Biocomp 12:367–378
1200 Maglia AM, Pyles RA. 1995. Modulation of prey-capture behavior in *Plethodon cinereus*
1201 (Green) (Amphibia: Caudata). J Exp Zool 272:167–183.
1202 McShea DW. 1996. Complexity and homoplasy. In: Sanderson MJ, Hufford L, editors.
1203 Homoplasy: The Recurrence of Similarity in Evolution. San Diego: Academic Press.
1204 p 207–225.
1205 Mead LS, Clayton DR, Nauman RS, Olson DH, Pfrender ME. 2005. Newly discovered
1206 populations of salamanders from Siskiyou County California represent a species
1207 distinct from *Plethodon stormi*. Herpetologica 61:158–177.
1208 Means DB. 1974. The status of *Desmognathus brimleyorum* Stejneger and an analysis of
1209 the genus *Desmognathus* (Amphibia: Urodela) in Florida. Bull Florida State Mus
1210 18:1–100.

- 1211 Min MS, Yang SY, Bonett RM, Vieites DR, Brandon RA, Wake DB. 2005. Discovery of
1212 the first Asian plethodontid salamander. *Nature* 435:87–90.
- 1213 Monath T. 1965. The opercular apparatus of salamanders. *J Morphol* 116:149–170.
- 1214 Mueller R, Macey JR, Jaekel M, Wake DB, Boore JL. 2004. Morphological homoplasy,
1215 life history evolution, and historical biogeography of plethodontid salamanders
1216 inferred from complete mitochondrial genomes. *Proc Natl Acad Sci USA* 101:13820–
1217 13825.
- 1218 Noble GK. 1931. *The Biology of the Amphibia*. New York: McGraw-Hill. p 577.
- 1219 Noble GK, Brady MK. 1930. The courtship of the plethodontid salamanders. *Copeia*
1220 1930:52–54.
- 1221 Noble GK, Pope SH. 1929. The modification of the cloaca and teeth of the adult
1222 salamander, *Desmognathus*, by testicular transplants and by castration. *J Exp Biol*
1223 6:401–411.
- 1224 Parra-Olea G, Wake DB. 2001. Extreme morphological and ecological homoplasy in
1225 tropical salamanders. *Proc Natl Acad Sci USA* 98:7888–7891.
- 1226 Rasband WS. 2008. *ImageJ*. Bethesda, Maryland: U. S. National Institutes of Health.
1227 <http://rsb.info.nih.gov/ij/>
- 1228 Roth G. 1976. Experimental analysis of the prey catching behavior of *Hydromantes italicus*
1229 Dunn (Amphibia, Plethodontidae). *J Comp Physiol A* 109:47–58.
- 1230 Roth G. 1987. *Visual Behavior in Salamanders*. Berlin: Springer-Verlag.
- 1231 Roth G, Wake DB. 1985. Trends in the functional morphology and sensorimotor control of
1232 feeding behavior in salamanders: an example of the role of internal dynamics in
1233 evolution. *Acta Biotheoretica* 34:175–192.

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1234 Schwenk K, Wake DB. 1993. Prey processing in *Leurognathus marmoratus* and the
1235 evolution of form and function in desmognathine salamanders (Plethodontidae). Biol
1236 J Linn Soc 49:141–162.

1237 Sessions SK, Stöck M, Vieites DR, Quarles R, Min MS, Wake DB. 2008. Cytogenetic
1238 analysis of the Asian plethodontid salamander, *Karsenia koreana*: Evidence for
1239 karyotypic conservation, chromosome repatterning, and genome size evolution.
1240 Chromosome Res 16:563–574.

1241 Staub NL. 1993. Intraespecific agonistic behavior of the salamander *Aneides flavipunctatus*
1242 (Amphibia: Plethodontidae) with comparisons to other species. Herpetologica
1243 49:271–282.

1244 van der Meijden A, Chiari Y, Mucedda M, Carranza S, Corti C, Veith M. 2009.
1245 Phylogenetic relationships of Sardinian cave salamanders, genus *Hydromantes*, based
1246 on mitochondrial and nuclear DNA sequence data. Mol Phylogen Evol 51:399–404.

1247 Vieites DR, Min MS, Wake DB. 2007. Rapid diversification and dispersal during periods
1248 of global warming by plethodontid salamanders. Proc Natl Acad Sci USA
1249 104:19903–19907.

1250 Vieites DR, Zhang P, Wake DB. 2009. Salamanders (Caudata). In: Hedges SB, Kumar S,
1251 editors. The Timetree of Life. Oxford: Oxford University Press. p 365–368.

1252 Wake DB. 1963. Comparative osteology of the plethodontid salamander genus *Aneides*. J
1253 Morphol 113:77–118.

1254 Wake DB. 1966. Comparative osteology and evolution of the lungless salamanders, family
1255 Plethodontidae. Mem South Cal Acad Sci 4:1–111.

- 1256 Wake DB. 1987. Adaptive radiation of salamanders in Middle American cloud forests. Ann
1257 Missouri Bot Gard 74:242–264.
- 1258 Wake DB. 1991. Homoplasy: the result of natural selection, or evidence of design
1259 limitations? Am Nat 138:543–567.
- 1260 Wake DB, Deban SM. 2000. Terrestrial feeding in salamanders. In: Schwenk K, editor.
1261 Feeding: Form, Function, Phylogeny. San Diego: Academic Press. p 95–116.
- 1262 Wake DB, Hanken J. 1996. Direct development in the lungless salamanders: what are the
1263 consequences for developmental biology, evolution and phylogenesis? Int J Dev Biol
1264 40:859–869.
- 1265 Wake DB, Larson A. 1987. Multidimensional analysis of an evolving lineage. Science
1266 238:42–48.
- 1267 Wake DB, Roth G. 1989. The linkage between ontogeny and phylogeny in the evolution of
1268 complex systems. In: Wake DB, Roth G, editors. Complex Organismal Functions:
1269 Integration and Evolution in Vertebrates. New York: Jon Wiley & Sons Ltd. p 361–
1270 377.
- 1271 Wake DB, Roth G, Wake MH. 1983. On the problem of stasis in organismal evolution. J
1272 Theor Biol 101:211–224.
- 1273 Wake DB, Salvador A, Alonso-Zarazaga MA. 2005. Taxonomy of the plethodontid
1274 salamander genus *Hydromantes* (Caudata: Plethodontidae). Amphibia-Reptilia
1275 26:543–548.
- 1276 Wake TA, Wake DB, Wake MH. 1983. The ossification sequence of *Aneides lugubris*, with
1277 comments on heterochrony. J Herpetol 17:10–22.

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1278 Wells KD. 2007. The Ecology & Behavior of Amphibians. Chicago: University of Chicago
1279 Press. xi + 1148 p.

1280 Wiedersheim R. 1875. *Salamandrina perspicillata* und *Geotriton fuscus*. Versuch einer
1281 vergleichenden Anatomie der Salamandrinen. Ann Musc Civ Stor Nat Genova 7:1-
1282 207, 17pls.

1283 Wiens JJ, Bonett RM, Chippindale PT. 2005. Ontogeny discombulates phylogeny:
1284 paedomorphosis and higher-level salamander relationships. Syst Biol 564:91–105.

1285 Wilder I, Dunn ER. 1920. The correlation of lunglessness in salamanders with a mountain
1286 brook habitat. Copeia 1920:63–68.

1287 Wiltenmuth EB. 1996. Agonistic and sensory behaviour of the salamander *Ensatina*
1288 *eschscholtzii* during asymmetrical contests. Anim Behav 52:841–850.

1289 Wiltenmuth EB, Nishikawa KC. 1998. Geographical variation in agonistic behaviour in a
1290 ring species of salamander, *Ensatina eschscholtzii*. Anim Behav 55:1595–1606.

1291 Zhang P, Wake DB. 2009. Higher-level salamander relationships and divergence dates
1292 inferred from complete mitochondrial genomes. Mol Phylogen Evol 53: 492–508.

Legends:

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 hemydactyliine species are distributed in the New World from southern Canada to Bolivia.

Fig. 2. General overview of the skull of *Karsenia koreana* and *Hydromantes platycephalus* 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).

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 and 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 and G: lateral and medial views of the maxilla in *K. koreana*, respectively; F and H: lateral and medial views of the maxilla in *H. platycephalus*, respectively; appm, alary process of the premaxilla; m, maxillae; 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; ppm, *pars palatina* of the premaxilla; ppm, *pars palatina* of the maxilla; pm,

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1315 premaxilla; sm, septomaxilla; v, vomer (scale bars: **A, C, E, G** = 1 mm; **B, D, F, H** = 2
1316 mm).

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1318 Fig. 4. Dorsolateral views of the skulls of *K. koreana* (**A**) and *H. platycephalus* (**B**),
1319 showing the articulations between, nasals, prefrontals, frontals, and parietals. An enlarged
1320 dorsal view of the nasal and prefrontal is provided. Prefrontals have been consistently
1321 reported absent in *Hydromantes*. In the specimen scanned, however, a differentiated
1322 element is found between frontals and nasal (pf?) (**B**). appm, alary process of the
1323 premaxilla; f, frontal; fpf, frontoparietal fontanelle; m, maxilla; n, nasal; nld, nasolacrimal
1324 duct; os, orbitosphenoid; p, parietal; pdm, *pars dentalis* of the maxilla; pdpm, *pars dentalis*
1325 of the premaxilla; pf, prefrontal; ps, parasphenoid; sm, septomaxilla (scale bars: **A** = 1 mm;
1326 **B** = 2 mm).

1327
1328 Fig. 5. Vomers in *K. koreana* (left panels, **A, C, E**) and *H. platycephalus* (right panels, **B,**
1329 **D, F**). **A** and **B**: frontal sections, showing the dorsal surfaces of the vomers and their
1330 articulation with premaxillae, maxillae, parasphenoid and orbitosphenoids. **C** and **D**: dorsal
1331 (d) and ventral (v) view of the isolated right vomers. **E** and **F**: ventral view of the skulls,
1332 showing the ventral disposition of the vomers, and the two posterior vomerine dental
1333 patches, attached to the parasphenoid. appm, alary process of the premaxilla; c, choana; dr,
1334 dentigerous ridge; m, maxillae; n, nasal; nld, nasolacrimal duct; pdm, *pars dentalis* of the
1335 maxilla; pdpm, *pars dentalis* of the premaxilla; pf, prefrontal; v, vomer; vb, anterior
1336 vomerine body; vdp, vomerine dental patch (scale bars: **A** = 1 mm; **B** = 2 mm; **C** = 0.5 mm;
1337 **D** = 1 mm; **E** = 1 mm; **F** = 2 mm).

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1339 Fig. 6. Braincase in *K. koreana* (**A, B**) and *H. platycephalus* (**C, D**). **A** and **C**: lateral view
 1340 of the skulls, showing the orbitosphenoids and their contact and articulation with vomers,
 1341 parasphenoid, frontals, and parietals. **B** and **D**: transverse section of the skulls, about level
 1342 of the anterior end of the orbitosphenoids. The braincases in the two species are very
 1343 different in shape. Note also the absence of contact between vomers and orbitosphenoids in
 1344 *H. platycephalus*. f, frontal; foc, *foramen oculomotorium*; fom, *foramen orbitonasale*
 1345 *mediale*; of, *optic fenestra*; os, orbitosphenoid; p, parietal; ps, parasphenoid; v, vomer
 1346 (scale bars: **A, B** = 1 mm; **C, D** = 2 mm).

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1348 Fig. 7. Occipito-otic complex in *K. koreana* (**A, C**) and *H. platycephalus* (**B, D**). **A** and **B**:
 1349 Dorsal view of the posterior part of the skull. Note the different articulations between
 1350 exoccipitals and atlas vertebrae, which correspond to differences in the relative size of the
 1351 tuberculum interglenoideum of the atlas in the two species. **C** and **D**: posterolateral view of
 1352 the skull. Note the different shape of the stylus of the *pars media plectri*. cr, crest; ex,
 1353 exoccipital; fo, *fenestra ovalis*; oc, otic capsule; occ, occipital condyle; p, parietal; pmp,
 1354 *pars media plectri*; st, stylus of the *pars media plectri*; ti, tuberculum interglenoideum (scale
 1355 bars: **A, C** = 1 mm; **B, D** = 2 mm).

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1357 Fig. 8. Anterior (a), lateral (l), posterior (p), and medial (m) views of the right squamosal
 1358 and the quadrate, the only ossified element in the suspensorium, in *K. koreana* (**A**) and *H.*
 1359 *platycephalus* (**B**). The medial view (m) corresponds to a sagittal section at the level of the
 1360 articulation between the otic capsule and the squamosal. Note the different articulation

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1361 between quadrate and articular in the two species. art, articular; den, dentary; prart,
1362 prearticular; oc, otic capsules; qu, quadrate; sq, squamosal; st, stylus of the *pars media*
1363 *plectri* (scale bars: **A** = 0.5 mm; **B** = 1 mm).
1364
1365 Fig. 9. Lateral (l), medial (m), and dorsal (d) views of the right lower jaw in *K. koreana* (**A**)
1366 and *H. platycephalus* (**B**). The posterior end of the mandible is not so robust in *H.*
1367 *platycephalus*. The coronoid process of the prearticular is less developed and the articular
1368 does not ossify. art, articular; cor, coronoid process of the prearticular; den, dentary; prart,
1369 prearticular; rid, ridge; sym, mandibular symphysis (scale bars: **A** = 1 mm; **B** = 2 mm).
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1371 Fig. 10. Ventral views of the hyobranchial apparatus in *Karsenia koreana* (**A**),
1372 *Hydromantes platycephalus* (**B**), *Desmognathus monticola* (**C**), *Plethodon neomexicanus*
1373 (**D**), and *Ensatina eschscholtzii* (**E**). Hyobranchial parts are largely cartilaginous, with the
1374 exception of the central portion of the basibranchial in *K. koreana* and the urohyal in all
1375 species except *H. platycephalus*, which lacks it. Ossified segments are shown in dark grey.
1376 **B, C, D, and E** have been modified from Deban and Wake (2000). bb, basibranchial; cbI,
1377 ceratobranchial I; cbII, ceratobranchial II; ch, ceratohyal; eb, epibranchial; r, radial; uh,
1378 urohyal (scale bars = 5 mm).
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