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Persistent Plethodontid Themes: Species, Phylogenies, and Biogeography

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ABSTRACT: From the time of Cope's first analysis of plethodontid systematics through Dunn's era of consolidation and integration to the present time, there have been persistent themes. Criteria for recognition of species continue to evolve as methods of data acquisition have progressed and new methods of analysis of data have been developed. Species descriptions continue, often based on new discoveries, even in well-studied areas, but taxon subdivision is more common. What do we, as a community, want our species to be? Understanding of phylogenetic relationships has advanced with voluminous new molecular data; for example, we know that desmognathines are not the sister-group of all other plethodontids and that the two great clades, plethodonines and hemidactyliines, are well supported. As we have added more data, however, resolution within Hemidactyliinae has dramatically improved while, ironically, resolution within Plethodontinae has decayed. Historical biogeographical thinking has changed dramatically; "Out of Appalachia!" has been replaced with "Into Appalachia?" Biogeography of tropical salamanders has advanced, and we recognize Mesoamerica as a major evolutionary arena. The dominant biogeographic mystery is, why are there so few Old World plethodontids, given that they have been there for so long and occur at opposite ends of Eurasia? Abundant challenges persist for students of plethodontids, offering opportunities for discovery and further research.

Key words: Biogeography; Phylogeny; Plethodontidae; Salamanders; Species criteria; Species recognition

MY STUDIES of salamanders of the family Plethodontidae began in 1958, my first year of graduate work in the lab of Jay Savage at the University of Southern California in Los Angeles. Arden H. Brame Jr., a senior graduate student in the lab, was dedicated to salamanders and his enthusiasm was contagious. I enrolled in a course in zoogeography, where I learned of the strange distribution of plethodontid salamanders (then known as an exclusively New World group, with the exception of a couple of species in the Mediterranean region). Plethodontids became the subject of my term paper and set the direction of my future research. I was warned not to specialize too narrowly, and my first couple of papers dealt with frogs and lizards, but plethodontids became the star of the show for me.

This was the dawn of the model-organism approach in biology (think *Drosophila*, Phage, etc.), and while I recognized that a plethodontid salamander would never be a model organism, this group gradually evolved in my mind into a model taxon (Griesemer 2013), and I have stuck with them, with some discursions, for nearly 60 years.

Throughout my career, certain persistent questions have resisted resolution. Here I focus on three of these: species, phylogenetics, and biogeography. I review how these themes have fared during my own career and conclude by laying out challenges for future research.

HOW MANY SPECIES OF PLETHODONTID SALAMANDERS SHOULD BE RECOGNIZED?

I am a Darwinian nominalist (Wake 2009; Kuchta and Wake 2016), in the framework of the unified species concept (de Queiroz 2011). Species are separately evolving lineage segments, in the spirit of de Queiroz, who advocated recognizing species at the point of the initial separation of descendant lineages. Because there are no contingent properties of species, in this view it is up to the taxonomic practitioners and the working biologists themselves to

determine when these lineages have separated. Before making a taxonomic decision, I think one should make a judgment call that the species are permanent and will not lose their identity as a result of subsequent gene flow. The ultimate evidence of permanence is sympatry without hybridization. There is also value in determining how they are identifiable and to what degree they have evolved some significant biological feature or set of features (i.e., differences in behavior, anatomy, ecology, genetics) in instances in which the putative descendent sister-species are first identified solely by a molecular feature. None of this is easy, leading me to ask—what do we want species to be?

The monographs of Cope (1889) and Dunn (1926, the first comprehensive account of Plethodontidae) serve as my points of departure in my quest to track both growth of knowledge and changing views of what species are, including what different scientists want them to be. Cope, although he dealt only with the United States and Canada, knew about plethodontids in Europe and that plethodontids ranged from Mexico at least as far south as Peru. Neither Cope nor Dunn knew about Asian plethodontids, but even today there is only one known, and it was a 21st Century discovery.

Dunn (1926) listed 72 species; the total would increase to 89 if one were to count subspecies, many of which were subsequently raised to full species rank. In contrast, AmphibiaWeb (2017) lists 458 species through the end of 2016 (I do not include 15 subspecies). I dropped *Plethodon ainsworthi* and *Pseudoeurycea tillicxtil*, taxa listed with queries by AmphibiaWeb and which I consider invalid, leaving 456 species. Regardless of any quibble over numbers, the question that concerns me is, how and why did this increase in species number occur?

I examined the history of each species-level taxon and scored it as follows: C (inferred from a combination of Cope 1889 and what that well-informed scientist likely would have known at that time based on his earlier publications and the literature of the time), D (either listed as a species or subspecies or as a synonym in Dunn 1926), F (taxa listed by Frost 1985, not known or recognized by Dunn), N (novelties,

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basically new discoveries that are distinct in morphology, typically recognized as unique upon discovery), S (subdivision of taxon known to Dunn and Frost), and M (taxa first recognized by molecular data alone). This is an imperfect exercise but it does provide a general overview.

I credited Cope with knowing 49 of the currently recognized species but sometimes as subspecies or segments of species. Dunn (1926) knew 79 currently recognized taxa. Some he recognized are no longer considered species (e.g., *Ensatina platensis*), others have different names (e.g., his *Desmognathus phoca* is now what he formerly had described as *Desmognathus monticola*), and some have since been raised in rank (e.g., his *Eurycea bislineata cirrigera*) or raised from synonymy (Dunn synonymized *Bolitoglossa palmata*, *Bolitoglossa peruviana*, and *Bolitoglossa sima* with *Bolitoglossa altamazonica*, but all four are valid). In the end I credited 73 species to him.

By the time of Frost's (1985) list there were 220 species-level taxa. All but six of these would qualify as novel, that is, identified by classical, nonmolecular criteria. Not all of Frost's listed taxa are still recognized (e.g., *Bolitoglossa arborescandens* is a synonym of *Bolitoglossa alvaradoi*). Removing species in Dunn, and taking taxonomic changes into account, I count 157 species in Frost.

Since 1985, I count 122 species that were diagnosed by classical criteria (N). I consider 25 of these to have received assists from molecular data and which thus qualify as somewhat cryptic. *Batrachoseps luciae* had first been identified as novel by Brame and Murray (1968) but assigned to *Batrachoseps relictus* before being formally named by Jockusch et al. (2001), who used molecular data to diagnose what had already been detected as something different. The 83 species represented in the S category qualify as cryptic, as do all taxa since 1985 diagnosed fundamentally by molecular characters (M). The 49 species credited to Cope are included in the 73 known to Dunn, another 157 were known to Frost, there have been 122 novelties since Frost, and finally the 22 new species identified by molecular data only result in a total of 456 species recognized by the end of 2016 (classification available on AmphibiaWeb 2017, under "Browse by Family"; this classification will be revised regularly in the light of new understandings). Of these I consider 136 (6 prior to 1985, 25 from N, 83 S, and 22 M) to be cryptic; thus 30% of currently recognized plethodontids are cryptic.

The two subfamilies of Plethodontidae differ greatly in species numbers. The Hemidactyliinae contains 362 species (79% of all plethodontids), dominated by tropical species, especially those classified as *Bolitoglossa* (131). However, it also includes such large extratropical taxa as *Eurycea* with 28 species in 2016. The Plethodontinae includes 96 species dominated by *Plethodon* (55).

Species categorized as N are mainly tropical, and tropical species dominate the additions since 1985. The morphologically cryptic species are primarily in the Plethodontinae. How can this be explained? I think a large factor is "taxonomic inflation," the accumulation of largely cryptic taxa arising from the introduction of molecular data: electrophoretic analysis of proteins starting in the 1970s moving on to DNA sequencing in the 1990s (see detailed evaluation in Vences and Wake 2007). A complementary, extended discussion of cryptic species and taxonomic

inflation with examples from plethodontids is given by Bernardo (2011).

Protein data were used to test species-recognition hypotheses, such as the demonstration by Feder et al. (1978) that *Plethodon gordonii* was not genetically distinct from the sympatric *Plethodon dunni* and it was reduced to its synonym, as well as being used to identify largely cryptic species which were then raised from synonymy (*Plethodon serratus* by Highton and Webster 1976, *Desmognathus imitator* by Tilley et al. 1978, and *Batrachoseps nigriventris* by Yanev 1980) or named as new taxa (*Plethodon websteri* by Highton 1979). It took a few years, but soon many new taxa were named, based largely on electrophoretic studies of proteins. Some of these were not new but raised from synonymy (e.g., *Plethodon grobmani*, *Plethodon cylandraeus*), but others were newly named (*Plethodon aureolus*, *Plethodon mississippi*; Highton 1989). Well-known taxa such as *Plethodon cinereus*, *Plethodon glutinosus*, and *Plethodon jordani* were subdivided (summarized by Highton and Peabody 2000). Protein studies also contributed to the descriptions of species in other genera including *Desmognathus*, *Eurycea*, *Nototriton*, *Pseudoeurycea*, *Oedipina*, and *Thorius* among others.

During the 1990s and into this century, DNA data began to have an impact on species with the application of mitochondrial (mt) genes, especially cytochrome b. Early examples are taxa of *Aneides* (Wake and Jackman 1998), *Batrachoseps* (Jockusch et al. 1998), and *Oedipina-Nototriton-Cryptotriton* (García-París and Wake 2000). More recently it has become commonplace for systematic studies to contain information on both mtDNA and nuclear (n) DNA as well as proteins (e.g., Kuchta et al. 2016). To date nuclear DNA has not been of much use at the level of species delimitation. When protein and mtDNA datasets are congruent, taxonomic revision and even new species descriptions may (e.g., *Batrachoseps bramei* and related taxa; Jockusch et al. 2012) or may not (e.g., *Batrachoseps major* complex; Martínez-Solano et al. 2012) follow. Cases of incongruence are common, the best-known among plethodontids likely being that of *Ensatina* (Pereira and Wake 2009; Kuchta and Wake 2016).

Without doubt there are thorny problems in delimiting species and various researchers apply criteria differently. For example, Highton (2014) reanalyzed the data from a fragment of the cytochrome b gene published by Martínez-Solano et al. (2007) and, ignoring their nuclear data, concluded that *Batrachoseps attenuatus* was a multispecies complex of "at least 39 species" (Highton 2014: 140). This is certainly the extreme in taxonomic splitting to date, and I know of no one else who is willing to delimit species on the basis of small amounts of mtDNA alone. Perhaps we can agree that species are hypotheses and the ultimate test is sympatry with a sister taxon. Camp and Wooten (2016) usefully examined cases of cryptic diversity including cases of allopatry, parapatry, and even sympatry, noting many instances in which cryptic segments of species taxa have been identified but not yet named.

Members of the herpetological community are not in agreement regarding species delimitation and identification. The issues discussed here are certainly not limited to plethodontids! A recent application of a phylogenetic species criterion to birds came to the surprising conclusion from a

carefully studied subset of 9159 then-recognized birds that 18,043 species could be recognized (Barrowclough et al. 2016). Species delimitation methods continue to be published regularly, but the situation has not changed much (except for many more methods) since I briefly reviewed the topic a few years ago (Wake 2009). My current view is neo-neodarwinian, a genetically, ecologically, and morphologically informed nominalism that accepts that, by selecting data to be analyzed and a species delimitation method, one can pretty much get the result one wants. Whatever one might think of taxonomic inflation, one beneficial outcome, insofar as the named taxa, is also historical biological entities, and conservation efforts can be more effective when all of the historical units within a larger taxon are recognized (Bernardo 2011).

I envision two broad categories of diversification patterns: Adaptive Radiation and Nonadaptive Radiation (Wake 2006). This is not a true dichotomy but more a categorization of convenience. I use adaptive radiation in a very general sense, unrestricted by time or taxonomic depth. Examples include the *Ensatina* complex, what we have long recognized as the “desmognathines,” and the genus *Thorius* and other tropical salamanders (bolitoglossines; Rovito et al. 2015). Adaptive radiations of this sort are active and expanding. Nonadaptive radiations, in contrast, are a mixed lot. In general they are derived from taxa that once spread out but that are now falling apart in time and across space, as they differentiate largely by passive processes (drift, vicariance; Jockusch and Wake 2002). Some plethodontid examples include *Batrachoseps*, the eastern small *Plethodon*, and the *Hydromantes* of Sardinia.

HOW ROBUST IS OUR CURRENT PHYLOGENETIC INFERENCE FOR PLETHODONTIDS?

Dunn (1926) discussed plethodontid relationships at some length but did not publish a tree. Whereas Cope (1889) had recognized Thoriidae (*Thorius* only) and Desmognathidae (*Desmognathus* only), Dunn included both in his Plethodontidae. In his view *Desmognathus* and *Leuognathus* formed a natural group distinct from the remaining genera. He thought *Plethodon*, *Ensatina*, *Aneides*, and *Hemidactylum* were close relatives and that *Batrachoseps* was “an elongate, degenerative form derived from *Plethodon*” (Dunn 1926: 26). *Stereochilus*, *Typhlomolge*, and *Typhlotriton* were thought to stand between the *Plethodon* and *Eurycea* (*Eurycea*, *Gyrinophilus*, *Pseudotriton*) groups. Finally, the tropical species (all in his *Oedipus*) and *Hydromantes* were seen as a natural assemblage allied with the *Eurycea* group.

Wake (1966) presented arguments illustrated with informal trees. I accepted a fundamental dichotomy of Desmognathinae and everything else (tribes Hemidactyliini, Plethodontini, Bolitoglossini). The potential paraphyly of the Hemidactyliini was implicitly recognized in its tree, which showed *Hemidactylum* dangling, unattached to the trunk. Another innovation over the Dunn hypothesis was the association of *Batrachoseps* with the tropical species and *Hydromantes* (Bolitoglossini of the time).

The above ideas, based on morphology with a bit of development, ecology, behavior, and biogeography thrown in, stood a kind of test of time. For example, a study by Lombard and Wake (1986), based mainly on hyobranchial

characters and using cladistics methodology, came basically to the same conclusions as Wake (1966) except that plethodontines and bolitoglossines were found to be sister taxa, resolving the earlier paraphyly.

The first major change in phylogenetic perspective came with the publications of Chippindale et al. (2004) and Mueller et al. (2004). Both used extensive DNA-sequence databases, the first relying on two mitochondrial genes (mtDNA) and the nuclear gene *Rag1* and some nonmolecular characters and the second using complete mitochondrial genomes. Leaving aside details, both found that *Desmognathus* and *Phaeognathus*, while sisters, were not sister to everything else but were within a Plethodon clade. Both found two main clades (the current subfamilies Plethodontidae and Hemidactyliidae; Wake 2012) but differed in topological details, notably the placement of *Hemidactylum*. A significant finding by both groups was that larval development had re-evolved within *Desmognathus*, a confirmation of the earlier work by Titus and Larson (1996). A second major finding (Mueller et al. 2004 only) was that *Hydromantes* is not allied with *Batrachoseps* and the tropical salamanders (the Bolitoglossini of Wake 1966) but instead belongs in the Plethodontinae. Together these two findings add substantially to the degree of morphological and life-history trait homoplasy in plethodontid phylogenesis. An important finding by Chippindale et al. (2004) was that Amphiumidae and Rhyacotritonidae were successive out-group taxa for plethodontids, a relationship seen in some earlier work and in all subsequent analyses. Chippindale et al. (2004) proposed taxonomic changes but, because of the uncertainty of placement of *Hemidactylum* and the absence of *Hydromantes* from their analysis, that effort was flawed from the start. Nevertheless, their proposal to recognize a subfamily Spelerpinae for Wake’s (1966) Hemidactyliinae, minus *Hemidactylum*, was used in a number of subsequent publications.

Vieites et al. (2007), using three nuclear genes, showed that *Karsenia*, the only known Asian plethodontid, is deeply nested within the plethodontine clade but has no clear sister-taxon, echoing findings by Min et al. (2005). Nevertheless, the most likely topology, albeit weakly supported, found *Karsenia* as sister to *Hydromantes*.

By combining data from three nuclear genes and complete mitochondrial genomes, Vieites et al. (2011) found a well-supported hemidactyline-plethodontine dichotomy with the Hemidactyliinae as a fully supported, fully resolved clade. Surprisingly to me, the Plethodontinae lost resolution relative to Vieites et al. (2007). A statistically unsupported topology found *Karsenia* sister to *Hydromantes*.

Pyron and Wiens (2011) performed the most species-rich analysis using available molecular data (they lacked new molecular data from Vieites et al. 2011). They found the now-expected plethodontine-hemidactyline split, with *Hemidactylum* sister to all other hemidactyline and *Plethodon* sister to all other plethodontines. *Karsenia* was in a polytomy with *Desmognathus-Phaeognathus*, *Aneides*, and *Ensatina*. *Hydromantes* was the sister-taxon of the polytomy. These authors recognized the four subfamilies of Chippindale et al. (2004).

The most recent effort (Shen et al. 2016) used 48,482 base pairs obtained from 50 identified and independent nuclear genes. The sampled taxa included 25 species selected from

clades spread across the plethodontid tree. From the Plethodontinae there were four species of *Plethodon* (including two from each subgenus), three each of *Desmognathus* and *Aneides*, two *Hydromantes* (European and American species), and one each of *Karsenia* and *Ensatina*. From the Hemidactyliinae came two species each of *Batrachoseps* (both subgenera) and *Eurycea*, a single species each of *Hemidactylum*, *Pseudotriton*, and *Stereochilus*, and a single species each of three genera of tropical salamanders (*Bolitoglossa*, *Pseudoeurycea*, and *Thorius*). Accordingly, all major taxonomic subdivisions and biogeographic regions were represented.

A maximum-likelihood (ML) analysis (Fig. 2a of Shen et al. 2016) recovered Plethodontinae and Hemidactyliini (100 bootstrap support, bs) and a fully resolved Hemidactyliini with Spelerpini (*Eurycea*, *Pseudotriton*, *Stereochilus*) that is sister to a combined clade of Hemidactyliini-(*Hemidactylum*)-*Batrachoseps* (*Batrachoseps*)-*Bolitoglossini* (*Bolitoglossa*, *Pseudoeurycea*, *Thorius*), with *Batrachoseps* sister to the *Bolitoglossini*.

The Plethodontinae lacks resolution near the base. A well-supported (100 bs) *Plethodon* (with the two subgenera also supported at 100 bs) is sister to the remaining taxa, but there is little support for these as a clade. The tree has *Karsenia* sister to all but *Plethodon* but with low support (42 bs). A subclade of *Hydromantes* + *Ensatina* (52 bs) is sister to an *Aneides* + *Desmognathus* subclade (90 bs) but also with poor support (58 bs). The nodes for the backbone of the tree are very short.

A Bayesian inference (BI) tree (Fig. 2b of Shen et al. 2016) has identical structure for the Hemidactyliini, but again has an unresolved Plethodontinae. *Plethodon* is sister to the remaining taxa, which topologically are a clade but with no support (0.61 posterior probability, pp). This topology is slightly different from the ML tree: *Karsenia* is sister to a fully supported subclade of *Aneides* + *Desmognathus* (1 pp) but this topology has low support (0.59 pp). *Hydromantes* and *Ensatina* are well-supported (0.99 pp) as sister taxa.

When Shen et al. (Fig S3, 2016) excluded *Ensatina* from the analysis, the BI tree was fully resolved and supported (1 pp) with *Hydromantes*, *Karsenia*, and *Plethodon* as successive sister taxa to *Aneides* + *Desmognathus*. However, the ML tree, with the same topology, had less support (0.81, 0.78, 0.57 bs for the sequence). These authors also did an analysis excluding *Karsenia* (Fig. S4 of Shen et al. 2016). Now *Ensatina* was sister to *Hydromantes* but with little support (50 bs, 0.93 pp).

In an attempt to resolve the Plethodontinae, Shen et al. (Fig. S5, 2016, reproduced here as Fig. 1) combined complete mitochondrial genomes with the nuclear data. Loss of resolution resulted. While the Hemidactyliini remains fully resolved and well supported (all nodes 100 bs) in the ML analysis, the Plethodontinae is less supported than in the analyses of nuclear data alone. *Aneides* and *Desmognathus* are sister (83 bs), and *Hydromantes* and *Ensatina* are sister (46 bs), and these two poorly supported clades are sister (76 bs). *Karsenia* is sister to the above taxa (51 bs) and *Plethodon* is sister to everything.

Ironically, adding more data led to loss of resolution within the plethodontines, whether it is nuclear or mitochondrial data or all data combined. The possibility of a star

phylogeny seems increasingly likely, with a near-simultaneous origin of the generic-level taxa studied.

The basal split of Plethodontinae and Hemidactyliinae, as well as the topology of the latter, are robust topological features. Whereas the genera of the Plethodontinae represented by multiple samples are individually robust as clades, the topology as a whole is not. *Aneides* and *Desmognathus* (and by inference *Phaeognathus*) form a clade, albeit with variable support, in all treatments and using different data partitions. *Plethodon* is a clade that is sister to the remaining taxa. With respect to the findings of Vieites et al. (2007) regarding *Karsenia*, Shen et al. (2016) failed to find support for a sister-taxon relationship with *Hydromantes*; they statistically rejected the possibility of a sister-taxon relationship to *Hydromantes* as well as to *Ensatina*, *Aneides*, and *Desmognathus* but not to *Plethodon* (Table S2 of Shen et al. 2016).

A final phylogenetic issue is the tropical radiation, Tribe *Bolitoglossini* (sensu Wake 2012). Whereas Cope (1869) placed *Thorius* in its own family *Thoriidae*, it only ever included *Thorius*, which Dunn (1926) placed in his *Oedipus* (subsequently found to be a preoccupied name). All relevant molecular analyses (cited above) find *Bolitoglossini* (now restricted to the tropical taxa) to be a clade with *Batrachoseps* as the sister taxon. Only recently have relationships within *Bolitoglossini* been nearly resolved (Rovito et al. 2015). That study sampled 58–63 taxa (all genera and major clades) for three nuclear genes and a portion of the mitochondrial genome. Two large clades were fully supported: a Central American clade (*Bradytriton*, *Cryptotriton*, *Dendrotriton*, *Nototriton*, *Nyctanolis*, *Oedipina*) and a Mexican/widespread clade (*Aquiloerycea*, *Bolitoglossa*, *Chiropterotriton*, *Isthmura*, *Ixalotriton*, *Parvimolge*, *Pseudoeurycea*, *Thorius*). *Thorius* is the sister taxon of other members of its clade. *Bolitoglossini* is nearly fully resolved at the level of generic-level clades, although a few nodes remain weakly supported.

PERSISTENT BIOGEOGRAPHIC PUZZLES

Historical biogeography of plethodontids is where I started, and the questions that first piqued my interest persist. Plethodontids remain fundamentally a North American taxon, with three notable exceptions: *Karsenia* on the Korean Peninsula, populations of *Hydromantes* in West-Mediterranean Europe, and the large tropical American clade. How can these exceptions be explained?

How Old Are Plethodontids and Where Did they Originate?

The latest time trees for Plethodontidae (Shen et al. 2016) find it much younger than hypothesized by Vieites et al. (2007). Instead of a date of about 94 million years ago (mya) for the basal split in Plethodontidae, the new estimate is 62–67 mya, or approximately the Cretaceous–Paleocene border (Shen et al. 2016). The earliest divergence within the Plethodontinae is now 42–49 mya.

How and When Did *Hydromantes* Reach the Mediterranean Region?

The European species of *Hydromantes* were long recognized as plethodontids (e.g., Gray 1850, who placed them in the genus *Geotriton*). Herpetologists were surprised

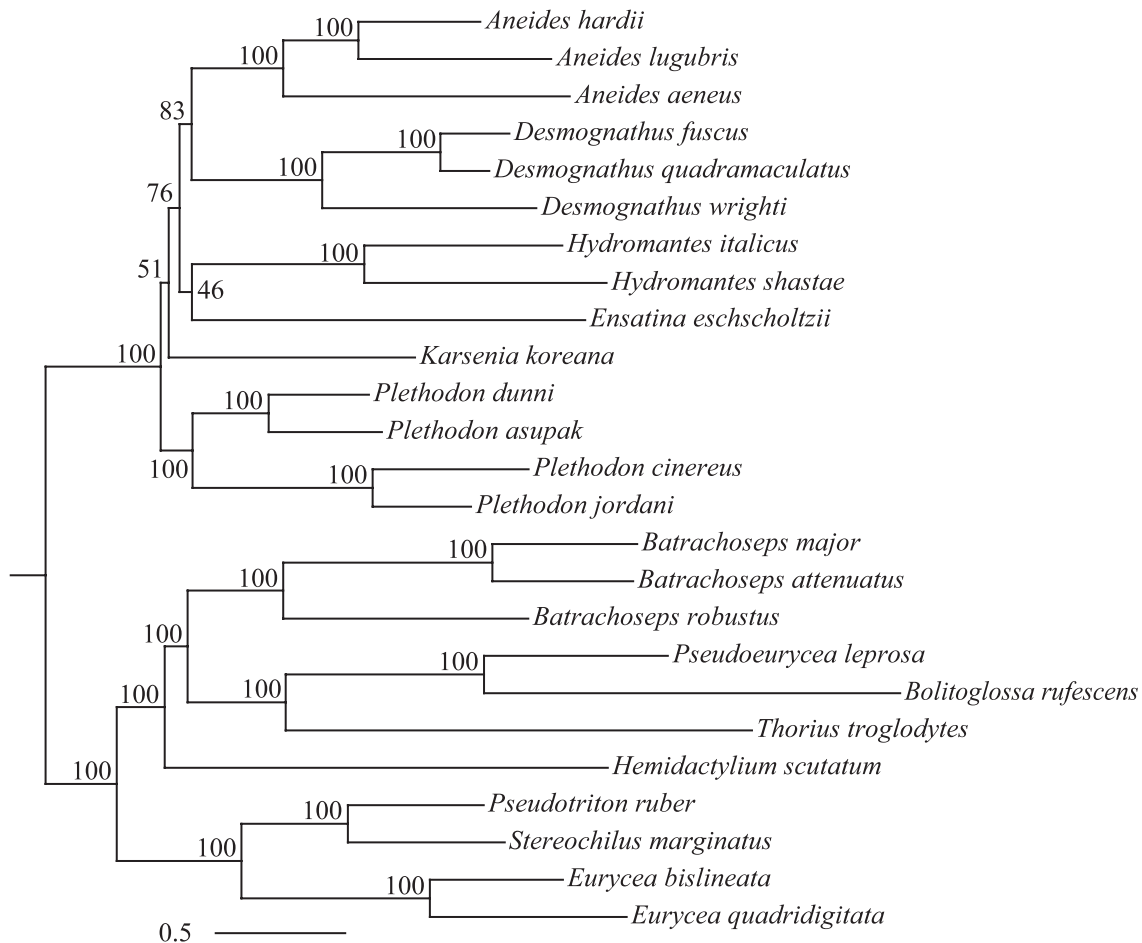


FIG. 1.—Maximum likelihood phylogram inferred from analyses of 50 nuclear genes + complete mitochondrial genome. Support values beside each node correspond to bootstrap percentages. For details, see Shen et al. (2016); reprinted with permission.

by Camp's (1916) discovery of a close relative in the Sierra Nevada of California. Since the time of Dunn (1923, 1926), a small clade with Californian and European species, clearly plethodontids, has been universally recognized.

I have argued (Wake 2013) that ancestral *Hydromantes* arrived in present-day Europe from the New World by foot by crossing from Western North America to Eastern Eurasia. The timing of their arrival has long been in contention, but until recently there only have been guesses. No serious worker has suggested human introduction. But until the discovery of fossils assigned to *Hydromantes* from Slovakia, dated at 13.75 (± 2.25) mya (Venczel and Sanchíz 2005), there was no direct evidence that the clade was once more widespread as suggested by Dunn (1926).

The discovery of the first Asian plethodontid, *Karsenia koreana*, on the Korean Peninsula (Min et al. 2005) might have illuminated the past history of plethodontids, and Vieites et al. (2007) developed hypotheses concerning the date and pattern of plethodontid excursions into Eurasia. They considered *Karsenia* and *Hydromantes* to be sister-taxa, whose clade diverged from other plethodontines approximately 74–77 mya. The two genera were thought to have split about 67–69 mya. They postulated a joint clade entering Eurasia at a time when the planet was warm and the distance across the top of the globe between the landmasses was relatively short. Subsequent differentiation, divergence,

migration, and extinction isolated ancestors of *Karsenia* in eastern Asia and *Hydromantes* in western Europe. The morphologically apomorphic American *Hydromantes* clade was postulated to have returned to its ancestral roots in western North America. The split within *Hydromantes* was estimated at 41 mya.

The analyses of recently generated DNA-sequences necessitate rethinking these hypotheses. The *Hydromantes-Karsenia* sister-taxon relationship hypothesized earlier has been rejected, but the weak resolution of the Plethodontinae remains a challenge. Ancestors of the two genera still might have entered Eurasia almost simultaneously and ended up at opposite ends of the megacontinent. Timing of events has also changed as a result of the new analyses. The divergence of the two subclades of *Hydromantes* is about 23–25 mya (Shen et al. 2016). This latter date is surprisingly close to the estimate of 28 mya derived from analysis of immunological data in the very first molecular study of *Hydromantes* (Wake et al. 1978).

How Did *Karsenia* End Up in Korea?

The discovery of *Karsenia* was a great surprise to me; I consider it to be the most significant zoogeographical discovery of this century. When I first saw living specimens, I was struck with how similar specimens are morphologically to *Plethodon* (*Hightonia*; Fig. 2), and I thought about



FIG. 2.—Adult female paratype (MVZ 246033) of *Karsenia koreana*, photographed by R.M. Brown in October 2004 and published with his permission.

connections that have existed between eastern Eurasia and western North America (Beringia), long considered to be the dispersal route of plethodontids to Europe. This discovery only heightened my interest in that route. We know that Earth was profoundly affected by events at the end of the Cretaceous, and when the rapid diversification of the present-day Plethodontinae took place in early Tertiary times, an extended period of global warming likely had left ancestral forms far to the North where distances to Eurasia, especially eastern Eurasia, would be relatively much shorter than potential dispersal routes in the late Pleistocene and Holocene. I think it was at this time that ancestors of both *Karsenia* and *Hydromantes* reached the Eurasian continent. I can only hypothesize that expansion followed by massive and probably long-continued extinctions left the current Eurasian species in fortunately favorable habitats, basically as relics of past distributions.

Into or Out of Appalachia?

What has been termed the “Out of Appalachia” hypothesis (Mueller et al. 2004) originated with Wilder and Dunn (1920) and was further elaborated by Dunn (1926, 1928). There are two lines of evidence that plethodontids arose in present-day Appalachia: (1) Evolution of lunglessness is thought to be related to well-aerated mountain brooks, and the Appalachians are ancient mountains that have long had such habitats; and (2) *Desmognathus* was thought to be a distinct clade, sister to all other plethodontids. Furthermore, *Desmognathus* is mainly associated with the Appalachian region. The long-standing hypothesis of Appalachian origins has slowly unraveled. Although *Phaeognathus*, a fully terrestrial sister-taxon of *Desmognathus*, was known at the time of Wake (1966), only more recently have we known that it develops directly with no larval stage. Furthermore, early mtDNA sequence analyses (Titus and Larson 1996) found that direct-developing species of *Desmognathus* (most species have an aquatic larval stage) were sequential sister-taxa to nested *Desmognathus* with larval stages, indicating that larvae might have re-evolved from ancestors that developed directly. This is a serious problem for the Beachy-Bruce (1992) hypothesis that lunglessness is a rheotropic adaptation to larval life (an

extension of the older idea: lungs would buoy the larvae into flowing water, causing them to be swept away). *Desmognathus* is deeply nested, not sister to all remaining plethodontids, and Bonett et al. (2014) argued that direct development may well have been ancestral for Plethodontidae. Finally, based on arguments by Bonett et al. (2013) and Shen et al. (2016), plethodontids might have arisen in northwestern North America, not Appalachia, or it is at least as probable as Appalachia. Among the successive sister-taxa of plethodontids, amphiumids were in the Pacific Northwest at the right time (Bonett et al. 2013), and there is no evidence that rhyacotritonids have ever been any place else. Moreover, the earliest plethodontid fossils are from a western Montana site (Tihen and Wake 1981) now known to be Oligocene, 23–29.5 mya.

I have little to add to related, persistent questions: (1) What factors led to the evolution of lunglessness in plethodontids; and (2) what were the circumstances leading to the re-evolution of larvae in plethodontids? I have always found the idea that lungs were lost as rheotropic specialization in brooks to be attractive, but I can also accept that specialization for tongue projection might have led to lung loss in terrestrial settings (debate succinctly summarized by Wells 2007: 69–70). What seems likeliest to me at present is that lung loss evolved as ancestors of plethodontids became increasingly terrestrial and began using significant tongue protrusion and finally projection (because of functional conflicts on the hyoid apparatus for pumping air to inflate lungs and serving as biomechanical tongue projectors). Species in other families that have relatively extreme (for their clades) tongue specialization are *Chioglossa* and *Salamandrina* (Salamandridae) and *Onychodactylus* (Hynobiidae), all lacking lungs and associated with brooks and streams. Direct development has clear adaptive value for terrestrial salamanders, freeing them from reliance on standing or flowing water. *Desmognathines* likely re-evolved larvae relatively late (in comparison to spelerpines, for example), and they show gradations in size and shape in relation to stream use, with the most stream-specialized being the largest and among the most-deeply nested taxa. Camp et al. (2007) argued that the long-continued uplift of the southern Appalachians during the late Cenozoic provided new habitats to be exploited by the contemporaneously radiating *desmognathines*. They downgraded the role of interclade competition, disagreeing with Chippindale et al. (2004) who suggested that competition avoidance with terrestrial salamanders may have been a major factor promoting re-evolution of larvae and occupation of diverse aquatic habitats (see also Bruce 2007).

When and Where Did the Tropical Radiation of Bolitoglossines Begin?

Dunn (1926) argued from the limited geological information then available that salamanders reached South America from Late Miocene to Pliocene times, but this was based in large part on the supposed presence of *Ensatina* in Uruguay and Argentina. Brame and Wake (1963) used botanical and paleobotanical evidence in arguing that plethodontids became tropical and moved south in early Tertiary, probably in western North America. The tropical border was then far north, and salamanders moved southward associated with later cooling trends from the

Oligocene through Miocene, ideas elaborated further by Vieites et al. (2007) and Shen et al. (2016). The latter authors estimated the age of the bolitoglossine clade at 47 (41–53) mya, or mid-Eocene. Rovito et al. (2015) estimated the date of the start of divergence within the clade at about 42 mya.

When Did Bolitoglossines Reach South America?

Whereas Brame and Wake (1963) assumed that salamanders did not reach South America until formation of the Isthmus of Panama, which finally closed the marine strait that separated southern Middle America from South America, Hanken and Wake (1982), using allozyme data, found relatively great differentiation of species of *Bolitoglossa* (*Eladinea*) within South America, about as great as that between species from Panama/Costa Rica compared with those from Colombia. They thought that invasion of the continent had occurred prior to the closure because of the lack of concordance between estimated dates of divergence (10–15 mya) and the estimated age of portal closure (roughly 3–5 mya). Elmer et al. (2013) studied timing of divergence of South American salamanders, mainly using Ecuadorian samples, and revisited the question. Using mtDNA and nuclear DNA, they estimated the entrance of *Bolitoglossa* into South America as having taken place roughly 23.6 (15.9–30.3) mya, or Early Miocene. This implies over-water migration, but recent and still controversial geological studies have found evidence for short-lived land connections on the order of 23–25 mya (Montes et al. 2012); a more recent paper states baldly that the marine barrier did not exist after mid-Miocene, 10–15 mya (Montes et al. 2015). However, these findings are hotly debated (e.g., O’Dea et al. 2016). I find it highly unlikely that plethodontids would have been able to cross marine barriers and I look forward to resolution of this controversy.

Where Were the Plethodontids in the Southeast US during the Pleistocene?

For the largest plethodontid genera of eastern North America, most species are more than 2 million years (Ma) old (e.g., Kozak et al. 2006). Communities of plethodontid salamanders, especially in the southern parts of Appalachia, are notably species-rich. Yet, during the last 2 Ma there has been profound climate change in the region, which has the highest species density of salamanders in the world. What happened? We are almost completely ignorant of what took place. Plants found a refuge in Cuba, among other places, but there is no evidence that salamanders went anywhere (south of the glacial borders). Yet many of them are montane forms. Where did they go? Many species display great phylogeographic genetic diversity, which implies relatively great age. I think it unlikely that such species experienced extreme reduction in population size or that they moved very far.

Did Plethodontids Really Reach the Caribbean Area? Are they Still There?

There is no evidence that plethodontids engage in “island-hopping,” for example, in their movements southward in Middle and South America. Thus, the discovery of *Paleoplethodon hispaniolae* in amber deposits of Hispaniola, tentatively dated at between 15–40 mya (Poinar and Wake 2015), poses several questions. How sure are we that it is a

plethodontid? The fossil appears to be an empty shell—little evidence of any bone. Yet, it seems nearly certain to be a salamander, perhaps even a bolitoglossine and possibly *Bolitoglossa*. It has costal grooves which number in the range of bolitoglossines. The lone specimen is probably a hatchling, 18 mm total length, 9 mm snout–vent length, about the size of hatchlings of the smallest known *Bolitoglossa* (*B. diminuta*). Based on what can be seen of the manus and pes, the anatomy resembles that of a *Bolitoglossa* with webbed digits. It probably was arboreal, as are many bolitoglossines. I envision a hatchling flipping out of its egg and landing in a puddle of sap! How it reached Hispaniola remains a mystery.

Can We Make Taxonomy Better Serve Biogeography?

The cladistic revolution convinced most taxonomists that, at taxonomic levels above species, we should only ever name clades. The current tendency among taxonomists is to split genera when internal clades (even in the absence of parphyly) are uncovered by molecular evidence, whatever their morphology, behavior, ecology, etc. (many examples in squamates including anoline lizards, skinks, and snakes). A case of useful splitting is that of Dunn’s (1926) *Oedipus*. He recognized the diversity (many species and even clades have since been discovered) but stated, “The extremes are quite different but there are many connecting links” (1926: 32). The subsequent subdivision of this genus into many genera has had a positive effect (for the latest classification, see Rovito et al. 2015). However, I argue against going further for bolitoglossines in the absence of new discoveries. *Bolitoglossa*, *Oedipina*, and *Nototriton* all have been divided into subgenera. This taxonomy clusters related, phenotypically similar clades and is useful for biogeographic analyses without confusing taxonomic changes. With respect to *Hydromantes*, using a single genus for this remarkably distinct clade heightens attention to the biogeographic questions it poses while subgenera highlight patterns of endemism (Wake 2013). The same arguments apply to other genera with subgenera: *Plethodon*, *Aneides*, *Batrachoseps*, *Eurycea*. Use of subgenera is a positive way to communicate phylogenetic information without disrupting prevailing nomenclature.

Another category increasingly used in herpetology is a rank between genus and subfamily: tribe. My classification made extensive use of clades (Wake 2012). Tribe Bolitoglossini for the tropical salamanders drove the recognition of Tribe Batrachosepini, the sister-taxon. I also recognized a Tribe Hemidactyliini and a Tribe Spelerpini. Shen et al. (2016) showed that Hemidactyliini is the sister of Bolitoglossini + Batrachosepini and that Spelerpini is sister of the three of them. This taxonomic compromise of four tribes avoids adding more taxonomic layers. Tribe-level taxonomy of the Plethodontinae is less satisfactory. *Plethodon* seems to be sister to all remaining plethodontines, but the oldest available name for this latter group is *Desmognathini*. Because “desmogs” has a clear meaning to plethodontid specialists and herpetologists in general, I felt it more appropriate to use Tribe *Desmognathini* for *Desmognathus* and *Phaeognathus*. This necessitated recognizing a Tribe Aneidini for its likely sister-taxon. Because I thought *Karsenia* and *Hydromantes* were possible sister-taxa, I proposed Tribe Hydromantini for them. This left *Ensatina*, which then got its own

Tribe Ensatinini. Now that the hypothesis that *Karsenia* is the sister taxon of *Hydromantes* has been rejected (Shen et al. 2016), each genus (except *Phaeognathus*) is in its own tribe, a useless classification. I propose that tribes no longer be used within Plethodontinae.

For common parlance, members of the family are plethodontids, members of Hemidactyliinae, hemidactylines, of Bolitoglossini, bolitoglossines, of Spelerpini, spelerspines, and of Plethodontinae, plethodontines. Because *Desmognathus* and *Phaeognathus* lack an exclusive suprageneric category, I suggest the informal “desmognathans” (as used by Bruce 2007).

CONCLUSION: PERSISTENT THEMES

Have We Lost Control of the Species Category?

At the dawn of the age of molecular systematics, salamander biologists were among the first to apply new techniques and generate new data. Today, almost everything that can be sequenced has been sequenced, and now the problem is interpretation (e.g., Camp and Wootton 2016). With the ever-growing number of so-called species delimitation methods, and the general acceptance that species are independently evolving units, taxonomic splitting is rampant. I have done my share but, despite having named more than 100 new species of salamanders, I am often considered a taxonomic conservative (perhaps largely because of my reticence to split *Ensatina* into multiple species). But I have to ask: Where will this end? Has the species category outlived its usefulness? My prime example is Highton's (2014) proposal to name as many as 39 species from what is now *Batrachoseps attenuatus*, which has already been split (starting with Brame and Murray 1968). I asked, what do we want salamander species to be? (Wake 2009). It is naïve and impractical to ask what a species is when it is perfectly apparent that, by choosing what one wants to emphasize, one can find an approach to justify whatever one wants to do.

Will We Ever Resolve Plethodontine Phylogeny?

My guess is that we will not. We may have a true polytomy, with a generalized *Plethodon*-like ancestral form rapidly evolving great phenotypic and ecological diversity, weak-jawed, long-tongued *Hydromantes*, strong-jawed, large-toothed *Aneides*, and stiff-necked, bony-skulled *Desmognathus* all being close relatives.

How Will We Resolve Biogeographic Puzzles?

Find more fossils!—We badly need well-placed (in time, space, and with respect to tree topology), identifiable fossils.

Find more Eurasian Plethodontids!—I doubt the discovery of *Karsenia* was a fluke. Here is an appropriate challenge for ecological modeling approaches and for enterprising young (because they might have to explore what are literally the last wild places on Earth's largest continent) herpetological explorers.

Find Hispaniolan or Cuban Plethodontids!—I think *Paleoplethodon* is a plethodontid and I think that it did occur on Hispaniola. That island, and perhaps to a lesser extent Cuba, has been pretty thoroughly explored by herpetologists who have found tiny, range-restricted geckos, xantusiid lizards, and eleutherodactylid frogs. But tiny arboreal salamanders still might have been overlooked. I need only

point out the recent discovery of a new species of giant anoline lizard (*Anolis landestoyi*) from the canopy of an isolated hispaniolan cloud forest (Mahler et al. 2016), the kind of habitat in which an arboreal *Bolitoglossa* might be found.

We have learned a lot about plethodontid salamanders since the days of Cope and Dunn, but challenges remain and questions persist.

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