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A new genus and species of lungless salamander (family Plethodontidae) from the Appalachian highlands of the south-eastern United States

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Abstract

We describe a striking new species of the lungless salamander family Plethodontidae from the Appalachian foothills of northern Georgia, USA. This miniature species, *c*. 25–26 mm (adult standard length), is so distinctive genetically and morphologically that we erect a new genus, the first new genus of amphibian described from the US in nearly 50 years. It is unique among plethodontids from eastern North America in displaying sexual colour dimorphism. Although certain miniaturized plethodontids exhibit a reduced number (four) of digits on the pes, this species possesses a full complement of five toes. A plethodontid phylogeny derived from mitochondrial and nuclear DNA sequences places it in the tribe Spelerpini as the sister taxon to *Eurycea*. Genetic divergence between the new species and *Eurycea* for the nuclear gene *Rag*-1 (4.7%) is among the higher levels observed between long-established spelerpine genera (2.6–5.3%). This new form appears to be rare and is of immediate conservation concern.

Introduction

Amphibians exhibit higher rates of new-species description than any other tetrapod group (Köhler *et al*., 2005) due, in part, to routine phylogenetic analyses of DNA sequence data, which often reveal substantive evolutionary divergence among otherwise morphologically similar lineages (Vences *et al*., 2005; Vences & Wake, 2007), a pattern particularly prevalent in the lungless salamander family Plethodontidae (e.g. Highton, 1995; Kozak, Blaine & Larson, 2006; Beamer & Lamb, 2008). Although morphologically distinct plethodontids continue to be described in remote or poorly surveyed regions (e.g. Parra-Olea, Canseco-Márquez & García-Paris, 2004; Min *et al*., 2005), the diverse plethodontid fauna of temperate North America has been subject to intense systematic study for over 100 years (e.g. Cope, 1869), and reports of distinctly new taxa are rare (Wynn, Highton & Jacobs, 1988; Wake, 1996). In fact, a half century has passed since the discovery of a previously unknown plethodontid – indeed, any amphibian – so distinct as to warrant its description as a new genus (Highton, 1961).

In the spring of 2007, we discovered a tiny plethodontid in the Appalachian foothills of northern Georgia, USA, that resembles certain members of the genus *Eurycea* (brook salamanders). However, this new species is significantly smaller than the average adult sizes of the smallest species of *Eurycea* and is morphologically distinct in other substantial ways. Moreover, the level of genetic divergence observed between the new form and *Eurycea* exceeds pair-wise values between several long-accepted plethodontid genera. Therefore, we describe this salamander as a new species and genus to reflect its substantive evolutionary divergence and phylogenetic placement within the Plethodontidae.

Materials and methods

Following the discovery of the first specimen in March 2007, we searched the study stream at least once every other week through June 2008. We also searched streams in the immediate vicinity. Pertinent morphological data, including standard length (SL, snout tip to posterior end of cloacal opening), tail length, colour, pattern, presence of secondary sex characteristics, costal-groove number, etc., were recorded for all specimens before preservation (fixed in 10% formalin and stored in 70% ethanol) or release. Measurements were made under magnification by a Dyna-Lume® (Skokie, IL, USA) illuminator with the use of a dial caliper. Detailed morphological measurements of the holotype were taken following its preservation using a dissecting

microscope with an ocular micrometer. The type series was deposited in the US National Museum (USNM) in Washington, DC and in the Museum of Vertebrate Zoology (MVZ) at the University of California, Berkeley. Two specimens of the type series at MVZ, an adult male (25.7 mm SL before preservation) and an adult female (25.8 mm SL), were cleared and stained for osteological analysis using Alcian blue for cartilage and alizarin red S for bone. Four additional specimens (two larvae, one gravid female and one male) were collected and measured.

Molecular phylogenetic analysis

We sequenced two genes routinely used in amphibian phylogenetic analysis, mitochondrial cytochrome *b* (*cob*) and the nuclear encoded recombination-activating gene 1 (*Rag*-1). *Rag*-1 has proven particularly informative in resolving generic-level relationships within Plethodontidae (Chippindale *et al*., 2004; Min *et al*., 2005; Vieites, Min & Wake, 2007). Genomic DNA was extracted from tail tips of two males, one female and a larva using Qiagen's DNeasy kit (Valencia, CA, USA). We used the primers MVZ15 (Moritz, Schneider & Wake, 1992) and HEMTHRREV (Hillis *et al*., 2001) for *cob* and the primers 5′-AAC TGG ACG RCA GAT TTT CCA GCC CTT ACA TGC-3′ and 5′-TTT AGA AGT GTA CAG CCA GTG GTG CTT TAG CAC A-3′ (this study) for *Rag*-1. Amplifications involved denaturation at 94 °C (60 s), annealing at 51 °C (45 s) and extension at 68 °C (90 s) for a total of 32 (*cob*) or 38 (*Rag*-1) cycles. Amplification products were sequenced on an Applied Biosystems 377 automated sequencer (Carlsbad, CA, USA). Sequences were assembled in Sequencher 3.0 (Gene Codes Corp., Ann Arbor, MI, USA), corrected manually and translated to ensure an appropriate reading frame.

We compiled sequence data for eight species in the genus *Eurycea*, representing all major lineages (Chippindale *et al*., 2000; Bonett & Chippindale, 2004; Kozak *et al*., 2006) as well as the type species (*Eurycea lucifuga*). We secured sequence data for species of *Gyrinophilus*, *Pseudotriton* and *Stereochilus*, genera which, together with *Eurycea*, constitute the Spelerpini (Vieites *et al*., 2007) and sequences for other representative plethodontids chosen on the basis of recent systematic surveys (Min *et al.,* 2005; Vieites *et al*., 2007). Two species in the family Amphiumidae, sister taxon to the Plethodontidae, served as outgroup taxa. We obtained sequence data for the majority of these species from GenBank. Table 1 provides a complete list of species used for analysis, with GenBank accession numbers for new and previously published sequences.

Bayesian phylogenetic analysis was implemented in MrBayes 3 (Ronquist & Huelsenbeck, 2003). We partitioned the dataset by gene and codon position, using MrModeltest 2.2 (Nylander, 2004) to identify appropriate models of DNA substitution by the Akaike information criterion. Bayesian analysis consisted of two concurrent runs with four simultaneous chains for 5 000 000 generations with a sample frequency of 100. To ensure convergence on the same topology, we allowed the analysis to run until the split standard deviation was <0.01 and examined additional parameters (e.g. branch lengths) using the program Tracer ver. 1.3 (Rambaut & Drummond, 2003) to confirm that all parameters had converged. Trees sampled before convergence were discarded; those trees remaining were used to calculate posterior probabilities (P_p) and to create a 50% majority-rule consensus tree.

Table 1 GenBank accession numbers for the DNA sequences used in the Bayesian analysis

Species	*Rag*-1	*cob*
Amphiuma means	AY650127	AY691722
Amphiuma pholeter	AY650128	AY691723
Aneides aeneus	AY691701	AY691742
Batrachoseps major	AY650126	AY691754
Bolitoglossa helmrichi	AY650124	AY691755
Desmognathus wrighti	AY691699	AY728225
Ensatina eschscholtzii	AY691702	AY728216
Eurycea bislineata	AY691706	AY728217
Eurycea guttolineata	FJ917631	FJ917635
Eurycea longicauda	AY650121	AY528403
Eurycea lucifuga	FJ917632	FJ917636
Eurycea multiplicata	AY691707	AY528343
Eurycea neotenes	AY650122	AY528400
Eurycea quadridigitata	FJ917633	FJ917637
Eurycea tonkawae	AY691709	AY014842
Gyrinophilus porphyriticus	AY691710	AY728230
Hemidactylium scutatum	AY691712	AY728231
Hydromantes brunus	AY887134	AY728234
Nyctanolis pernix	AY691714	AY691756
Phaeognathus hubrichti	AY691700	AY728233
Plethodon cinereus	AY691703	AY728232
Plethodon elongatus	AY650120	AY728223
Pseudotriton ruber	AY650123	AY728220
Stereochilus marginatus	AY691713	AY728212
Urspelerpes brucei	FJ917630	FJ917634

cob, cytochrome *b*; *Rag*-1, recombination-activating gene 1.

Description of the new genus and species

Urspelerpes brucei **gen. et sp. nov.**

Suggested common name

Patch-nosed salamander.

Holotype

USNM 558253, adult female collected by W. E. Peterman, J. R. Milanovich, K. Holcomb, D. Sollenberger, A. Grosse and S. Sterrett on 30 March 2007.

Allotype

USNM 558254, adult male collected by C. D. Camp on 21 April 2007 at type locality.

Paratypes

USNM 558255, adult male collected on 15 April 2007; USNM 558256, adult male collected on 11 May 2007; USNM 558257–558259 and MVZ 257762, larvae collected on 19 April 2008; MVZ 258038 (adult female collected on 17 May 2007) and 258039 (adult male collected on 15 April 2007), specimens cleared and stained for osteological analysis. All specimens were collected at the type locality.

Type locality

Small, first-order stream located at the foot of the Blue Ridge escarpment in Stephens County, GA, USA (34°39′N; 83°18′W). Exact locality data to the scale of seconds is kept by the museums (USNM and MVZ) holding the type specimens.

Etymology

Urspelerpes is derived from the Greek *ur-*, meaning 'original', and the genus name *Spelerpes*, a primary synonym for *Eurycea*. *Spelerpes* is from the Greek *spelaion*, meaning 'cave', and *herpes*, meaning 'crawler', having been originally applied to the cave salamander, *E. lucifuga*, the type species of the genus. A modification of the name *Spelerpes* persists as the tribe (Spelerpini) comprising the genera *Eurycea*, *Gyrinophilus*, *Pseudotriton* and *Stereochilus* (Vieites *et al.*, 2007). The prefix *ur-*, then, indicates the basal relationship this new species has to *Eurycea*. The specific epithet *brucei* is in honour of Dr Richard C. Bruce, Professor Emeritus of Western Carolina University and retired director of the Highlands Biological Station in North Carolina.

Diagnosis

Urspelerpes brucei resembles certain species of *Eurycea* but differs in several important morphological features. All specimens (Figs 1–3) possess, on the superior surface of the snout, a distinctive patch that is saffron yellow in adults and white in larvae. A thin but distinct dorsal line runs down the centre of the tail that again, is saffron yellow in adults and white in larvae. Adults have a yellow venter. Adult SL (25 mm) is significantly less than that of any known non-paedomorphic spelerpine except for the smallest adults of the dwarf salamanders, *Eurycea quadridigitata* and *Eurycea chamberlaini*. *Urspelerpes* differs from dwarf salamanders in having a full complement of five digits on the pes instead of four and a tail length approximately equal to SL (tail length in both dwarf species is significantly greater). *Urspelerpes* exhibits sexual dimorphism in colour and pattern, a condition previously unreported in spelerpines. Adult male *U. brucei* (Fig. 1) resemble two-lined salamanders (*Eurycea bislineata* complex), having a pair of distinct, dark dorsolateral stripes and a yellow dorsum. As in many populations of the *E. bislineata* complex, the males possess enlarged nasal cirri and a conspicuous, circular mental gland (Fig. 1). Adult *Urspelerpes* are significantly smaller, however, approximating the size of newly metamorphosed two-lined

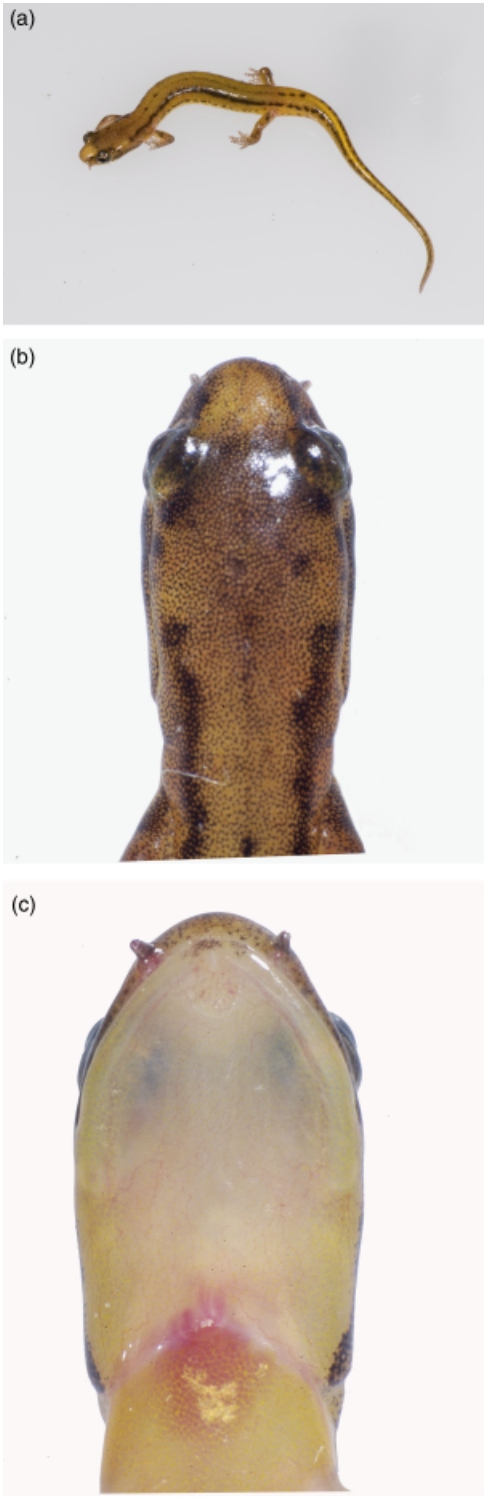

Figure 1 Photographs of male (allotype, USNM 558254) *Urspelerpes brucei* sp. nov.: (a) entire aspect in life; close-ups of (b) dorsal and (c) ventral views of head, respectively. USNM, US National Museum.

salamanders. Adult female *U. brucei* (Fig. 2) have a more muted colour and lack dorsolateral stripes.

Description of the holotype

Before preservation, SL measured 25.7 mm, and the tail measured 25.2 mm. The dorsum, suffused with dark melanophores, was dull brownish yellow except for the yellow snout-patch and bright-yellow tail stripe (Fig. 2); the sides were slightly more yellow; the venter was yellow, spotless, with the ventral aspect of the tail bright yellow. There was the suggestion of a dark, lateral stripe immediately anterior and posterior to the orbit, terminating at a point anterior to the forelimb. There were 15 costal grooves, counting those in the axilla and groin, and 14 enlarged, yolked ova visible through the venter. Detailed measurements (mm) of the holotype following preservation are given in Table 2.

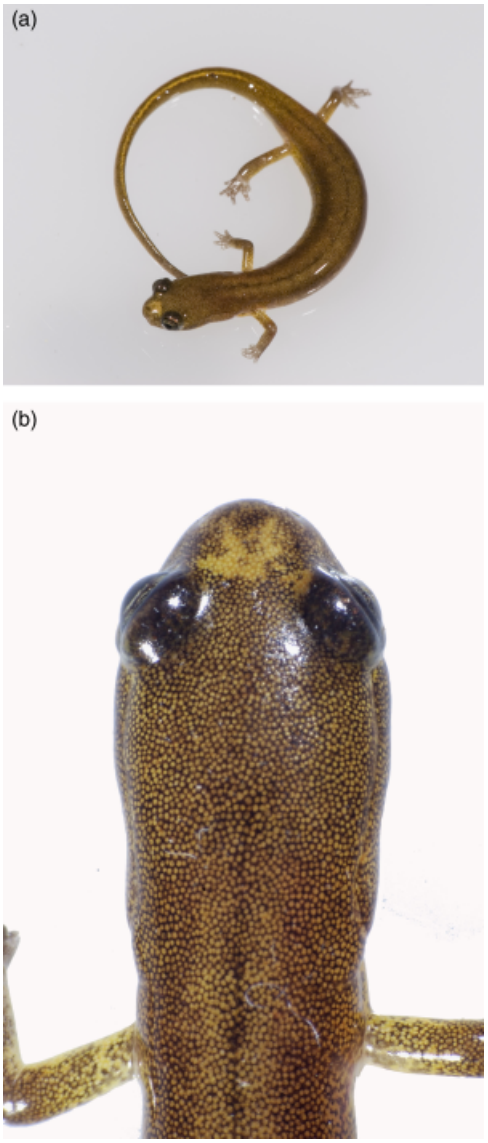

Figure 2 Photographs of female (holotype, USNM 558253) *Urspelerpes brucei* sp. nov.: (a) entire aspect in life; (b) close-up of dorsal view of head.

Table 2 Detailed measurements (mm) of holotype of *Urspelerpes brucei*

Standard length	24.8
Head width	2.3
Head length (snout to gular fold)	4.5
Head depth at posterior angle of jaw	1.3
Eye to nostril (left)	0.8
Anterior rim of eye to snout (left)	0.9
Eye diameter (horizontal)	1.4
Interorbital distance	1.4
Snout to forelimb insertion	6.4
Axilla to groin (left)	17.0
Width at mid-body	3.0
Depth at mid-body	3.0
Tail length	24.1
Tail width at base	1.9
Tail depth at base	1.7
Forelimb length to tip of longest digit (left)	5.3
Hind-limb length to tip of longest digit (left)	5.8
Width of forelimb foot (left)	0.6
Width of hind-limb foot (left)	0.8
Free length of longest digit on forelimb (left)	0.8
Free length of longest digit on hind limb (left)	1.1

Variation

Members of the type series and additional adults (one male, two females) showed little variation in SL (mean $\pm$ 1 SD = 25.37 $\pm$ 0.60 mm, $n = 8$) with no significant difference between males (25.37 $\pm$ 0.58 mm, $n = 5$) and females (25.38 $\pm$ 0.77 mm, $n = 3$; $F_{1,6} = 0.0004$, $P = 0.984$). The ratio tail length/SL varied from 0.94 to 1.00. The distribution of colour morphs (bright yellow with stripes vs. brownish yellow without stripes) was not random ($\chi^2 = 8.0$, d.f. = 3, $P < 0.05$) but was clearly associated with sex. Males exhibited minor variation in the degree of cranial spotting, number of ventrolateral caudal spots and the degree of black edging of the yellow tail stripe. Females exhibited minor variation in the number of tiny black spots along the dorsal midline of the trunk.

Body size (7.9–22.4 mm in SL) varied greatly among the five larvae. Except for the smallest individual, larvae were similar in colour; small, brownish melanophores densely covered the entire dorsal surface except for the white, pigmentless snout patch (Fig. 3), and the white, dorsal tail stripe, on which the number of melanophores was greatly reduced. There were no indications of black spots or stripes characteristic of adults. The venter was pigmentless and white. The smallest larva (7.9 mm SL) appeared largely without pigment but had no yolk in the gut, indicating that it was not newly hatched. Larval gills were relatively short with teardrop-shaped secondary fimbriae. The tail fin was low (2.3 mm maximum height in the largest larva), originating well posterior to the vent, and ranged between 54 and

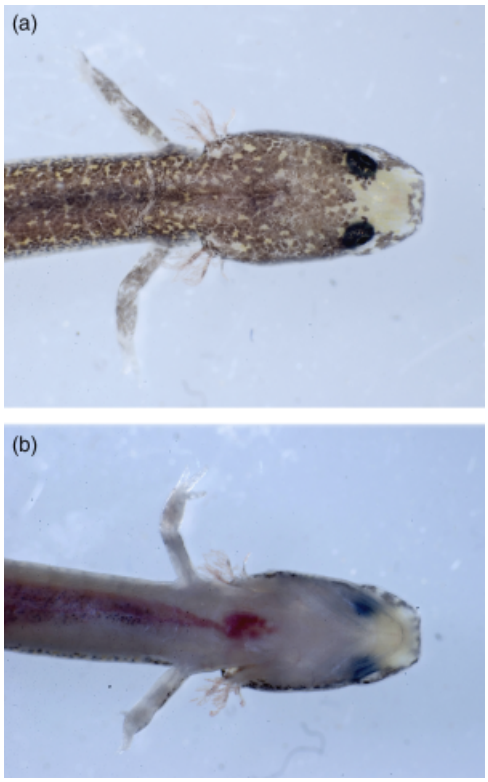

Figure 3 Close-up photographs of head of larval *Urspelerpes brucei* sp. nov.: above, dorsal aspect; below, ventral aspect. USNM, US National Museum.

70% of overall tail length, with the largest larva having proportionally the longest tail fin.

Osteology

Both male and female *Urspelerpes* have skeletons that generally resemble those of similar-sized specimens of *Eurycea* with which they were compared, that is, *Eurycea wilderae*, *E. bislineata*, *Eurycea guttolineata* and *E. quadridigitata*. Curiously the bones appear to be more completely developed in terms of expansion of membrane bones but weakly mineralized. The dorsal roof of the skull is complete with no frontoparietal foramen. The bones of the snout region are thin and delicate. The septomaxillaries are especially small. The prefrontals and nasals contact the ascending process of the maxilla, and the nasolacrimal duct passes through a gap at the three-way intersection of these bones. The vomers bear very short preorbital processes. Vomerine teeth are discontinuous from the small paravomerine tooth patches. The occipito-otic complex lacks dorsal crests. A round operculum bears a slender, short columella. Both jaws are slender and weakly articulated to other bones, and the teeth are small and few in number. The male, with long, spiny premaxillary teeth, has larger teeth than the female. The mandible articulates with a small quadrate that is overlapped by a slender, flattened squamosal.

The hyobranchial apparatus is typical of spelerpines in general (cf. Lombard & Wake, 1976). The only ossified element is a large, triradiate urohyal, which has long, slender arms extending posterolaterally and a smaller medioposterior spine. The basibranchial is broad and robust, widening immediately posterior to the attachment of the cornua. The anterior tip of the basibranchial is broad and flat and bears a pair of laterally located and vertically oriented cornua and a discrete lingual cartilage that is separated from the basibranchial. The first ceratobranchials are distinctly stouter and straighter than the second. The first ceratobranchial is the same length as the basibranchial, and the second ceratobranchial is 0.75 times this length. The epibranchial, the longest hyobranchial element, is 1.3 times the length of the basibranchial. The basibranchial is much broader (0.40 times its length) than the other elements but becomes narrower and more round posteriorly, at and behind the attachment of the first ceratobranchial. The cornua are 0.11 times the length of the basibranchial.

The male has 15 trunk vertebrae and the female 16, typical in form to those of *Eurycea* (Wake & Lawson, 1973). The limbs are small but well formed. A stout spur is attached to the proximal end of the tibia. The manus and pes are diminutive, and the digits, while short, exhibit the standard plethodontid phalangeal formulae: 1–2–3–2, 1–2–3–3–2. The carpus and tarsus have the default ancestral pattern (Shubin & Wake, 2003), including eight carpal and nine tarsal cartilages. Distal tarsal five is much smaller than distal tarsal four and does not articulate with the centrale. The first and last digits are particularly short, and the fifth digit of the pes is especially slender (Fig. 4). All terminal phalanges are pointed with no expansion. The phalanges are well ossified, with small cartilaginous caps. The metapodials are slender, especially at the midpoint. Free digital length is greatly reduced relative to the underlying skeletal structure than in other small *Eurycea* (Fig. 4).

Urspelerpes has an osteology typical of spelerpines and is especially similar to species of *Eurycea* (cf. Wake, 1966). Synapomorphies of *Eurycea* and *Urspelerpes* include fused

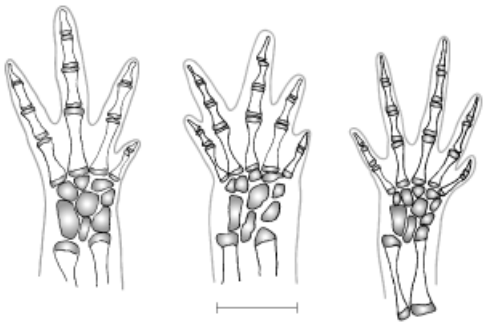

Figure 4 Skeletal structure of left hind feet of *Urspelerpes brucei* and similarly sized specimens of *Eurycea*. From left to right, *Eurycea quadridigitata* (MVZ 184321, Emmanuel County, GA, 25 mm SL), *U. brucei* (MVZ 258039), and larval *Eurycea wilderae* (MVZ 218480, Macon County, NC, 20 mm SVL). Shaded elements represent cartilage. The scale bar represents 1 mm.

premaxillaries and reduced or absent preorbital processes of the vomer. Proportions of the hyobranchial apparatus are in general similar to those of *Eurycea*, but the cartilages are especially well formed for such small individuals. The distinctive basibranchial resembles that of adult *Eurycea cirrigera* and even *Gyrinophilus*, especially in comparison with small individuals of other *Eurycea*, which typically have cornua and lingual cartilages that are poorly developed or absent and basibranchials that are only weakly expanded laterally (e.g. metamorphosed *E. bislineata*; Wilder, 1924). The epibranchial is the longest element in the hyobranchial apparatus and is relatively longer than that of juvenile individuals of *E. wilderae* (20.0 mm SL) or *E. guttolineata* (29.3 mm SL). However, the epibranchial is much shorter that that of adult specimens of available species of *Eurycea*; relative lengths are as follows: *E. cirrigera*, 1.7; *Eurycea longicauda*, 1.9–2.0; *Eurycea spelaeus*, 1.6–1.7; *E. quadridigitata*, 1.6. A metamorphosed specimen of *Eurycea neotenes* illustrated by Sweet (1977) has a relative epibranchial length of 1.7. The basibranchial of that specimen resembles that of *Urspelerpes* but is slightly broader. Species of related genera also have relatively longer epibranchials than *Urspelerpes*: *Pseudotriton*, 1.5; *Gyrinophilus,* 1.6; *Stereochilus*, 1.75.

Although some individuals of *E. quadridigitata* achieve sexual maturity at sizes equivalent to *Urspelerpes* (Trauth, 1983), small specimens of the former species are not yet well ossified. Perhaps a more appropriate comparison is with a metamorphosed specimen (32 mm SL) of *E. neotenes*, a mainly non-metamorphosing species, illustrated by Sweet (1977). The hyobranchial apparatus of the specimen of *E. neotenes* is fully developed and closely resembles that of *Urspelerpes*. The basibranchials are broad and flattened in both, with well-developed cornua and a distinct lingual cartilage; however, the ceratobranchials and epibranchial are substantially longer in *E. neotenes*. The urohyals are similar, but that of *Urspelerpes* is longer and more slender. The skull of *E. neotenes* is less ossified but appears to be more robust than that of *Urspelerpes*. The frontals and parietals do not articulate with their bilateral counterparts, in contrast to the situation in *Urspelerpes*.

Phylogeny reconstruction

No nucleotide sequence variation was observed among the four sampled individuals of *Urspelerpes* for either the *cob* or *Rag*-1 gene segments. The combined sequence dataset totaled 2633 nucleotides (*cob* = 1108 nts; *Rag*-1 = 1525 nts), for which the best fitting evolutionary models for gene partitions were: *cob* first, second and third positions = GTR + I + G; *Rag*-1 first & third = GTR + G; and *Rag*-1 second = HKY + I. A consensus phylogram generated from Bayesian analysis of these combined sequence data is illustrated in Fig. 5. Overall, our resulting topology is congruent with plethodontid phylogenies generated from analyses of mitochondrial genomic (Mueller *et al*., 2004), nuclear gene (Vieites *et al*., 2007) and mitochondrial/nuclear gene (Chippindale *et al*., 2004) datasets. We recovered two major clades, the Plethodontinae and Hemidactyliinae (*sensu* Vieites *et al*., 2007); the latter contains two subclades, one of which comprises the spelerpine genera, among which *Urspelerpes* is nested as the sister taxon to *Eurycea*. Each clade received strong statistical support ($P_p = 1.0$, Fig. 5). The level of sequence divergence between *Urspelerpes* and *Eurycea* for *Rag*-1, at 4.7%, exceeds all pairwise comparisons between the spelerpine genera *Gyrinophilus*, *Pseudotriton* and *Stereochilus* and is comparable to values for each of these genera versus *Eurycea* (Table 3).

Our tree places *Hemidactylium* outside the remaining plethodontids, though with little support ($P_p = 0.67$). This unexpected (indeed, unlikely) topology was recovered in a small set of Bayesian analyses by Chippindale *et al*. (2004). However, the most recent plethodontid molecular phylogeny, based on three nuclear genes, depicts *Hemidactylium* within a strongly supported Hemidactyliinae (Vieites *et al*., 2007). Our Bayesian tree is otherwise congruent with previous plethodontid molecular phylogenies.

Ecology and life history

The ecology of *U. brucei* remains largely unknown. We collected only eight adults, all from a single, first-order stream, either within or along the banks of the non-inundated part of the streambed. Four individuals were collected under rocks whereas the others were found in loose-leaf litter. Our collecting efforts largely coincided with a severe regional drought; it is possible that this species may occupy more terrestrial microhabitats under suitably mesic conditions. Its relative scarcity indicates a secretive, fossorial proclivity. Jaw structure, tooth size and number, and hyobranchial structure suggest *U. brucei* captures small, terrestrial prey using a projectile tongue similar to that of *Eurycea* (Lombard & Wake, 1977).

All three adult females were gravid, each with ova visible through a translucent venter. Two had 14 eggs each; the third had six. Egg diameters (estimated through the ventral walls) were 1.5–2.0 mm. The adult males, all collected between 15 April 2007 and 29 May 2007, had pronounced nasal cirri and mental glands associated with breeding condition.

Minimal variation in adult body size versus the large variation in larval body size indicates that most growth occurs during the larval stage. At the time of capture, the largest larva was nearly adult size, indicating that sexual maturity may occur during or shortly following metamorphosis, a situation observed in certain populations of the spelerpine *Gyrinophilus porphyriticus* (Bruce, 1972).

Discussion

The overall morphology of adult *U. brucei* resembles the typical habitus of historically conceived *Eurycea* (Bishop, 1947). Recent phylogenetic analyses, however, demonstrate that genera with radically different morphologies nest deeply within *Eurycea* (Chippindale *et al*., 2000; Hillis *et al*., 2001; Bonett & Chippindale, 2004). As now constituted, the genus *Eurycea* encompasses a variety of morphological

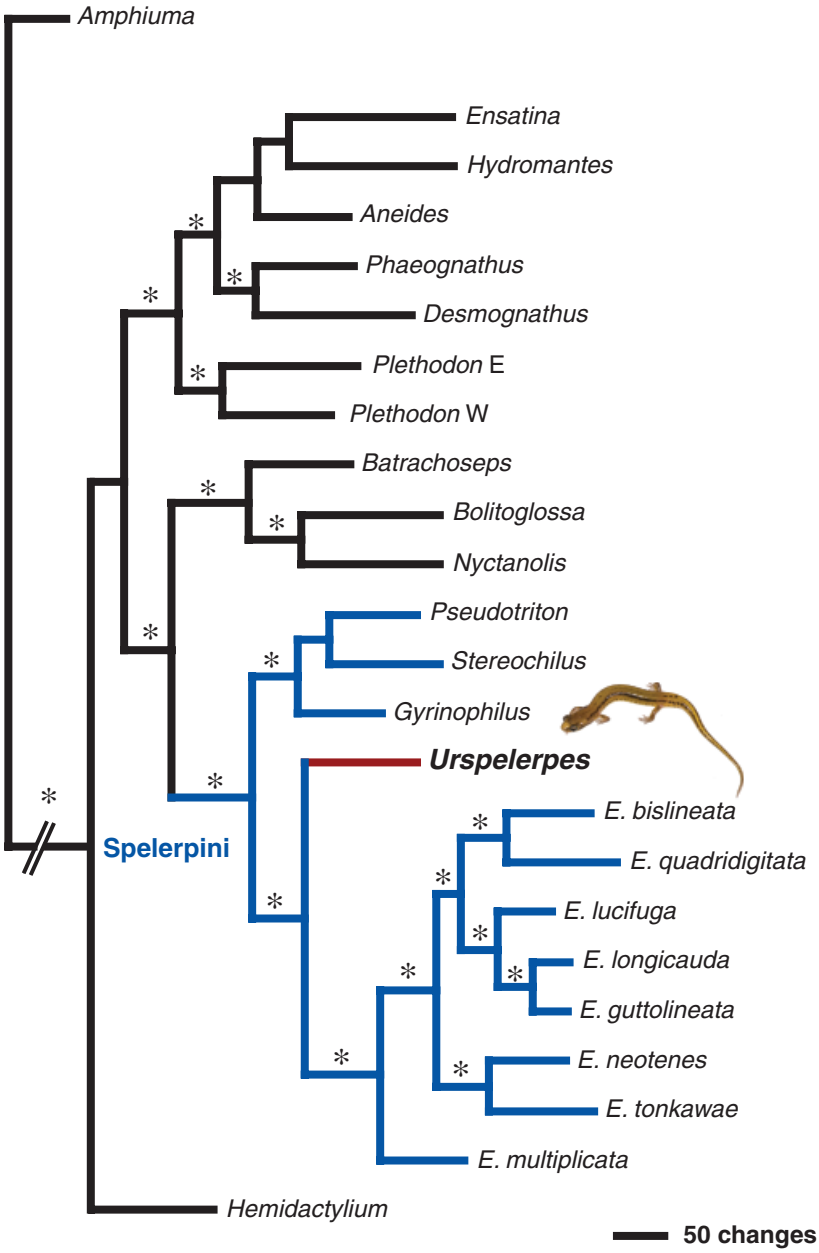

Figure 5 Bayesian phylogram depicting the placement of *Urspelerpes* relative to other plethodontid taxa. The branch for *Urspelerpes* is highlighted in red and those for remaining members of the Spelerpini in blue. Nodes with posterior probabilities ≥98% are indicated by a star symbol.

Table 3 Uncorrected pair-wise distance (%) comparisons between spelerpine genera for the nuclear gene *Rag*-1

	Eurycea	*Gyrinophilus*	*Pseudotriton*	*Stereochilus*	*Urspelerpes*
Eurycea	–				
Gyrinophilus	4.329	–			
Pseudotriton	5.249	2.557	–		
Stereochilus	5.010	2.754	2.623	–	
Urspelerpes	4.677	4.354	4.982	5.269	–

Rag-1, recombination-activating gene 1.

forms, and the 'traditional' eurycean morphology has little taxonomic meaning. Moreover, uncorrected pair-wise distances for *Rag*-1 indicate that divergence between *Urspelerpes* and *Eurycea* is at least comparable to other pair-wise comparisons of spelerpine genera (Table 3). Therefore, we place *U. brucei* in its own genus as the taxonomic arrangement most informative of recovered evolutionary history. The evolutionary relationship between *Eurycea* and

Urspelerpes parallels that between the species-rich plethodontid genus *Desmognathus* and its monotypic sister taxon *Phaeognathus* (Chippindale *et al*., 2004).

A striking morphological feature of *Urspelerpes* is its sexual dimorphism in colour and pattern. Although plethodontids often exhibit sexual size dimorphism (Bruce, 2000), intersexual differences in colour or pattern are rare (Wake & Lynch, 1976; McCranie & Wilson, 1995) and heretofore unreported for any plethodontid in temperate North America. Conversely, *Urspelerpes* does not exhibit the sexual size dimorphism common in other plethodontids (Bruce, 2000).

Miniaturization (*sensu* Wake, 1991) has evolved multiple times independently among plethodontids with developmental consequences that often include a reduction from five to four digits on the pes (Hanken & Wake, 1990; Wiens & Hoveman, 2008). The smallest metamorphosing species of *Eurycea* exhibit toe reduction, so it is significant that a salamander as tiny as *Urspelerpes* retains a full complement of five toes.

Nearly a century ago, Wilder & Dunn (1920) hypothesized that plethodontids originated in the Appalachian Mountains, an idea based largely on the region's plethodontid species richness and adaptive diversity. Recent phylogenetic analyses (Mueller *et al*., 2004; Vieites *et al*., 2007) have offered compelling evidence for a North American origin of plethodontids, with the Appalachian region as an area of significant diversification for several lineages. Certain plethodontid clades, including the Spelerpini, date to divergence times coincident with an Appalachian uplift in the Cenozoic (Vieites *et al*., 2007). The discovery of *Urspelerpes* at the Blue Ridge escarpment and the relative basal phylogenetic position of the genus within the Spelerpini are consistent with an Appalachian origin for the clade.

It is possible that we found so few specimens of *U. brucei* because it is highly secretive. However, our failed effort to capture additional larvae – so easily collected (five within 45 min) in the study stream – in nearby streams is disturbing. It seems likely that this species may be extremely rare, perhaps occurring in such few numbers as to be in danger of extinction. Protection of this remarkable new species should be of paramount concern.

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