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SALAMANDERS

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Salamanders are a diverse group of vertebrates, exploiting moist cool habitats in a variety of ways. Several lineages have colonized subterranean habitats, particularly in regions of climatic extremes. Salamanders associated with karst exhibit differences in the amount of time they spend in subterranean habitats, their dependence on these resources, and morphological and behavioral adaptations to life underground (troglomorphisms). Differences are manifested within and across taxonomic groups as well as between geographical regions. North America and Europe host the greatest number of known cave-dwelling species. Cave-dwelling salamanders may inhabit subterranean environments for significant portions of their life cycle but not all of it, others migrate to the surface periodically, and still others are exclusively found underground. Sources of general information on salamanders are Amphibia Web (2011), Grossenbacher and Thiesmeier (1999), and Lannoo (2005).

Salamanders are the only tetrapods that exhibit an exclusive subterranean existence. Such troglobitic salamanders belong to the families Plethodontidae (in North America) and Proteidae (in Europe). Troglomorphisms seen in troglobitic salamanders vary from species to species and even from locality to locality, but they frequently include reduced eyes and pigmentation, reduced number of trunk vertebrae, elongated appendages, and modified head shape with increased dentition. Salamanders are opportunistic predators, feeding on a variety of small invertebrates and will often also consume their own kind. There are records of notable dietary oddities in troglobitic salamanders, including feeding on bat guano and silt. Troglobitic salamanders are usually found in caves where fishes do not occur, and function as top predators in those subterranean waters. Because of the constant water temperatures in their habitat, they are likely active year-round. For orientation, feeding, and mating, they rely on mechano- and chemosensory cues, which corresponds to progressive development of extraoptic sensory systems. Salamanders from subterranean environments usually have lower metabolism, efficient energy (fat) storage, and a longer life span than their surface counterparts. They have fewer, larger eggs and offspring compared to related surface-dwelling species. The rate of development in amphibians strongly depends on environmental factors, most notably ambient temperature. When compared with related epigean species that reproduce at the same water temperature, troglobitic salamanders usually develop more slowly. All begin their life inside a gelatinous egg capsule, deposited in water. Eggs hatch into free-swimming aquatic larvae, with visible bushy gills and a tailfin. Instead of metamorphosing into mature adults, however, most troglobitic salamanders attain sexual maturity while retaining these and other larval characteristics—a condition known as *neoteny* or *paedomorphism*.

We can only speculate about the origins and time required for the evolution of obligate subterranean existence. While the ancestors of some troglobitic species may have colonized caves as early as in the Miocene, it is more likely that subterranean colonization occurred much later, even as late as during the Pleistocene. Salamander fossils are rare and the oldest found that may belong to a cave-dwelling species date to the Pleistocene.

The following sections portray all ten currently described troglobitic salamanders. Accounts also are provided for their close relatives that show affinities to subterranean habitats.

OLMS AND WATERDOGS (PROTEIDAE)

The family includes six species of surface-dwelling mudpuppies or waterdogs (*Necturus* sp.) from eastern and central North America and the troglobitic olm (*Proteus anguinus*) found in southeastern Europe. These

FIGURE 1 The olm (*Proteus anguinus*). (A) Described in 1768, this blind salamander was the first scientifically documented cave-dwelling animal, but its existence had already been known long before. Its earliest representation may be a Venetian stone carving from the tenth or eleventh century. (B) A rare, pigmented individual with eyes, from southeastern Slovenia. This unique population was discovered toward the end of the twentieth century. (C) Female protecting her clutch of eggs from predation by conspecifics. *Photos courtesy of G. Aljančič, Tular Cave Laboratory. Used with permission.*

Olm (*Proteus anguinus*)

The olm (*Proteus anguinus*) is the only European obligate cave-dwelling vertebrate. It has been found in over 250 springs and caves in the Dinaric karst on the western Balkan Peninsula, between the rivers Isonzo/Soča (northeastern Italy) and Trebišnjica (Bosnia and Herzegovina). Several populations, however, have been destroyed or severely diminished by dam construction, earth fill, or pollution. The salamander inhabits karst waters from close to the surface to deep in fissures. During flooding or at night it may be found in cave entrances or short distances away from springs.

This elongated salamander reaches 25 cm in total length, although 40-cm-long individuals have also been found. The salamander's predominant mode of locomotion is swimming with eel-like undulations of the body ending in a short, finned, laterally compressed tail (Fig. 1A). The very short limbs, with digits reduced in number to three on the forelegs and two on the hindlegs, are used for crawling on the water bottom and occasionally outside the water. Its muscular head ends with a flattened, blunt snout.

The species is currently divided into two subspecies based on the extent of troglomorphism. Analyses of molecular data suggest, however, that *P. anguinus* may be a complex of several species that independently evolved troglomorphic traits. Mitochondrial DNA data suggest that *P. anguinus* is divided into six divergent lineages. These are about 5 to about 15 million years old, that is, they originated in the Pliocene or Miocene. Conversely, it has been hypothesized that troglomorphism might have evolved in less than 500,000 years (Trontelj *et al.*, 2007).

Morphologically, the extremely rare subspecies *Proteus anguinus parkelj*, found only in the springs of two streams near Jelševnik in Bela Krajina (southeastern Slovenia), is believed to resemble the supposed epigean ancestor of both forms. Despite having an entirely subterranean life, this salamander is darkly pigmented (Fig. 1B). Its small but functional eyes are covered with a transparent skin and have well-differentiated lens and retina. Conversely, the pineal gland, which in amphibians controls circadian rhythms, gamete development, and pigmentation changes, is greatly reduced.

In contrast, the widespread, troglomorphic *Proteus anguinus anguinus* is characterized by a yellowish to pinkish-white skin, with few scattered melanophores (pigment cells) invisible to the naked eye (Fig. 1A). Melanin synthesis can be light-induced, and after a prolonged exposure to light, the animals turn dark. The skin is thinner than in the pigmented form, and contains fewer multicellular glands. The head and snout of *P. a. anguinus* are elongated and flattened, and

salamanders are aquatic throughout their life, retaining external gills, but are also able to breathe air through their skin and with their sac-like lungs. All representatives of this family are obligate paedomorphs; paedomorphism in *Proteus* is thus seemingly not a result of conditions particular to the subterranean environment. Thyroid glands, which control metamorphosis, are functional in all species of this family. Metamorphosis cannot be induced artificially, although changes in the skin structure toward a metamorphosed form have been observed in *Proteus*. Furthermore, it has been shown that *Necturus* possesses functional thyroid hormone receptors and its tissues are not generally irresponsive to the hormone (Safi *et al.*, 2006). Rather, the absence of metamorphosis may be due to loss of function of thyroid hormone-dependent genes required for tissue transformation.

the cranial bones are longer and the teeth more numerous than in the pigmented form. Cervical vertebrae are often elongated as well. Compared to the pigmented form, *P. a. anguinus* has a shorter trunk, but a proportionately longer tail and extremities. Morphometric variability among genetically and hydrologically isolated populations can be quite high.

The eyes of this salamander are greatly reduced in size and structure, and lie embedded deep in the hypodermal tissue. While vision is lost, the eye is still capable of detecting light. The pineal gland is also greatly reduced, but may still be capable of light detection and hormonal activity. As is common in salamanders, the skin and midbrain are also photosensitive. Extraoptic sensory systems, including the lateral line, inner ear, ampullary electroreceptors, olfactory epithelium, and taste buds, are well developed and believed to play a crucial role in prey detection and communication among individuals. Furthermore, behavioral experiments suggest that the animals may use the Earth's magnetic field to orient themselves.

Because of its very low metabolic rate and efficient fat storage, predominantly in the liver and tail, *P. anguinus* can survive prolonged periods of food deprivation. When food in the form of small subterranean crustaceans, mollusks, or surface insect larvae is abundant, it becomes a voracious and efficient predator.

P. anguinus is a social animal, recognizing and communicating with conspecifics through scent. Both males and females use water-borne chemical signals to locate each other, but in order to recognize sex and reproductive state, they need to come into direct contact. Outside of the breeding period, aggressive behavior is reduced and the animals aggregate at communal resting places under stones and in cracks. Long periods of inactivity are punctuated by sessions of foraging or exploratory behavior. The animals show no apparent daily rhythm of activity and resting.

Little is known about timing of reproduction. A female is thought to reproduce only every 12 years on average. Up to 70 eggs are attached under rocks, and are guarded by the female until hatching (Fig. 1C). Larvae hatch after 4 to 6 months. The newly hatched larvae are 2.5 cm long and have incompletely formed limbs, but well-differentiated eyes and scattered, visible melanophores in the skin. During early larval development, the eyes only slightly increase in size without further differentiation, and then gradually sink into the surrounding tissue. After four months, major degenerative changes appear which cannot be prevented by illumination. Sexual maturity is reached at a size of about 20 cm, which is attained after about 15 years. *P. anguinus* is thought to have the longest life span of all amphibians, living for 70 years or more.

LUNGLESS SALAMANDERS (PLETHODONTIDAE)

Salamanders of the family Plethodontidae are widely distributed in North and Central America, with populations also in South America, southern Europe, and on the Korean Peninsula. Lacking lungs, these salamanders breathe primarily through their skin (cutaneous respiration). Pheromones play an important role in communication, and the characteristic nasolabial grooves present in metamorphosed adults allow fine-tuning in chemoreception. A great diversity of lifestyles has evolved in this group, from terrestrial, arboreal, to semi-aquatic and aquatic; life histories range from biphasic and larval reproduction to direct development and possibly even vivipary. It is no surprise to find in this family species associated with caves. The family includes nine described troglobitic species, all found in central and eastern North America. They are grouped in the genera *Eurycea*, *Haideotriton*, and *Gyrinophilus*. Formerly used genera *Typhlotriton* and *Typhlomolge* have been synonymized with *Eurycea*.

Brook Salamanders (*Eurycea*)

This is a diverse genus of 26 recognized species, five of which are troglobites. Except for *Eurycea spelaea*, the troglobitic species inhabit subterranean waters of the Edwards Plateau and Balcones Escarpment in central Texas, in the United States. Many species of *Eurycea* in this region are spring-dwelling troglophiles, and additional troglophilic *Eurycea* are encountered in caves outside the region. Paedomorphism is common in both cave- and spring-dwelling salamanders. *Eurycea spelaea* is the only metamorphosing troglobitic member of the genus.

Texas Cave and Spring-Dwelling *Eurycea*

All species of *Eurycea* from the Edwards Plateau in Texas share a common ancestor. It has been hypothesized that about 15 million years ago a split into two lineages occurred, corresponding to the geographic divide imposed by the Colorado River. The resulting lineage south of the divide later gave rise to a group of extremely troglomorphic cave-dwelling species collectively called Typhlomolge and its sister lineage comprised of less troglomorphic cave- and spring-dwelling species called Blepsimolge. For the spring-dwelling species north of the Colorado River the name Septentriomolge has been coined. Each troglobitic species has a surface counterpart inhabiting the spring outflows of the same watershed or geological formation. The complex interspecies relationships along with
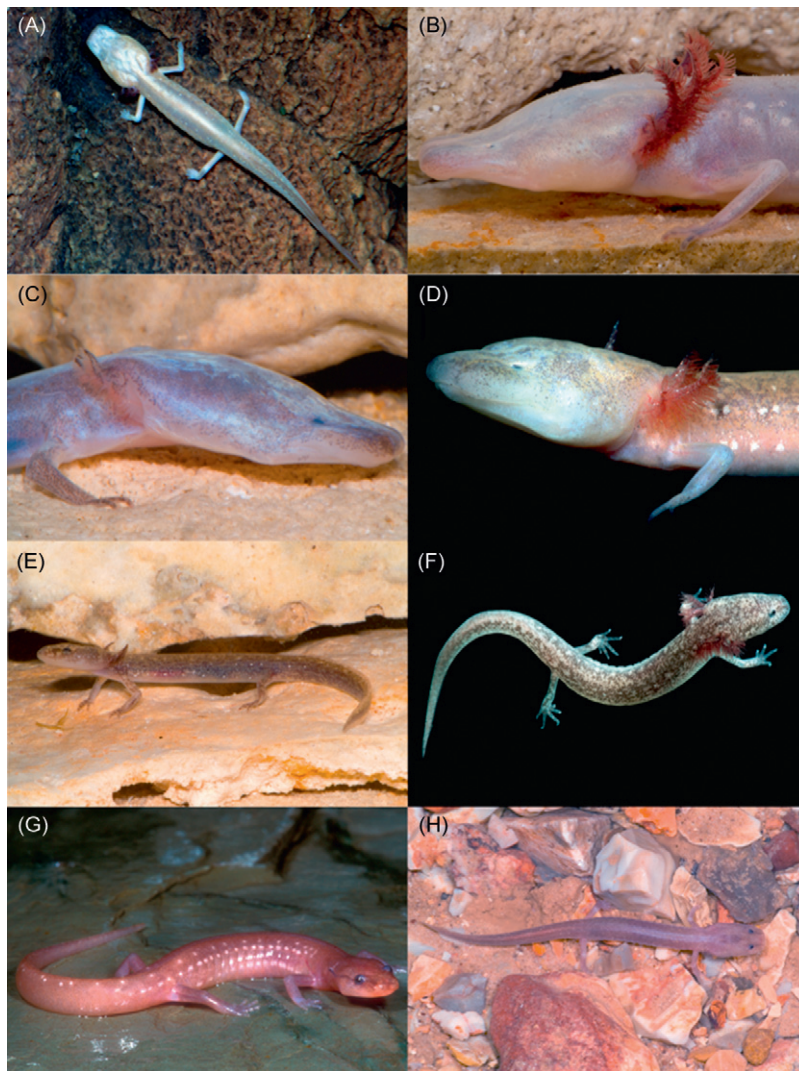
FIGURE 2 Brook salamanders (*Eurycea*). Troglobitic *E. rathbuni* (A) and (B), *E. waterlooensis* (C), and *E. tridentifera* (D) from the Edwards Plateau have degenerate eyes and reduced pigmentation compared to spring-dwelling populations of *E. sosorum* (E) and *E. latitans* (F). Adult (G) and larva (H) of *Eurycea spelaea* from the Ozark Plateau. This species is the only troglobitic brook salamander to readily undergo meta-morphosis in nature. *Photos by D. Fenolio.*

their geographic and ecological segregation indicate that an ancient speciation event giving rise to the sub-terranean and largely epigean species was followed by a rapid adaptive radiation and more recent coloniza-tion of subterranean habitats by still more species. Evidence of intermittent gene flow between species that diverged millions of years ago has been detected. Conversely, in some of the species with relatively broad ranges, there is generally very little migration between geographically distant populations, with "dis-tant" sometimes referring to as little as a few hundred meters (Chippindale *et al.*, 2009; Lucas *et al.*, 2009).

Typhlomolge (Blind Salamanders)

All species are neotenic troglobites with very long and thin limbs, short trunk, broad and flattened snout, and a virtually pigmentless and translucent skin (Fig. 2A). They have a shimmering white appearance due to the reflective connective tissue that lies beneath the skin. The rudimentary eyes may be visi-ble as tiny dark spots under the skin, and possibly still detect light. These salamanders are moderately small with the total length of adults ranging from 7−14 cm. Their diet consists of a variety of small aquatic subterranean invertebrates, including crusta-ceans and snails. When feeding, the salamanders usu-ally probe the bottom using lateral movements of their spatulate head.

The Texas blind salamander (*Eurycea rathbuni*) has been found in caves, wells, and pipes that intersect the San Marcos Pool of the Edwards Aquifer, although it may have a wider geographic range than previously thought. Molecular analyses revealed high levels of genetic variation and the possibility that two distinct species occur within *E. rathbuni*. In springs immedi-ately above the subterranean waters occupied by *E. rathbuni*, *E. nana* (see below) is locally abundant. A captive breeding program has been established for both species.

Artificial induction of transformation in *E. rathbuni* resulted in only partial metamorphosis, with some tissues (*e.g.*, skin) continuing to show only larval features. This salamander has an extremely depressed and broad anterior part of the head (Fig. 2B), with elongated anterior cranial bones, and the longest limbs of all troglomorphic *Eurycea*. The eyespots are barely visible. Juveniles have darker pigmentation and proportionally larger eyes.

In contrast, the olfactory system is well developed and plays a very important role in the social behavior of this salamander (Epp *et al.*, 2010). Nothing is known about social interactions or breeding of this species in nature; however, they have been observed in captivity. These salamanders rarely show aggressive or social behavior. The male of *E. rathbuni* lacks mental (chin) and caudal hedonic glands used during courtship in other *Eurycea* salamanders. Nevertheless, the species has an elaborate tail-straddling walk similar to that which has been observed in other plethodontid salamanders, albeit somewhat simplified. In captivity, these salamanders can live for over 10 years.

The Blanco blind salamander (*Eurycea robusta*) was described based on a single specimen collected from a well drilled in the bed of the Blanco River east of San Marcos. More specimens had been collected at the time, but birds consumed two individuals while they were in a bucket and one preserved specimen has vanished. Molecular data are not available for this species, but the geological formation in which it occurs is hydrologically isolated from that in which the geographically proximal *E. rathbuni* is found. The epigean salamander found in the springs of the Blanco River drainage is *E. pterophila* (see below). Compared to *E. rathbuni*, the body of *E. robusta* is stockier, with longer trunk, and shorter, relatively robust limbs. The head also is wider posteriorly and more massive. The eyes are extremely reduced and invisible through the skin.

The Austin blind salamander (*Eurycea waterlooensis*) is known only from the outflows of Barton Springs in Austin, where individuals presumably are washed out from the Barton Springs segment of the Edwards Aquifer. This species is much more rarely encountered than the sympatric surface-dwelling *E. sosorum* (see below), both of which are included in a captive breeding program. This species is similar to *E. rathbuni*, but has a wider head (Fig. 2C). The eyespots are visible and superficially resemble those of *E. rathbuni*. The limbs are shorter and the skin is slightly more pigmented than in *E. rathbuni* and *E. robusta*. The tail fin is weakly developed.

Blepsimolge (Sighted Salamanders)

A large majority of these salamanders are neotenic and completely aquatic. Besides those listed below, the group includes several populations of yet undescribed species. The salamanders of this group are primarily surface-dwelling, found in the immediate vicinity of spring outflows, but they also inhabit caves, fissures, and sinkholes. Various species have independently evolved cave-associated morphological traits, which has become more apparent as new populations are discovered and genetically analyzed (Bendik *et al.*, 2009).

These small salamanders (6−10 cm long) usually have a dark, finely patterned dorsal coloration and translucent ventral parts (Figs. 2E,F). The intensity and pattern of dorsal coloration may vary due to an irregular pattern of melanophores and reflective white iridophores. Single or double dorsolateral rows of light spots extend along the body. The species differ in eye size, head shape, tail fin shape, and color pattern on the tail and flanks. Certain cave-dwelling populations exhibit marked reduction of eyes and pigmentation. The skin of the troglomorphic animals has a light yellowish color, with diffuse gray or brown spots dorsally and lighter spots laterally (Fig. 2D). Juveniles are darker than adults. The snout is flattened but truncated. The eyes are regressed, sometimes lacking a lens, but are usually still visible through the skin as dark spots; they are larger than the rudimentary eyes of *E. rathbuni* and other members of the Typhlomolge group. The trunk is short, containing fewer vertebrae, and the limbs are somewhat elongated.

The life history of most species is poorly known and based predominantly on observations of *E. nana* and *E. sosorum* in captive breeding programs (e.g., Najvar *et al.*, 2007). The spring-dwelling *Eurycea* are fairly sedentary, although they may seasonally move between surface and cave habitats. On the surface, these secretive salamanders usually hide under rocks, in gravel substrate, or among aquatic plants. They can survive temporary drying of ephemeral springs, presumably by retreating to subsurface refugia. They are active throughout the year and appear to reproduce during all seasons. Up to 70 eggs are laid singly and do not receive parental care. In captivity, the salamanders usually live for about 4 years, but an individual of *E. sosorum* survived to at least 12 years.

Historically, many spring-dwelling populations from throughout the Edwards Plateau were assigned to the Texas salamander (*Eurycea neotenes*) based on morphological similarity, but they were found to differ genetically. This species is now restricted to the springs in the vicinity of Helotes, near San Antonio. A disjunct and genetically divergent population inhabits the area of Comal Springs in New Braunfels.

One salamander that has been elevated to species from *E. neotenes* is the Barton Springs salamander (*Eurycea sosorum*), known from the Barton Springs pool and adjacent springs in Austin. This species is

primarily surface-dwelling, but appears to reproduce in subterranean conduits. Skin pigmentation in *E. sosorum* varies considerably, but all individuals have very small eyes and somewhat elongated limbs. The flattened, slightly elongated head ends with a truncated snout (Fig. 2E). The subterranean portion of the Barton Springs Aquifer is inhabited by the troglobitic *E. waterlooensis* (see above).

The San Marcos salamander (*Eurycea nana*) is abundant in the Spring Lake pool at the source of San Marcos River, the only site where it occurs. Metamorphosis has been induced artificially in this species.

The Fern Bank salamander (*Eurycea pterophila*) inhabits springs and caves of the Blanco and Guadalupe River drainages. Most populations are morphologically similar to *E. neotenes*, to which they were formerly assigned, but cave-dwelling individuals with reduced eyes and pigmentation have also been identified. This species is genetically different from *E. neotenes*, but similar to, perhaps even conspecific with, *E. latitans* and/or *E. tridentifera* (see below).

The Cascade Caverns salamander (*Eurycea latitans*) is possibly a complex of several species found in caves and springs of the Cibolo Creek basin and south of the Guadalupe River in the southeastern part of the Edwards Plateau. For some time before initial molecular analyses were completed, most populations were regarded as *E. neotenes*, and the population at the type locality (Cascade Caverns) was considered to be hybrid between *E. neotenes* and *E. tridentifera* (see below). This population includes individuals with a spectrum of morphological features, ranging from highly troglomorphic, most similar to those of *E. tridentifera*, to surface-like (Fig. 2F), most similar to what was historically considered *E. neotenes*. Recent molecular analyses suggest that at least part of *E. latitans*, including the Cascade Caverns population, is conspecific with *E. tridentifera*. Furthermore, a few cave populations were determined genetically to be hybrids between *E. latitans* and *E. neotenes*, and in two cases individuals of both species were located within the same cave.

As currently recognized, the Comal blind salamander (*Eurycea tridentifera*) is a troglobitic species. It exhibits morphological modifications similar to the blind salamanders of the Typhlomolge group, although generally not to the same extreme extent (Fig. 2D). The salamander is found in caves of the Cibolo Creek basin and south of the Guadalupe River, in the southeastern part of the Edwards Plateau. These populations may actually be cave populations of *E. latitans* that have become troglomorphic. At the entrance to the type locality (Honey Creek Cave), individuals intermediate in morphology between *E. tridentifera* and the surface species have been found.

The Valdina Farms salamander (*Eurycea troglodytes*) is likely a complex of several species found in the southwestern part of the Edwards Plateau. Most populations in this species complex are neotenic. However, natural metamorphosis has been observed in populations from several springs and caves. Two groups of populations within this complex are troglomorphic, with enlarged head, and reduced eyes and pigmentation. The morphologically variable population from the type locality (Valdina Farms Sinkhole) was for some time considered a hybrid between *E. neotenes* and *E. tridentifera*. This population is now extinct, but analyses of others have revealed that *E. troglodytes* is genetically distinct from both *E. neotenes* and *E. tridentifera*.

Septentriomolge (Northern Salamanders)

Most populations of these spring-dwelling species from north of the Colorado River were discovered after 1995. The few known prior to 2000, when these species were formally described, had been considered peripheral isolates of *E. neotenes*. Morphologically they resemble spring-dwelling species south of the Colorado River (Blepsimolge), described above. They can be seen year-round, most easily during the spring and summer months, but small juveniles are rarely observed.

The San Gabriel Springs salamander (*Eurycea naufragia*) is known from springs and caves in the drainage of the San Gabriel River, in the vicinity of Georgetown. The adults have prominent eyes with melanophores concentrated around them. The range of the Jollyville Plateau salamander (*Eurycea tonkawae*) is limited to a few drainages on the Jollyville Plateau segment of the Edwards Aquifer. This species makes extensive use of subterranean aquatic habitat, especially when surface spring flow decreases. Adults have well-developed eyes, broad jaws, and blunt snouts. The rare Chisholm Trail salamander (*Eurycea chisholmensis*) is known only from springs at Salado. Compared to *E. tonkawae* and *E. naufragia*, the eyes of this salamander are reduced, and the head is flattened and elongated.

Other Troglobitic and Troglophilic *Eurycea*

Several species of *Eurycea* with overlapping ranges occur syntopically in caves and spring-fed headwaters within the Ozark and Appalachian Highlands and the Interior Lowlands of North America. These include *E. tynerensis*, *E. lucifuga*, *E. longicauda*, and the troglobitic *E. spelaea*.

The grotto salamander (*Eurycea spelaea*) is restricted to the Springfield and Salem Plateaus in the Ozark

region of southern Missouri, extreme southeastern Kansas, and adjacent areas in Arkansas and Oklahoma (U.S.A.). DNA sequence divergence within *E. spelaea* is relatively large, and some of the populations may represent distinct species. This is the only known troglobitic member of the genus that undergoes complete metamorphosis. Adults are known only from caves, but a large fraction of the larval population develops in surface springs and streams. Here they displace the larvae of sympatric *Eurycea* species to more ephemeral parts of the stream and where predatory fishes and crayfish are present. Both adults and larvae are sensitive to floods, which can extirpate a significant proportion of a population.

Adults grow to 7−14 cm in length and are white, pinkish white, or light brown in color (Fig. 2G). The eyes are reduced in size and structure, visible as raised dark spots through the fused eyelids. The larvae are tan dorsally, often spotted or mottled; those developing inside caves can be pale to pink (Fig. 2H). They have relatively small but functional eyes, which degenerate in old larvae or during metamorphosis. The extent of retinal degeneration in adults is related to postmetamorphic age but there is variability in each age group. In its terminal stage, reduction encompasses the entire area of the retina and vision is lost. Individuals raised in light maintain some pigmentation or become pigmented. The larvae may retain vision longer than those kept in darkness and the eyelids in adults do not fuse.

Adults congregate in the main caverns where colonial bats roost, most likely for the purposes of feeding and breeding. They are most active during spring and summer, when moisture levels are high and food is abundant. They may be found climbing moist vertical rock walls outside water, but commonly return to the water to hunt aquatic invertebrates. Besides aquatic and terrestrial invertebrates they also feed on bat guano (Fenolio et al., 2006). In caves where colonial bats have declined, emaciated individuals have been observed. Mating in late spring is followed by oviposition through summer and fall. Larvae metamorphose 2−3 years after hatching, at the size of 5−12 cm, and mature soon afterward.

The sister species of *E. spelaea*, the troglophilic Oklahoma salamander (*Eurycea tynerensis*), is also found on the Ozark Plateau. The species comprises both strictly aquatic neotenic populations and more terrestrial metamorphic populations. Neotenic animals that inhabit caves are often pale, whereas those on the surface retain normal larval pigment patterns. The metamorphs rarely venture far away from water. Usually they inhabit cool, moist habitats both on the surface and in the twilight zone of caves. Larvae may use the karst cavities to move within or between streams.

The troglophilic cave salamander (*Eurycea lucifuga*) occurs in the broad region between southern Indiana, northwestern Virginia, northern Alabama, and northeastern Oklahoma (U.S.A.); this includes the karst areas of the Ozarks, Interior Low Plateau, and the Appalachians. The twilight zone of caves is the preferred habitat of this species, although it can also be found deeper into caves and, despite its name, outside of caves in forested limestone ravines, springs, and spring-fed streams. The species is broadly sympatric with the long-tailed salamander (*Eurycea longicauda*), which is distributed throughout a similar area. This species is also frequently associated with caves and mines. These slender salamanders have very long tails and grow to 18−19 cm. They are very conspicuous, colored yellow or orange, and covered with black spots (Figs. 3A,B). *E. lucifuga* has long limbs and a prehensile tail used in climbing rock walls and ledges. The eyes of this salamander are large and capable of twilight vision. Adults can be found in caves year-round, but their numbers fluctuate seasonally. They aggregate there between spring and fall, when surface temperatures are high. During this time the females deposit their eggs in subterranean waters. Larvae hatch after rains, when water flow increases and more food becomes available (Ringia and Lips, 2007).

Georgia Blind Salamander (*Haideotriton wallacei*)

The geographically isolated troglobitic Georgia blind salamander (*Haideotriton wallacei*) has been found in a few caves pertaining to the Floridan Aquifer below Georgia, Florida, and Alabama (U.S.A.). At least two historic localities have been destroyed by human activities, but cave divers have since spotted this species in the corresponding portion of the aquifer. This species is sometimes included in the genus *Eurycea*.

Adults of this neotenic salamander measure 5−8 cm in length and have little pigmentation, being mostly pink or whitish with scattered melanophores on the back and sides. The head is broad and the snout is long, but not flattened. The limbs are slender and the eyes are almost invisible, embedded in a mass of adipose tissue below the skin. Juveniles are slightly more pigmented and the eyespots are visible.

Haideotriton wallacei can be seen in cave pools and streams especially in caves where bats defecate over or near the water. Salamanders move about slowly, resting on bottom sediments or climbing on submerged limestone sidewalls and ledges. Deeper in subterranean tunnels the salamanders are much less common, presumably because their food (benthic invertebrates) is

FIGURE 3   A few salamanders frequently found in caves. In eastern North America, *Eurycea lucifuga* (A) and *E. longicauda* (B) frequently inhabit the same cave. (C) *Plethodon petraeus* is associated with karst and caves on Pigeon Mountain in Georgia. (D) *Pseudotriton ruber* also frequently uses caves for reproduction (Miller *et al.*, 2008). (E) This undescribed species of *Paramesotriton* inhabits pools in the twilight zone of caves in China. *Photos (A)−(C) by D. Fenolio; (D)−(E) by M. Niemiller.*

scarce. Cave stream sediments have been found in the digestive tracts of *H. wallacei*. One hypothesis attempting to explain silt feeding holds that *Haideotriton* intentionally ingests the material to digest biofilm and microorganisms in the sediments (the same has been observed in young *Proteus anguinus*). Another hypothesis posits that the silt represents failed feeding attempts.

Spring Salamanders (*Gyrinophilus*)

Four North American species are currently recognized. Three species are troglobites, found in caves of southern and central Appalachian Highlands (U.S.A.). In *Gyrinophilus*, paedomorphosis is not universal and has appeared after colonization of caves, although the metamorphosing nontroglobitic species already has an extremely long larval period.

The troglophilic spring salamander (*Gyrinophilus porphyriticus*) is common in and around small streams and springs in eastern North America. Next to *Eurycea lucifuga* this is the most frequently encountered salamander in caves. Both larvae and adults can be found in caves throughout the Appalachian Highlands, even considerable distances from the entrance. These large salamanders, which can grow over 20 cm in total length, are notorious for supplementing their invertebrate diet with conspecifics and other salamanders. Metamorphosed animals are usually orangish-red to salmon and turn darker with age (Fig. 4B), but some individuals of cave populations are pale. The eyes of cave-dwelling individuals do not differ from those from surface populations. A much higher occurrence of ingested debris found in cave-dwelling individuals compared to individuals from surface streams may indicate a lower feeding efficiency in the dark. Larvae, which are also often pale in color, can grow up to 16 cm. They metamorphose after 3−5 years in surface populations but perhaps after 10 years or more in cave populations.

FIGURE 4 Spring salamanders (*Gyrinophilus*). Metamorphosed adults of *G. palleucus* (A) and left in (B) are pale and have smaller, degenerate eyes compared to the troglophile *G. porphyriticus* (right in (B)). The larvae of *G. subterraneus* (C), *G. palleucus palleucus* (D), *G. palleucus necturoides* (E), and *G. gulolineatus* (F) have small but functional eyes. In addition, the numerous neuromast mechanoreceptors on the head and flanks enable them to detect vibrations in the water. *Photos (A)–(B) and (D)–(F) by M. Niemiller; photo (C) by D. Fenolio.*

The three troglobitic species are believed to have arisen independently from a single epigean ancestor similar to *G. porphyriticus* as early as 2.5 million (Pliocene) to as recently as 60,000 years ago (Pleistocene), but precise phylogenetic relationships are obscured due to recent speciation (Niemiller et al., 2008, 2009). Nonetheless, other evidence supports their independent origins and DNA data indicate that divergence has occurred in the presence of continuous or periodic gene flow between subterranean populations and their surface-dwelling progenitor.

The Tennessee cave salamander (*Gyrinophilus palleucus*) is found in caves in the Central Basin, Highland Rim, and Cumberland Plateau of central Tennessee, northern Alabama, and extreme northwestern Georgia. Its range is on the periphery of that of *G. porphyriticus*. Two subspecies of *G. palleucus* are recognized: the pale salamander (*Gyrinophilus palleucus palleucus*; Fig. 4D), which inhabits caves in the Lower Tennessee River watershed in Alabama and Tennessee, and the big mouth cave salamander (*Gyrinophilus palleucus necturoides*), found in the

Collins, Duck, Elk, and Stones River drainages in Tennessee. This form is tan with dark spots dorsally and laterally (Fig. 4E). The subspecies also differ slightly in head width, leg length, eye size, and the number of trunk vertebrae. Except for the lost ability for eye accommodation (focusing), vision is not greatly impaired in *G. palleucus* (Besharse and Brandon, 1973). Exposure to light has no effect on eye development and does not induce any change in skin pigmentation.

G. palleucus inhabits sinkhole-type caves that are rich in nutrients, which support its invertebrate prey base. Besides cave streams, where it is easily observed, the salamander is believed to inhabit subterranean waters inaccessible to humans. It has occasionally been found in springs outside caves. Little is known about the life history of this species. Breeding is most likely seasonal, occurring in late autumn or early winter. This salamander is typically paedomorphic, although metamorphosed individuals have been encountered in at least four caves. Metamorphosed individuals of

G. palleucus differ from adult *G. porphyriticus* in their pale skin, gaunt appearance, longer and narrower snout and smaller eyes (Fig. 4A,B) and in retaining the larval characteristic of an undivided premaxillary bone.

The Berry Cave salamander (*Gyrinophilus gulolineatus*) is known from caves in the Ridge and Valley of eastern Tennessee. Its range is entirely contained within the range of *G. porphyriticus* and the two species sometimes occur in the same cave (Miller and Niemiller, 2008). Like *G. palleucus*, *G. gulolineatus* is usually paedomorphic and generally resembles the former (Fig. 4F), but can be distinguished from it by having a darker pigmentation, wider head, more spatulate snout, and by attaining a greater adult size. The eyes are small and degenerate, comparable in size to the eyes of *G. palleucus*. Unlike *G. palleucus*, however, transformed individuals have fully divided premaxillae, in which they resemble *G. porphyriticus*.

The West Virginia spring salamander (*Gyrinophilus subterraneus*) is known only from General Davis Cave in West Virginia where it co-occurs with *G. porphyriticus*. Unlike *G. palleucus* and *G. gulolineatus*, *G. subterraneus* regularly metamorphoses, although at an exceedingly large size (up to 18 cm). Large larvae have fully developed, mature gonads. Both larvae and adults have a light brownish-pink skin color, overlaid dorsally and laterally by a darker reticulate pattern (Fig. 4C). The head of larval *G. subterraneus* is broader than the heads of either *G. porphyriticus* or *G. palleucus*, but does not have the spatulate snout typical of *G. gulolineatus*. Metamorphosed adults retain fused premaxillae (as in *G. palleucus*). Both larvae and adults have smaller eyes than *G. porphyriticus* and, unlike *G. palleucus*, only weak visual perception.

Although *G. porphyriticus* also occurs in General Davis Cave, only adults of this species have been found (Niemiller et al., 2010). *G. subterraneus* has been observed as far as 2 km into the cave; however, in the first 300 m of the stream passage, predominantly larvae have been encountered, suggesting that its main habitat may be located deeper into the cave. The larvae prefer shallow, calm pools and are found in water depths of 1−20 cm. Adults can be found in shallow water as well, whereas *G. porphyriticus* almost never enters the water. The banks of the cave stream contain thick deposits of decaying leaf litter, washed into the cave by floods. This leaf litter is the source of nutrients for cave invertebrates that adults of both species putatively prey on.

DIVERSITY PATTERNS OF SALAMANDERS FOUND IN CAVES

Including the salamanders described above, over 90 species (approximately 15% of all known salamander species) belonging to five families have been reported from natural or manmade subterranean environments. Their dependence on the cave environment varies not only between species, but also among populations of the same species.

Over half of all species found in caves are reported from eastern North America. Several woodland salamanders (*Plethodon* sp.), especially *Plethodon albagula*, *P. glutinosus*, *P. dorsalis*, and *P. petraeus* (Fig. 3C) are frequently associated with caves. Also reliant on caves are the red salamander (*Pseudotriton ruber*; Fig. 3D), a few dusky salamander species (*Desmognathus* sp.), and the green salamander (*Aneides aeneus*). To the south, in the Mexican Sierra del Madre Oriental and Sierra Madre del Sur, two splayfoot salamanders (*Chiropterotriton* sp.) and two false brook salamanders (*Pseudoeurycea* sp.) occur in caves. Of the salamanders that occur in western North America, the web-toed salamanders (*Hydromantes* sp.) in California, especially the shasta salamander (*Hydromantes shastae*), are often found in caves. Apart from the plethodontids, the mole salamanders (*Ambystoma* sp.) of the family Ambystomatidae are regularly encountered in caves, particularly in the region from Arizona to Alabama and Tennessee. Neotenic cave-dwelling populations of the barred tiger salamander (*Ambystoma mavortium*) have been reported from New Mexico and in gypsum caves of northeastern Oklahoma.

Europe's salamander fauna is dominated by the family Salamandridae, but seven species of the plethodontids also occur here. The European cave salamanders (*Speleomantes* sp. and *Atylodes* sp.) of Sardinia, northwestern mainland Italy, and southern France are closely related to the Californian *Hydromantes* and share many life history features with them. They are strongly associated with caves, and will aggregate in the twilight zone. Although they have prominent eyes, they can also detect prey in darkness using olfactory information. Several surface species of the family Salamandridae frequent natural or manmade subterranean refugia in Europe, especially in the arid circum-Mediterranean region. The widespread fire salamander (*Salamandra salamandra*) not only seeks refuge in caves but also frequently favors them as reproduction sites even when surface water is available nearby. The Pyrenean mountain newt (*Calotriton asper*) inhabits streams, lakes, and ponds along the Pyrenees Mountains of France and Spain, but is also found in subterranean waters, especially on the periphery of its range. Except for one pale-colored cave population, no morphological modifications have been described in this species. Instead, modifications related to cave life involve the reproductive cycle and its periodicity, including prolonged or continuous gamete production, slower egg deposition, and delayed metamorphosis. Neotenic individuals have also been found.

Despite extensive karst areas in Central America and Asia, and great salamander diversity (especially in Central America), the number of salamanders that inhabit caves and other subterranean habitats is low. The caves on the Yucatán Peninsula in Mexico and adjacent Guatemala are the temporal habitat of the plethodontid Yucatán salamander (*Bolitoglossa yucatana*), (perhaps) the nimble long-limbed salamander (*Nyctanolis penix*), and potentially two more species. In western Asia, only one species, of the family Hynobiidae, has been found in a cave. The almost fully aquatic Gorgan mountain salamander (*Paradactylodon gorganensis*) is known from two localities in the Alborz Mountains of northern Iran, one of which is a cave. In eastern Asia, another hynobiid species, the oki salamander (*Hynobius okiensis*) from the Dogo Island of Japan, is believed to reproduce in hypogean waters. The salamandrid wart newts (*Paramesotriton* sp.) have been observed in the twilight zone of caves in Guizhou Province, China.

CONCLUSION

Very few salamanders are obligate cave-dwellers, which is in contrast to the great number of species that temporarily inhabit caves. Ranges of troglobitic salamanders are usually small, limited by hydrological or geological barriers. Due to the limitations such as inaccessibility or rarity, only the more common species have been studied in detail. A fair amount of attention has been given to their phylogenetic relationships, observations on regressive morphological modifications associated with cave life, development, and behavior, but limited knowledge exists of their life histories and ecology. Continuing research in these areas is essential for gaining insight into the origins and processes involved in evolution of obligate subterranean existence.

Because of suspected small population sizes, limited distribution, and high specialization, troglobitic salamanders are threatened by habitat and water quality degradation, caused mainly by urbanization, agriculture, and deforestation. Their position in the food web and the tendency to accumulate energy reserves in their bodies make them particularly vulnerable to the effects of toxic chemicals in contaminated water. Most troglobitic salamanders are legally protected. However, in the face of increasing demands for groundwater, pollution, and alterations to the landscape above cave systems, their numbers continue to decline. Not only troglobitic species but also all salamanders that use caves during part of their life history, are important components of subterranean ecosystems and greatly depend on the preservation of these special habitats.

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SALTPETRE MINING

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DEFINITIONS AND IMPORTANCE

Historically, saltpetre is one of the most strategic of commodities. It occurs naturally in caves and rockshelters, but it is rare. A suite of related nitrates occurs in many caves. The mining and processing of cave nitrate-enriched sediments is a relatively simple endeavor, although labor intensive. The tendency of these sediments to contain a suite of nitrates rather than just potassium nitrate is one reason the archaic spelling *saltpetre* is used in reference to the mining of cave nitrates and the caves in which they occur. This convention is followed throughout this article.

The invention of gunpowder revolutionized weaponry and warfare. Gunpowder, also referred to as black powder, consisted of a mixture of saltpetre, sulfur, and charcoal. Although saltpetre was used in the preservation of meats, the greatest historic demand for saltpetre was during times of insurrection and war. Nowhere has the quest for saltpetre contributed to historic events more than in the United States, where this commodity contributed to both the formation of a country and almost its destruction.

The mineral *niter* (synonym, *saltpeter*) is potassium nitrate (KNO_3). Like many other nitrate compounds, niter is deliquescent; that is, it has a natural tendency to draw water to itself and dissolve into a solution. Although deliquescent minerals can absorb moisture from humid air, they occur naturally in sheltered locations under conditions of low humidity or during periods of reduced humidity. The deliquescent nature of saltpeter is the reason for the old warning of soldiers and frontiersmen, who depended on their firearms for survival, to "keep your powder dry!"

Caves and rockshelters, also termed *rockcastles*, are locations where nitrates may accumulate. Analyses of cave sediments, which were mined historically for saltpetre, commonly reveal no nitrate minerals. The reason is that most of the classical saltpetre caves are in regions where the humidity typically is too high for niter and the even more deliquescent minerals *nitromagnesite*,

$Mg(NO_3)_2 \cdot 6H_2O$, and *nitrocalcite*, $Ca(NO_3)_2 \cdot 4H_2O$, to crystallize into their solid mineral forms. Instead, the saltpetre-rich sediments, historically termed *petre dirt*, contain concentrated viscose nitrate solutions in the form of sediment moisture.

Because nitrate minerals rarely crystallize in most of the known saltpetre caves, other clues to the accumulation of nitrates in cave sediments are important. In the absence of niter, the foremost evidence of significant nitrate concentrations in cave sediment is the presence of *efflorescent crusts*. These white or light-colored powdery crusts commonly are composed of a mixture of soluble salts and minerals, such as gypsum and calcite, that accumulate on cave sediment and rock surfaces as a result of evaporation. Efflorescent incrustations signify locations where periodic atmospheric conditions allow evaporation and the concentration of the minute amounts of dissolved solids in interstitial soil and rock moisture. Precipitation and concentration drive the wicking action of the dissolved solids through soil and rock pores from their respective remote sources. The sources of most saltpetre cave nitrates are the surface ecosystems overlying saltpetre caves.

Recent microbiological work in caves and karst has shown that bacteria are important in cave development (*speleogenesis*) and in the development of the secondary cave mineral forms (*speleothems*) that were thought to be the result of physiochemical reactions (Taylor, 1999). The importance of nitrifying and other bacteria in the accumulation of efflorescent crusts and nitrate accumulations in sediments (petre dirt) is unknown but probably is not trivial.

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The mineral niter (KNO_3) is rarely found in caves, but when observed it occurs as clear to white lint-like fibers, acicular (needle-like) crystals, powder, crusts, coralloid, or flowstone forms. The most extensive form observed in saltpetre caves is the lint-like fibers that occur in dense carpets on bedrock walls (Fig. 1) and sediment-covered walls and floors. These niter fiber occurrences can be harvested with the use of a thin wooden spatula or paddle-like scraper, leaving little or no evidence of extraction. Such a wooden scraper was observed high on a Virginia saltpetre cave ledge before this author had observed an efflorescent niter occurrence.

The majority of the documented saltpetre caves does not normally contain crystalline niter. At the humidities typically found in these caves, the deliquescent nitrate accumulations occur as viscose nitrate solutions in efflorescent crusts on rock and sediment