A new maximum body size record for the Berry Cave Salamander (*Gyrinophilus gulolineatus*) and genus *Gyrinophilus* (Caudata, Plethodontidae) with a comment on body size in plethodontid salamanders

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Abstract

Lungless salamanders in the family Plethodontidae exhibit an impressive array of life history strategies and occur in a diversity of habitats, including caves. However, relationships between life history, habitat, and body size remain largely unresolved. During an ongoing study on the demography and life history of the paedomorphic, cave-obligate Berry Cave Salamander (*Gyrinophilus gulolineatus*, Brandon 1965), we discovered an exceptionally large individual from the type locality, Berry Cave, Roane County, Tennessee, USA. This salamander measured 145 mm in body length and represents not only the largest *G. gulolineatus* and *Gyrinophilus* ever reported, but also the largest plethodontid salamander in the United States. We discuss large body size in *G. gulolineatus* and compare body size in other large plethodontid salamanders in relation to life history and habitat.

Keywords

amphibian, habitat, life history, paedomorphosis, subterranean
Introduction

Body size in amphibians is driven by strong selective pressures, because it interacts with many aspects of life history (Whitford and Hutchison 1967, Blueweiss et al. 1978, Hairston and Hairston 1987, Stearns 1992). Although several ecological and evolutionary mechanisms can be responsible for body size variation in amphibians, overarching patterns are elusive (e.g., Bernardo and Reagan-Wallin 2002, Adams and Church 2008, Slavenko and Meiri 2015). In response to Tilley and Bernardo (1993), Beachy (1995) argues that a primary influence on body size in amphibians is a delay in larval and juvenile period. In general, $K$-selected characteristics are correlated with increased longevity and a shift toward larger propagule size in stable environments. Prolonged developmental periods may promote neoteny (or prolonged maturation) and can be associated with reduced energy demand (McNamara and McNamara 1997). This suggests a possible correlation between increased body size and both paedomorphic and $K$-selected life history strategies. However, the relationship between amphibian body size and these life history strategies is largely unresolved (Yeh 2002, Wiens and Hoverman 2008).

While the reduction of body can be associated with paedomorphic traits (e.g., Alberch and Alberch 1981, Yeh 2002), Wiens and Hoverman (2008) concluded that obligately paedomorphic salamanders (Amphiumidae, Cryptobranchidae, Proteidae, Sirenidae) exhibit larger body sizes compared to those within clades that undergo metamorphosis. This pattern does not seem to translate to paedomorphic species within clades that possess metamorphic or direct-developing species (Wiens and Hoverman 2008). In fact, paedomorphic *Eurycea* (Plethodontidae) associated with springs and caves of the Edward’s Plateau in Texas are characterized by reduced body size relative to their obligately metamorphic congeners, while both metamorphic and paedomorphic *Ambystoma* (Ambystomatidae) share similar body size (Ryan and Bruce 2000, AmphibiaWeb 2018).

Caves and other subterranean habitats are often viewed as extreme and inhospitable environments characterized by an absence of primary production and limited resources (Culver and Pipan 2009). Salamanders are one of only two vertebrate groups to have successfully colonized and obligately live in subterranean habitats. Fourteen species from two families (Plethodontidae and Proteidae) occur exclusively in caves, and most have evolved paedomorphosis (Goricki et al. 2012, in press, Niemiller et al. unpubl. data), which may be a response to limited food resources within terrestrial cave habitats (Brandon 1971, Wilbur and Collins 1973, Ryan and Bruce 2000). Few studies have examined the relationship between cave inhabitation and body size, and changes in body size may not necessarily be associated with shifts from surface to subterranean habitats (Romero 2009, Pipan and Culver 2017). However, many cave-obligate species (i.e., troglobites) exhibit $K$-selected life history traits such as reduced growth rate, delayed sexual maturity, and increased longevity (Brandon 1971, Culver and Pipan 2009, Hüppop 2012), and some troglobites and stygobites are larger than their surface congeners, such as in amblyopsid cavefishes (Poulson 1963, 1985, Niemiller and Poulson 2010).
The plethodontid genus *Gyrinophilus* Cope, 1869 includes four semi-aquatic to paedomorphic species endemic to the highlands of eastern North America. Three species are paedomorphic stygobionts found in caves of the Interior Low Plateau and Appalachians karst regions of Alabama, Tennessee, Georgia, and West Virginia in the United States (Niemiller et al. 2009, Goricki et al. 2012). Here, we report on a Berry Cave Salamander, *G. gulolineatus* Brandon, 1965, from the type locality in Roane Co., Tennessee that exceeds the current maximum body size record for the species and represents the largest *Gyrinophilus* and plethodontid salamander reported in the United States. *Gyrinophilus gulolineatus* is known from just ten localities in the Clinch and Tennessee River watersheds in the Appalachians karst region of eastern Tennessee (Figure 1). The largest *G. gulolineatus* previously reported measured 136 mm snout-vent length (SVL; tip of the snout to the posterior margin of the vent) from the type locality (Brandon 1965, 1966).

**Methods**

As part of an ongoing study on the demography and life history of *Gyrinophilus gulolineatus*, we captured a large *G. gulolineatus* at the type locality, Berry Cave (Tennessee Cave Survey no. TRN3), on 12 August 2018. Berry Cave is located 0.37 km west of the Tennessee River near Wright Bend in Roane County, Tennessee. The main entrance is in a large sink, with the passage from the entrance steeply sloping down to the main stream passage. The passage can be followed downstream to the northeast for ~160 m along the stream until large debris and sediment buildup block further exploration. The stream is characterized by a series of riffles and shallow (<0.5 m) pools with primarily chert, cobble, and coarse gravel substrate and significant amounts of coarse woody debris, detritus, and fine mud and sediment in some areas. The salamander was observed and captured in the margin of a shallow (<0.5 m deep) pool located in a small passage upstream from the main entrance chamber. When first encountered, all but the salamander’s head was out of the water, as it appeared to be moving partially over land to continue upstream.

The salamander was captured with a handheld dip net and immediately transferred to a clear plastic bag for processing. We massed to the nearest 0.5 g using a Pesola® spring scale and measured to the nearest 0.5 mm snout-vent length (SVL; tip of the snout to the posterior margin of the vent) and total length (TL; tip of the snout to the end of the tail) using a metric caliper. The salamander was measured four times by MLN, confirmed by NSG and ETC, and then photographed using an Olympus Tough TG-5 Camera. We also noted any physical abnormalities and the overall health of the salamander. Finally, we marked the salamander by injecting a 1.2 × 2.7 mm visible implant (VI) alpha tag (Northwest Marine Technology Inc., Shaw Island, WA) into the dermis of the tail. The salamander was released at its point of capture following processing.
Figure 1. Geographic distribution of the Berry Cave Salamander (*Gyrinophilus gulolineatus*) in relation to karst adapted from Weary and Doctor (2014). Blue circles represent cave localities from which the species has been reported, and the red star represents the location of Berry Cave. The top right image shows the main stream passage near the entrance of Berry Cave that continues throughout the entirety of our sampling area. The bottom right image shows the large individual captured on 12 August 2018. Photo credits: Matthew L. Niemiller.

To provide a comparison of body size relations across other large-bodied plethodontids, we later compiled a list of maximum body sizes, modes of development, and habitat for several plethodontid salamanders by conducting a search of the primary literature and relevant field guides (see Table 1 and references therein).

**Results**

The *Gyrinophilus gulolineatus* observed and captured at Berry Cave on 12 August 2018 measured 145 mm SVL and 238 mm TL, with a mass of 35 g (Figure 2). Head width measured 22 mm. There was notable damage to the posterior end of the tail, and it is likely that this individual was >250 mm TL before tail tissue loss. Additionally, the two distal-most gill rachises on the right side of the head were notably smaller than those on the left side, while the most proximal right gill rachis was enlarged relative to that on the left side of the head.

A list of maximum body size and total length for several large plethodontid salamanders is reported in Table 1. Based on our literature review, *G. gulolineatus* is the largest plethodontid based on body size (SVL) in the United States, while
Table 1. Mode of development (DD = direct development, m = metamorphic; OP = obligately paedomorphic, FP = facultatively paedomorphic), habitat (AQC = aquatic cave, SAC = semi-aquatic cave, SAT = semiaquatic terrestrial, SUT = surface terrestrial), maximum body size (SVL) and total length (TL) of select plethodontid salamanders based on literature sources and the current study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mode of development</th>
<th>Habitat</th>
<th>SVL (mm)</th>
<th>TL (mm)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolitoglossa dofleini</td>
<td>DD</td>
<td>SUT</td>
<td>130</td>
<td>205</td>
<td>Feder et al. (1982)</td>
</tr>
<tr>
<td>Desmognathus quadramaculatus</td>
<td>M</td>
<td>SAT</td>
<td>103</td>
<td>189</td>
<td>Bakkegard and Rhea (2012)</td>
</tr>
<tr>
<td>Gyrinophilus galolineatus</td>
<td>OP</td>
<td>AQC</td>
<td>145</td>
<td>238</td>
<td>Brandon (1965, 1966), this study</td>
</tr>
<tr>
<td>Gyrinophilus palleucus</td>
<td>OP</td>
<td>AQC</td>
<td>113</td>
<td>186</td>
<td>Lazell and Brandon (1962), Dent and Kirby-Smith (1963), Niemiller et al. (unpubl. data)</td>
</tr>
<tr>
<td>Gyrinophilus porphyriticus</td>
<td>M</td>
<td>SAT</td>
<td>134</td>
<td>221</td>
<td>Brandon (1966), Niemiller et al. (2010), Niemiller et al. (unpublished data)</td>
</tr>
<tr>
<td>Gyrinophilus subterraneus</td>
<td>FP</td>
<td>SAC</td>
<td>117</td>
<td>199</td>
<td>Niemiller et al. (2010)</td>
</tr>
<tr>
<td>Isthmura bellii</td>
<td>DD</td>
<td>SUT</td>
<td>146</td>
<td>327</td>
<td>Smith (1949), Feder et al. (1982), Raffaelli (2014)</td>
</tr>
<tr>
<td>Isthmura gigantea</td>
<td>DD</td>
<td>SUT</td>
<td>161</td>
<td>276</td>
<td>Taylor and Smith (1945)</td>
</tr>
<tr>
<td>Isthmura maxima</td>
<td>DD</td>
<td>SUT</td>
<td>128</td>
<td>244</td>
<td>Parra-Olea et al. (2005)</td>
</tr>
</tbody>
</table>

Figure 2. Dorsal view of the *Gyrinophilus galolineatus* captured at Berry Cave. Photo credit: Matthew L. Niemiller.
only *Phaeognathus hubrichti* attains a greater total length. Body size in *G. gulolineatus* rivals that observed in the direct-developing *Isthmura bellii* species complex endemic to Mexico.

## Discussion

Plethodontid salamanders exhibit considerable variation in life history strategies and habitat that has resulted in an extraordinary range of growth rates and age at maturity (Tilley and Bernardo 1993, Beachy 1995, Beachy et al. 2017). Representative species with notable larger body sizes included in Table 1 represent four primary modes of development in salamanders, with paedomorphic and direct-developing species exhibiting larger body sizes relative to metamorphosing species. Larger species also are correlated with aquatic habitats, apart from the *Isthmura bellii* species complex, which inhabits Neotropical montane forests in southern North America.

Larger plethodontids are likely to occur in well-oxygenated, moist to fully aquatic habitats, which largely relax allometric constraints on gas exchange. This is particularly relevant to those species that exhibit paedomorphic life history strategies. Paedomorphic individuals may be able to grow unimpeded in their permanently aquatic state owing to indeterminate growth. Obligate paedomorphosis has evolved multiple times within Plethodontidae, with the subfamily Spelerpinae having the greatest richness of paedomorphic species (Chippendale 1995; Ryan and Bruce 2000; Bonnet et al. 2014). Additionally, neoteny has been predicted to be the primary causal mechanism of paedomorphosis in salamanders (Duellman and Trueb 1986, Ryan and Bruce 2000). Larger amphibian body sizes are further associated with longer juvenile periods, which significantly covary with age at maturation (e.g., *Desmognathus quadramaculatus* and *Gyrinophilus porphyriticus*, Bruce 1988, Beachy 1995, Beachy et al. 2017).

Many of the largest plethodontid salamanders are direct-developing (e.g., *Phaeognathus hubrichti* in the United States; *Isthmura bellii* in Mexico). Direct-developing species are generally characterized by having larger eggs and longer embryonic development relative to metamorphic or paedomorphic species, and this may related to attaining larger body sizes (Wake and Hanken 2004). There are, however, tradeoffs related to larger body size in these terrestrial plethodontids. The habitat must support gas exchange through adequate temperature and moisture gradients, and these taxa have evolved physiological mechanisms, such as waxy secretions, to reduce water loss. Second, terrestrial environments typically have lower food availability, and, accordingly, terrestrial salamanders often experience more extended periods of inactivity (Jaeger 1979, 1981, Scott et al. 2007). *Phaeognathus*, for instance, has rarely (if ever) been observed outside of burrows in densely forested ravines. Larger body size in such species is in accordance with the ‘starvation hypothesis’ that predicts that greater mass is positively correlated to seasonality and periods of low resource availability (Lundberg 1986), because larger individuals can persist through low-resource events by having
greater energy stores and typically more efficient metabolism owing to positive allometry. The starvation hypothesis has received recent support in multiple amphibian taxa, where body size is positively related to extended inactivity (Valenzuela-Sánchez et al. 2015) and increased precipitation seasonality (Goldberg et al. 2018).

Cave environments are often characterized by low food resources and few natural predators, which likely shaped much of the evolution of many subterranean taxa (Gibert and Deharveng 2002). However, this archetype may not be representative of all subterranean systems, as many caves possess a high surface-environment connection with significant allochthonous organic input (i.e., higher influx of organic matter) driving both terrestrial and aquatic food webs. Cave obligate salamanders often exhibit reduced growth rates and low metabolic demand (e.g., Hervant et al. 2000), and they may also exhibit greater longevity owing to the slow pace of life and low predation pressure associated with subterranean environments (Brandon 1971, Culver and Pipan 2009, Voituron et al. 2011, Hüppop 2012). High resource environments may thus permit more rapid growth and sustain a larger overall body size. The exceptionally large *Gyrinophilus gulolineatus* reported here occurred within 10 m of the cave entrance in a high flow zone with an abundance of organic matter accumulated in the cave pool. Berry Cave is a diverse system relative to other caves in the Appalachian Valley and Ridge (Niemiller et al. 2016), likely due to the large influx of organic matter from the surface. There are a variety of invertebrate taxa that serve as prey for *G. gulolineatus* (e.g., isopods, amphipods, crayfish, flatworms, etc.).

While there has been much focus on life history evolution in salamanders, sampling biases may impact interpretations of the relationship between body size and mode of development. Paedomorphic species may be more difficult to capture, and they are often associated with extreme habitats such as underground springs and caves (Ryan and Bruce 2000, Bonnet et al. 2014). More thorough survey efforts and detailed life history observations within harsher or more isolated environments are necessary to better understand how paedomorphosis may relate to body size in amphibians.

Due to its subterranean existence and cryptic nature, many life history characteristics of *G. gulolineatus* have yet to be documented. Active survey efforts are continuing to assess the species’ demography in Berry Cave, as well as to better understand the growth of this species. Further biological inventory within the Appalachian Valley and Ridge is underway with the intent to uncover additional localities. Future directions for research include additional life history characterization and study of the species’ ecology.

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