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Body Size Is Positively Correlated With Conspicuous Coloration in *Ambystoma* Salamanders, but Negatively Correlated With Conspicuous Coloration in *Plethodon* Salamanders

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Conspicuous coloration coupled with secondary defensive mechanisms is known as aposematic coloration and is used in predator avoidance and defense. Classic theory argues that aposematic signals tend to be more effective in larger organisms as they are intrinsically more easily detected by predators and are also more profitable prey items. Thus, it has been hypothesized that conspicuous coloration evolves in tandem with increased body size in aposematic prey because this likely increases the visibility and efficacy of the signal. To test this hypothesis, we used a comparative phylogenetic approach to investigate associations between body size and conspicuous coloration in two North American salamander genera: *Ambystoma* and *Plethodon*, both of which secrete noxious compounds to deter predators. Our analyses showed that increased conspicuous coloration co-evolved with increased body size in *Ambystoma*, yet we found the opposite relationship in the *Plethodon* clade. We speculate this is likely because *Ambystoma* are generally larger than *Plethodon* and exhibit gregarious mating behavior. Future studies should explore the toxicity of slimy skin secretions and how this may contribute to aposematic signaling in salamanders.

Keywords: aposematism, comparative analysis, independent contrasts, Mesquite, phylogeny

INTRODUCTION

Animals use a variety of visual signals to convey information to one another, including conspicuous coloration (Wallace, 1867). When conspicuous coloration is coupled with a secondary defense, such as venom or poison, color is thought to serve as a signal of unpalatability to potential predators via aposematic signaling (Poulton, 1890). Cott (1940) suggested that aposematic signals typically involve red, yellow, or white patterns on a black background, likely because they are easily detected, recalled, and associated with the defense by predators (Gittleman and Harvey, 1980). It is also

hypothesized that larger animals are more likely to evolve conspicuous coloration compared to smaller animals for several reasons. First, larger animals are intrinsically more obvious to predators than smaller animals and are therefore more likely to evolve secondary defensive mechanisms (Hossie et al., 2015), and are less likely to benefit from cryptic coloration (Hagman and Forsman, 2003). Second, because smaller animals are less easily detected, they are less likely to benefit from conspicuous coloration (Hossie et al., 2015). Third, larger prey items are expected to be more profitable than smaller prey items and thus larger prey should experience stronger selection pressure for defensive mechanisms (Penney et al., 2012). Indeed, experimental evidence from a study examining the responses of naïve domestic chicks (*Gallus gallus domesticus*) to varying sizes of conspicuously colored ground bugs (Lygaeidae) suggests that aposematic signal efficacy increases with increasing body size (Gamberale and Tullberg, 1996a).

There is conflicting evidence in the literature, however, as to whether conspicuous coloration coevolves with increased body size across taxa that employ antipredator coloration. In the caterpillar subfamily Macroglossinae, a phylogenetically-controlled analysis found that conspicuous eye-like markings (eyespots), which are thought to deter predation, are associated with larger body size (Hossie et al., 2015). Using a comparative approach, Hagman and Forsman (2003) found that increased conspicuousness also evolved in tandem with increased body size in poison dart frogs (Family Dendrobatidae). These findings are supported in a subsequent study focusing on a single dendrobatid species, the Strawberry poison dart frog (*Oophaga pumilio*, Rudh, 2013). Strawberry poison dart frogs exhibit remarkable phenotypic diversity throughout their range, and more conspicuous populations also have a larger mean body size. Moreover, phylogenetic relationships suggest that a loss of conspicuous coloration co-evolved with a decrease in body size in several populations (Rudh, 2013). Similarly, a phylogenetic investigation of camouflage morphology in the crab superfamily Majoidea demonstrated that decreased camouflage decoration behavior is associated with an increase in body size as well as increases in alternative defensive strategies such as color change (Hultgren and Stachowicz, 2009). All the aforementioned studies support the hypothesis that conspicuous coloration is positively associated with body size. However, the opposite pattern occurs in nudibranchs, with conspicuous coloration decreasing with increasing body size (Cheney et al., 2014), despite the presence of secondary chemical defenses (indicating their color is used as an aposematic signal). Finally, in lepidopteran larvae there is no apparent relationship between body size and conspicuousness (Nilsson and Forsman, 2003).

In addition to body size, experimental evidence also suggests that the efficacy of an aposematic signal increases with the size of the conspicuous body pattern (see Gamberale and Tullberg, 1996b), and that avian predators (*Parus major*) are better able to discriminate between palatable and unpalatable prey when the pattern elements are larger (Lindstrom et al., 1999). Behavioral traits are also expected to influence the efficacy of aposematic signals. The efficacy of aposematic signals should be positively associated with group size for two potential reasons:

(1) because there is greater initial unconditioned aversion by predators (Gamberale and Tullberg, 1996b, 1998) and (2) because predators will learn more quickly to avoid unpalatable and conspicuous prey (Gagliardo and Guilford, 1993). Finally, conspicuous behavior may both increase the likelihood that the signal is detected and potentially help protect the prey by increasing the likelihood of engaging the predator with any defensive compounds (Brodie, 1977; Toledo and Jared, 1995). For example, in salamanders, many of the granular (defensive secretion) glands are located in the tail (Toledo and Jared, 1995), and most salamanders, when threatened, will position their bodies so that the tail is closest to the threatening stimuli (e.g., Brodie and Gibson, 1969).

Here, we used a comparative approach to investigate relationships between conspicuous coloration and body size in two genera of North American salamanders: *Plethodon* (family Plethodontidae) and *Ambystoma* (family Ambystomatidae). These two genera present an interesting contrast for this type of comparative study: *Ambystoma* salamanders are generally larger than *Plethodon* salamanders and nearly all *Ambystoma* species undertake annual breeding migrations resulting in aggregations, whereas no *Plethodon* taxa are known to migrate or form large aggregations (Petranka, 1998); referred to hereafter as "gregarious behavior." Species within both genera display a diversity of color patterns and body sizes (Petranka, 1998) and their integument secretions are unpalatable to some predators (Brodie, 1977).

The aim of this study is to use hypothesized evolutionary relationships in a comparative analysis of independent contrasts to investigate associations between the evolution of body size and conspicuousness in the *Ambystoma* and *Plethodon* genera. We predicted that larger-bodied species would have more conspicuous coloration. We expected the positive trends between body size and conspicuousness to be more pronounced in the *Ambystoma* genus for three reasons: (1) *Ambystoma* are larger than *Plethodon*, (2) there is more empirical support for toxicity in *Ambystoma* than *Plethodon*, and (3) because *Ambystoma* aggregate during a discrete breeding season (often as short as 1 month) whereas *Plethodon* opportunistically breed during a longer portion of the year (Spring to Fall).

MATERIALS AND METHODS

Data Collection

Our dataset included 50 *Plethodon* species from Wiens et al. (2006) and 17 *Ambystoma* species taken from Williams et al. (2013). To obtain information on conspicuousness for each species, we surveyed 43 and 46 undergraduate students for *Plethodon* and *Ambystoma*, respectively. We obtained photographs of one individual of each species from Amphibiaweb and removed backgrounds from each photograph. We chose this method for two reasons: (1) to prevent bias in conspicuousness ratings due to background color because salamanders may be found on numerous substrates of varying color (moss, leaf litter, soil, trees, rocks) and no data is available indicating the frequency with which each species may be found on each substrate, and (2) because comparable photographs

showing full dorsal coloration for each species were not available on one type of background. We then asked students to rate level of conspicuousness on a scale of 1–10 (1 being least conspicuous and 10 being most conspicuous), then averaged the responses (Summers and Clough, 2001). We acknowledge that attempts to classify coloration as cryptic or conspicuous without accounting for background can be prone to error (Endler, 1978), but felt removing backgrounds was the most objective method given available data and photographs. Although this method uses photographs and human observations to determine conspicuousness rather than the reflectance spectra of animals and visual systems of natural predators and conspecifics, we considered it sufficient for applying the aposematism theory to these taxa because birds are thought to be common predators in North America (Petranka, 1998) and evidence suggests that humans and birds see colors similarly enough that most organisms that appear colorful to humans also appear colorful to birds (Vorobyev et al., 2000). Because birds perceive a greater range of wavelengths (i.e., spectral purity or hue) and are better able to discriminate achromatic differences (i.e., brightness) between two objects (Vorobyev and Osorio, 1998) compared to humans, it is possible this method may underestimate the conspicuousness of integument coloration.

We also compiled a database of maximum and minimum total lengths for adults from each species from published literature (Petranka, 1998; Beane et al., 2010; Mitchell and Gibbons, 2010). We used total lengths rather than snout-vent lengths because tail length is variable between species and because granular cells are most abundant in tail integument tissues. We found that, in both genera, maximum total length and minimum total length were significantly correlated (*Ambystoma*: $r = 0.96$, $n = 17$, $p < 0.001$; *Plethodon*: $r = 0.82$, $n = 50$, $p < 0.001$), and thus used average total length in analyses. Appropriate phylogenetic trees were available for each genus and we obtained phylogenetic information for each species from the published literature (*Plethodon*: Wiens et al., 2006; *Ambystoma*: Williams et al., 2013).

Statistical Analysis
To investigate general relationships between body size and conspicuousness, we compared the average total length of each species with the average observer response. Because closely related taxa cannot be considered statistically independent because of shared ancestry, we calculated independent contrasts (Felsenstein, 1985) using the computer software Mesquite (Maddison and Maddison, 2011) using the PDAP module (Phenotypic Diversity Analysis Programs, Garland et al., 1992). For both *Ambystoma* and *Plethodon* species, we used a gradual model of character evolution and obtained branch length information from Williams et al. (2013) and Wiens et al. (2006), respectively, which was based on the number of substitutions per site in mitochondrial and nuclear DNA sequences. To ensure that the data met the statistical assumptions that body size and coloration evolution are related to phylogenetic data, we then regressed contrasts for conspicuousness on contrasts for body size through the origin for both species and found that the slope did not differ significantly from

zero (Harvey and Pagel, 1991; Garland et al., 1992; Pagel, 1993).

RESULTS

A Mann-Whitney Rank Sum Test demonstrated that *Ambystoma* salamanders are larger than *Plethodon* salamanders (*Mann-Whitney U statistic* = 315, $T = 774$, $n(Ambystoma) = 17$, $n(Plethodon) = 50$, $p = 0.049$; **Table 1**).

Ambystoma
Regressions of independent contrasts showed that conspicuousness was positively correlated to average total length ($R^2 = 0.75$, $n = 16$, $p < 0.001$; **Figure 1**). Because this analysis used independent contrasts, it is likely that these correlations are not simply the result of phylogenetic inertia.

Plethodon
Regression of independent contrasts showed conspicuousness was negatively correlated with average total length ($R^2 = 0.14$, $n = 49$, $p < 0.01$; **Figure 2**). It should be noted that when the largest species is removed (*Plethodon yonahlossee*), the relationship between average total length and conspicuousness changes from $R^2 = 0.14$ ($n = 49$), to $R^2 = 0.29$ ($n = 48$).

DISCUSSION

The results for *Ambystoma* salamanders are consistent with the hypothesis that conspicuousness is associated with body size; larger species tend to be more conspicuous than smaller species. Moreover, phylogenetic patterns suggest relatively recent evolution of conspicuousness in some species (**Figure 3**) and the co-evolution of larger body size and more conspicuous coloration in *Ambystoma*, likely as a consequence of ecological selection pressures. The results for *Plethodon* salamanders, however, are not consistent with this hypothesis; smaller species tend to be more conspicuous. The phylogeny suggests that the ancestral *Plethodon* state is more conspicuous and smaller in body size than current taxa (**Figure 4**), suggesting that evolution has favored increasing body size but not conspicuousness. Although initially these results suggest equivocal support for the hypothesis of coevolution between body size and conspicuousness in salamanders, differences between the genera may explain these discrepancies.

Aposematic colors signal information about unpalatability to potential predators; conventional theory posits that conspicuousness should be positively correlated with unprofitability (Summers and Clough, 2001). There is evidence to suggest that both *Ambystoma* and *Plethodon* are unpalatable to certain predators and that they exhibit behavior consistent with possession of noxious integument secretions (Brodie, 1977). Moreover, *Ambystoma* may be less profitable prey than *Plethodon*. Species of *Ambystoma* are known to produce integument secretions which have strong adhesive properties (Evans and Brodie, 1994), contain neurotoxic components (Hamning et al., 2000) and may be lethal to animals such as mice and voles (Brodie and Gibson, 1969). Although *Plethodon*

TABLE 1 | Average conspicuousness rating, maximum total length, and average total length ± 1 standard deviation for each species of *Ambystoma* and *Plethodon*.

Species	Average conspicuousness rating	Maximum total length (cm)	Average total length (cm)
A. annulatum	5.80	23.5	18.75 ± 4.75
A. barbouri	2.50	17.0	14.00 ± 3.00
A. bishopi	2.63	10.5	9.00 ± 1.50
A. californiense	5.67	22.0	18.50 ± 3.50
A. cingulatum	4.59	13.5	11.25 ± 2.25
A. gracile	2.83	22.0	18.00 ± 4.00
A. jeffersonianum	2.72	18.0	14.50 ± 3.50
A. laterale	4.80	14.0	11.00 ± 3.00
A. mabeei	4.74	12.0	10.00 ± 2.00
A. macrodactylum	8.50	17.0	13.50 ± 3.50
A. maculatum	7.15	25.0	22.50 ± 2.50
A. mexicanum	3.13	30.0	25.00 ± 5.00
A. opacum	6.78	10.7	9.85 ± 0.85
A. ordinarium	3.01	16.5	15.25 ± 1.25
A. talpoideum	3.17	12.0	10.00 ± 2.00
A. texanum	3.11	19.0	15.00 ± 4.00
A. tiginum	9.07	35.0	34.00 ± 1.00
P. albagula	3.10	20.0	15.75 ± 4.25
P. amplus	2.60	18.4	13.70 ± 4.70
P. angusticlavius	6.70	12.5	9.53 ± 3.00
P. aureolus	3.70	15.1	12.55 ± 2.55
P. caddoensis	5.20	11.0	10.00 ± 1.00
P. chattahoochee	3.10	20.0	15.75 ± 4.25
P. cheoah	5.20	18.4	13.70 ± 4.70
P. chlorobyronis	3.10	20.0	15.75 ± 4.23
P. cinereus	6.70	12.5	9.50 ± 3.00
P. cylindraceus	3.10	20.0	15.75 ± 4.25
P. dorsalis	6.70	11.0	8.75 ± 2.25
P. electromorphus	2.50	14.5	11.00 ± 3.50
P. elongatus	3.30	15.0	13.00 ± 2.00
P. fourchensis	3.10	17.8	14.65 ± 3.15
P. glutinosus	3.10	20.0	15.75 ± 4.25
P. grobmani	3.10	20.0	15.75 ± 4.25
P. hoffmani	2.50	13.0	10.40 ± 2.60
P. hubrichti	3.00	13.0	10.50 ± 2.50
P. idahoensis	5.20	13.0	9.00 ± 4.00
P. jordani	6.30	18.5	13.50 ± 5.00
P. kentucki	3.10	17.0	13.25 ± 3.75
P. kiamichi	3.10	20.0	15.75 ± 4.25
P. kisatchie	3.10	20.0	15.75 ± 4.25
P. larselli	5.10	10.5	9.00 ± 1.50
P. meridianus	2.60	18.4	13.70 ± 4.70
P. metcalfi	2.60	18.4	13.70 ± 4.70
P. mississppi	3.10	20.0	15.75 ± 4.25
P. montanus	2.60	18.4	13.70 ± 4.70
P. neomexicanus	2.30	14.3	11.90 ± 2.90
P. nettingi	2.50	11.0	9.50 ± 1.50

(Continued)

TABLE 1 | Continued

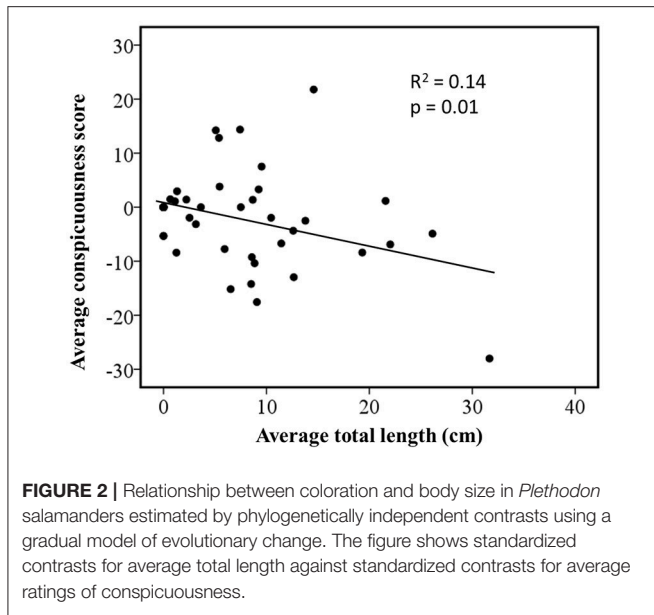| Species	Average conspicuousness rating	Maximum total length (cm)	Average total length (cm)
P. ocmulgee	3.10	20.0	15.75 ± 4.25
P. ouachitae	4.60	17.0	13.50 ± 3.50
P. petraeus	6.00	18.0	14.75 ± 3.25
P. punctatus	3.10	17.0	13.50 ± 3.50
P. richmondi	2.50	14.5	11.00 ± 3.50
P. savannah	3.10	20.0	15.75 ± 4.25
P. sequoyah	3.1	20.0	15.75 ± 4.25
P. serratus	6.7	10.5	8.50 ± 2.00
P. shenandoah	3.9	10.0	8.50 ± 1.50
P. shermani	5.2	18.4	13.70 ± 4.70
P. teyahalee	3.1	20.7	16.40 ± 4.30
P. vandykei	5.9	10.5	10.00 ± 0.50
P. variolatus	3.1	20.0	15.75 ± 4.25
P. vehiculum	5.5	11.0	9.25 ± 1.75
P. ventralis	6.7	11.0	8.75 ± 2.25
P. virginia	3.3	11.2	10.40 ± 0.80
P. websteri	5.9	8.2	7.55 ± 0.65
P. wehrlei	3.9	17.0	13.50 ± 3.50
P. welleri	3.8	9.2	7.80 ± 1.40
P. yonahlossee	8.2	22.0	16.50 ± 5.50
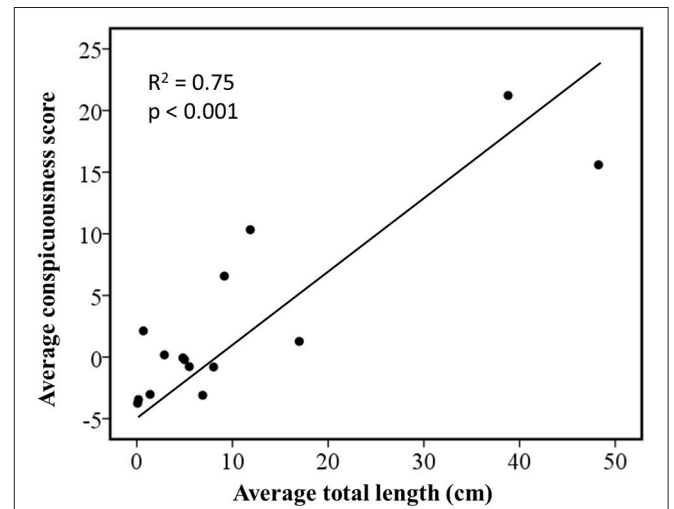
FIGURE 1 | Relationship between coloration and body size in *Ambystoma* salamanders estimated by phylogenetically independent contrasts using a gradual model of evolutionary change. The figure shows standardized contrasts for average total length against standardized contrasts for average ratings of conspicuousness.

species are typically avoided in feeding trials in comparison with other salamanders in Plethodontidae, particularly *Desmognathus* species, their integument secretions have not been shown to be lethal to any potential predators (Hensel and Brodie, 1976; Brodie et al., 1979). For aposematic traits to be adaptive,
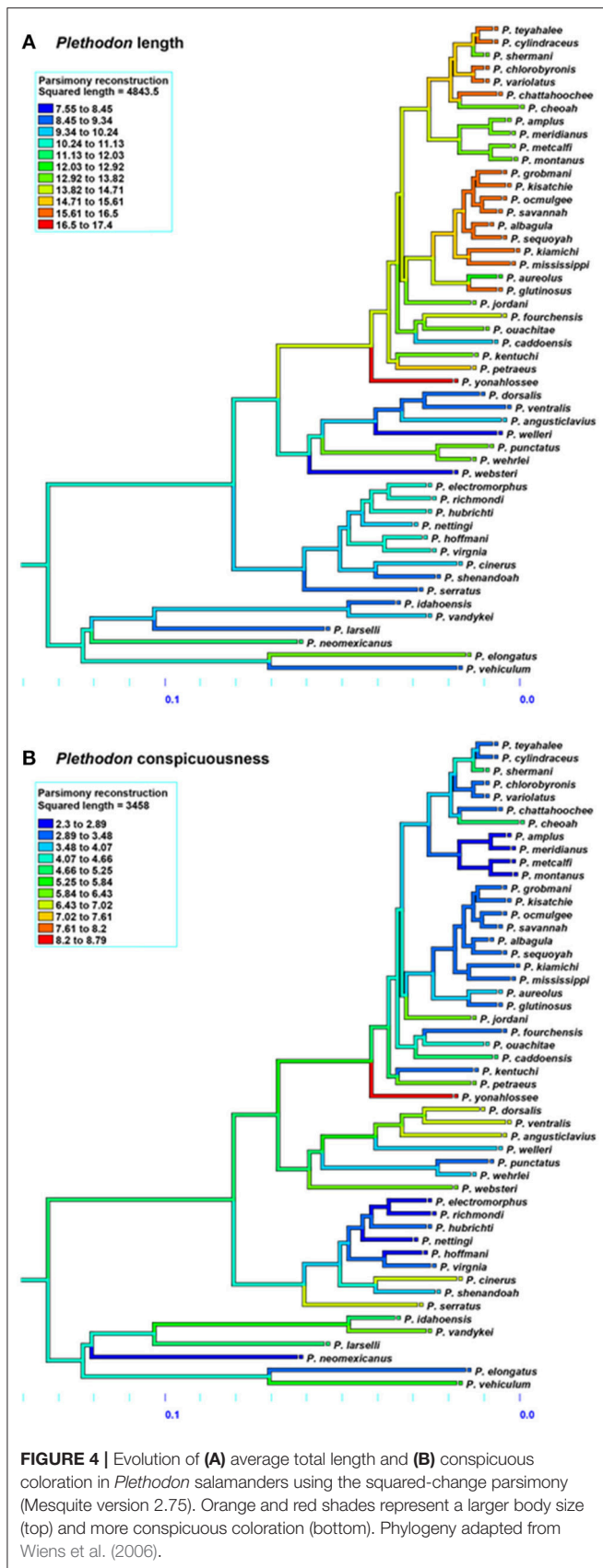
FIGURE 2 | Relationship between coloration and body size in *Plethodon* salamanders estimated by phylogenetically independent contrasts using a gradual model of evolutionary change. The figure shows standardized contrasts for average total length against standardized contrasts for average ratings of conspicuousness.

FIGURE 3 | Evolution of **(A)** average total length and **(B)** conspicuous coloration in *Ambystoma* salamanders using the squared-change parsimony Mesquite version 2.75. Orange and red shades represent a larger body size (top) and more conspicuous coloration (bottom). Phylogeny adapted from Williams et al. (2013).

the benefit of conspicuousness must outweigh the costs. Perhaps, among *Ambystoma,* greater unpalatability coupled with conspicuousness was more adaptive, while, among *Plethodons,* reduced unpalatability coupled with conspicuousness was less adaptive and thus conspicuousness was lost in *Plethodons.* Future work should focus on quantifying unpalatability of these groups.

The efficacy of warning signals increases with increasing body size, thus larger animals should be more likely to evolve conspicuous coloration than smaller animals (Gamberale and Tullberg, 1996a; Hagman and Forsman, 2003; Penney et al., 2012; Hossie et al., 2015). Additionally, larger prey items are associated with greater nutritional benefit and thus may need to allocate more resources to defense mechanisms than smaller prey items (Smith et al., 2016; Briolat et al., 2018). Generally, *Ambystoma* are larger than *Plethodon* (average *Ambystoma* length = 15.9 cm, average *Plethodon* length = 12.7 cm); *Ambystoma* typically have stout bodies with broad heads and tails, while *Plethodon* tend to be slender with narrow heads and tails (Petranka, 1998). Thus, the difference in direction of the covariation between body size and conspicuousness between *Ambystoma* and *Plethodon* genera may be influenced by differences in body size of species in these genera. Perhaps there is a size threshold wherein most *Plethodon* salamanders are too small to benefit from conspicuous coloration while larger *Ambystoma* benefit more from conspicuous coloration and secondary defense mechanisms as they are more distinguishable and likely more profitable prey. This idea is supported by the one exception to the negative relationship in *Plethodon* between body size and conspicuousness: *Plethodon yonahlossee.* The Yonahlossee Salamander (*P. yonahlossee)* is the largest species in the genus, as well as the most conspicuous, and, when removed from the analysis, changes the results rather dramatically. Though the data show that selection in *Plethodon* currently favors large body size and inconspicuous coloration, *P. yonahlossee* has
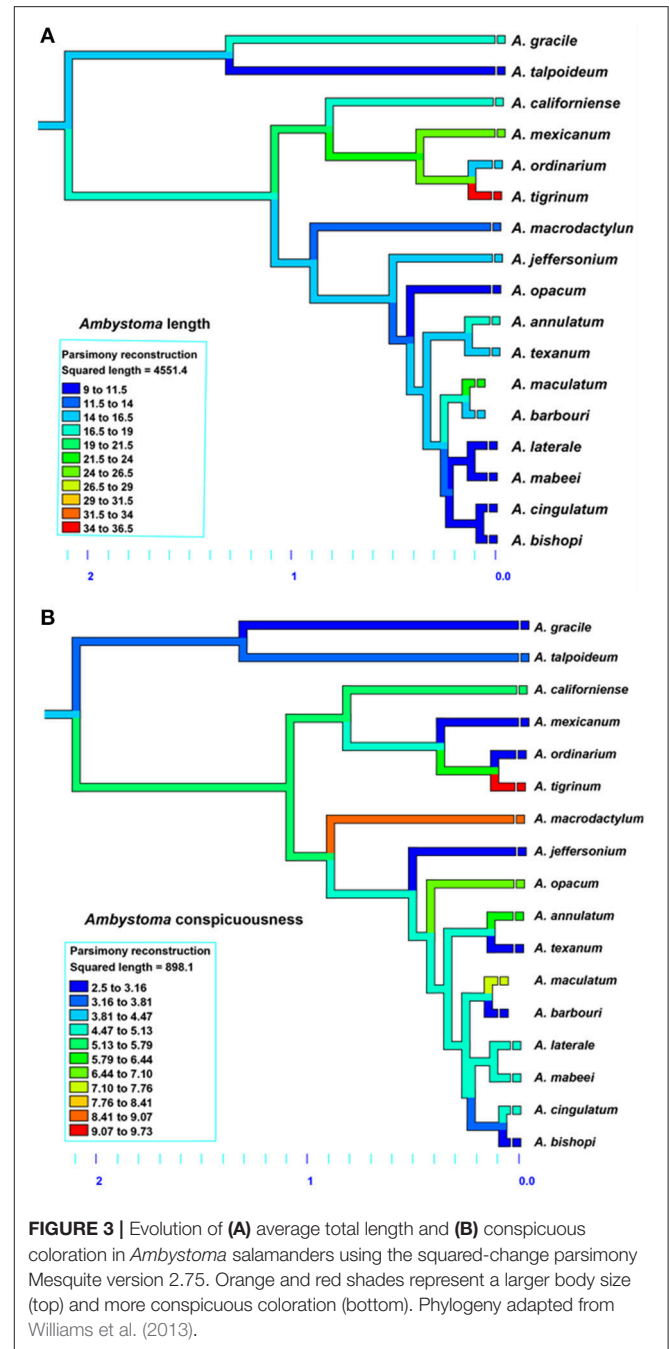
either not undergone the same selection pressures as other *Plethodon* species, or is large enough or has acquired/maintained a defense mechanism potent enough to benefit from conspicuous coloration.

Ambystoma and *Plethodon* also have important lifestyle differences; *Ambystoma* species are largely fossorial with aquatic larvae ($n = 17$) and a few taxa that are fully aquatic (*A. mexicanum, A. ordinarium*, and *A. dumerilii*-a data deficient species excluded from analyses) and inconspicuous (Petranka, 1998). Terrestrial *Ambystoma* exhibit explosive annual breeding

FIGURE 4 | Evolution of (A) average total length and (B) conspicuous coloration in *Plethodon* salamanders using the squared-change parsimony (Mesquite version 2.75). Orange and red shades represent a larger body size (top) and more conspicuous coloration (bottom). Phylogeny adapted from Wiens et al. (2006).

migrations where large numbers of individuals aggregate above ground at least once per year (Petranka, 1998). One exception is the inconspicuously-colored *A. barbouri*, a streamside species that does not travel long distances to breed. Gregarious behavior is thought to amplify aposematic signals, as large numbers of organisms gathered in one place increases the likelihood of being seen and encountered by predators (Poulton, 1890). Models of the evolution of aposematic coloration and gregariousness suggest that they should evolve in tandem in insects (Sillen-Tullberg, 1988), and that gregarious behavior evolved with both chemical defenses and warning coloration in Macrolepidopteran larvae (Tullberg and Hunter, 1996). This is further supported by studies using artificial prey (Alatalo and Mappes, 1996; Mappes and Alatalo, 1997; Riipi et al., 2001).

Species in the *Plethodon* clade, in contrast, are solitary and terrestrial throughout their life cycle but may occur in high densities. Most are active on warm, rainy nights, as well as under leaf litter and cover objects during the day (Brandon and Huheey, 1975; Petranka, 1998). Though they are rarely seen on the surface during the day, some potential predators (such as grouse and turkeys) scratch in the leaf litter and could potentially uncover individuals. Moreover, despite nocturnal activity patterns, species could have evolved warning coloration through selection pressure from being uncovered during the day or from nocturnal predators like owls. Among the largely fossorial caecilians (Amphibia: Gymnophiona), a slight propensity to surface activity coincided with the evolution of bright yellow integument patterns in three separate evolutionary events (Wollenberg and Measey, 2009).

In addition to aposematism, conspicuous coloration can evolve via mimicry of unprofitable organisms (Rudh and Qvarnstrom, 2013). There is evidence of mimicry in other plethodontid species; using clay model simulations, Kuchta (2005) found that the plethodontid salamander *Ensatina eschscholtzii xanthoptica* is likely a mimic of the highly toxic *Taricha* newts. Mimicry of the red eft stage of the eastern newt, *Notophthalmus viridescens viridescens* is the most often explored hypothesis for conspicuous coloration in *Plethodon* (Howard and Brodie, 1971; Brodie and Howard, 1973). Red coloration is common among the *Plethodon* species that received high conspicuousness ratings, and, though most species with red coloration are not entirely red (*N. viridescens* is entirely red), a red patch could be sufficient to induce avoidance in predators that have an established innate or learned avoidance response of *N. viridescens* efts.

Sexual signaling is another possible use of conspicuous coloration (Rudh and Qvarnstrom, 2013) and sexual dimorphism is a general indicator of sexual selection (reviewed in Andersson, 1994). Although there is some evidence of sexual dichromatism in *Ambystoma* (Morgan et al., 2014), there is no evidence in *Plethodon* (Petranka, 1998). Thus, sexual signaling among *Plethodons* seems unlikely. Many *Plethodon* species have evolved an "olfactory" approach to sexual communication, with males using a proteinaceous pheromone stored and dispensed from an enlarged mental gland to increase female receptivity during

courtship (Palmer et al., 2005). Finally, there may be physiological selection pressure for darker (less conspicuous) coloration in amphibians. Melanin pigmentation gives the body a darker color and aids in thermoregulation and UV protection (Trullas et al., 2007). Perhaps evolution favored the loss of conspicuousness in larger bodied *Plethodon* species via physiological pressures to increase melanin content in the integument.

Although salamanders are often conspicuously colored, to date, very little empirical work has addressed the signaling function of elaborate coloration in this group (but see Mochida, 2009, 2010; Kraemer et al., 2015). Using a comparative study, we show that increased body size and increased conspicuous coloration have evolved in tandem in *Ambystoma* salamanders, while increased body size has co-evolved with a loss of conspicuous coloration in *Plethodon* species. Differences between the genera help explain these trends; *Ambystoma* are larger, presumably more noxious, and exhibit gregarious behavior during annual breeding migrations. *Plethodon* are smaller, with evidence indicating they are less noxious than *Ambystoma*, are not known to exhibit gregarious behavior, and several species could potential be imperfect mimics of the toxic *N. viridescens*. More information on toxicity in each genus, and among salamanders in general, will increase understanding of antipredator defense mechanisms as well as the signal function of conspicuous coloration.

AUTHOR CONTRIBUTIONS

MW is the Master's student who compiled data for genus Plethodon, completed data analysis for both genera, and prepared the manuscript. MP assisted in data compilation and editing the manuscript. MO and MG assisted in the initial stages of the project design. LS is the Master's advisor and assisted in project design as well as manuscript edits.

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