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Colour and size reveal hidden diversity of *Necturus* (Caudata: Proteidae) from the Gulf Coastal Plain of the United States

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ABSTRACT

Recent data from the mitochondrial genome reveal six lineages of Gulf Coast Waterdogs traditionally classified as *Necturus beyeri*. Here, we use patterns of colour and body size, along with previously published data, to reveal a large, heavily spotted phenotype with an unstriped larva possessing numerous white spots; we re-describe *N. beyeri* to correspond to this phenotype. We also reveal a small, weakly spotted phenotype possessing an unstriped larva lacking numerous white spots. This phenotype characterises the Apalachicola and Escambia lineages, which current evidence suggests are paraphyletic. We reject taxonomies that place these two lineages in *N. lodingi* because the type specimen of this species appears to be a melanistic member of *N. beyeri*. Therefore, we describe the Apalachicola and Escambia lineages as independent new species.

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Taxonomy; morphology; waterdogs; biogeography; salamanders

Introduction

The genus *Necturus* traditionally contains five species of paedomorphic salamanders restricted to the eastern United States (Highton et al. 2017). Fossil and molecular evidences indicate the genus to be ancient (originating at least 60 mya) but with all extant species being derived within the southeastern United States about 5 mya (Bonett et al. 2013). Two species (*N. lewisi* and *N. punctatus*) inhabit drainages to the Atlantic Ocean and all others are found largely in drainages of the Gulf of Mexico [hereafter, Gulf drainages, with Gulf Coastal Plain referring to Southeastern Plains, Southern Coastal Plain, South Central Plains, and Western Gulf Coastal Plain units of Level III ecoregions of the continental United States (U.S. Environmental Protection Agency 2013) associated with Gulf drainages]. Waterdogs of the Gulf drainages currently include three named species, *N. alabamensis, N. beyeri*, and *N. maculosus* (Highton et al. 2017). These three species are inferred to share a common history, to the exclusion of those of the Atlantic drainages, because they share derived karyotypes possessing 3–6 pairs of telocentric chromosomes (Sessions and Wiley 1985), derived allelomorphs of glucose phosphate isomerase,

Supplemental data for this article can be accessed here

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isocitrate dehydrogenase, 1-lactate dehydrogenase, and malic enzyme (Guttman et al. 1990), and share sequence differentiation of the mitochondrial genome (Chabarria et al. 2017). These evolutionary changes suggest an origin of *Necturus* in the Atlantic Coastal Plain followed by dispersal of an ancestral population to the Gulf Coastal Plain that then diverged into the currently recognised Gulf coastal species.

Clarification of waterdog evolution within the Gulf Coastal Plain has been hampered by competing concepts of the content of *N. beyeri*, by faulty characterisation of the content and geographic extent of N. alabamensis, and by confusion regarding the taxonomic status of N. lodingi, an unspotted species described by Viosca (1937) based on the type series from the Dog River of Mobile Bay, and of problematic status since (Bart et al. 1997). Chabarria et al. (2017) recovered six monophyletic lineages of N. beyeri based on the mitochondrial genome (Figure 1) and the concept of that species presented in Highton et al. (2017), but this species was rendered paraphyletic by a sister-taxon relationship between N. alabamensis (as diagnosed by Bart et al. 1997) and the Mobile lineage of N. beyeri, and unresolved relationships of the Western and Pontchartrain lineages of N. beyeri with N. maculosus. Additionally, the Apalachicola and Escambia lineages of Chabarria et al. (2017) comprise waterdogs of the Gulf Coastal Plain that Hecht (1958) considered to be N. punctatus lodingi, for unspotted and weakly spotted coastal specimens, and to be *N. beyeri alabamensis*, for boldly spotted upland specimens. Likewise, Neill (1963) considered the region occupied by the Apalachicola and Escambia lineages to contain two taxa, a flattened species living under submerged rocks and logs (incorrectly placed in N. alabamensis) and a rounded species living in holes in stream banks (N. beyeri). However, Mount (1975) considered all waterdogs from the Ochlockonee River westward to belong to a single, variable species, N. beyeri. Recently, Dubois and Raffaëlli (2012) placed unspotted specimens from Mobile Bay eastward to the Ochlockonee River in N. lodingi, a taxonomy rejected by Highton et al. (2017), who considered N. lodingi to be a synonym for N. alabamensis. Thus, taxonomic confusion over the identity of waterdogs of the Gulf Coastal Plain, especially for specimens from the Ochlockonee River to the Mobile drainage, hinders study of these organisms.

Colour patterns, especially of larvae, play an important role in distinguishing species of *Necturus*. Two colour phenotypes involve striped larvae. In *N. lewisi*, a single light middorsal stripe is diagnostic of the species (Ashton and Braswell 1979). In *N. alabamensis* and *N. maculosus*, bold light dorsolateral stripes on larvae are diagnostic (Hecht 1958; Bart et al. 1997). All other forms, including all mitochondrial lineages of *N. beyeri*, possess an unstriped larva, but the status of larvae from the type locality of *N. lodingi* has not been described and variation of this phenotype among mitochondrial lineages of *N. beyeri* has not been characterised. Based on the relationships recovered by Chabarria et al. (2017) and parsimony, the unstriped phenotype is thought to have given rise, independently, to the two striped phenotypes. Discovery of additional larval colour phenotypes would expand diagnostic character states that might clarify the taxonomy of Gulf Coastal Plain waterdogs.

Characterisation of species of *Necturus* based on colour phenotypes of adults is less successful. Hecht (1958) used the size and number of dark dorsal, lateral, and ventral spotting to characterise species of waterdogs. Unfortunately, these data were not subjected to statistical analyses that diagnosed distinct populations. Thus, the degree to which adult colour patterns differentiate lineages deserves additional attention.

Body size and shape also can distinguish species within *Necturus*. Bart et al. (1997) used eight linear measurements of adult individuals to distinguish *N. alabamensis* from specimens classified as *N. beyeri*. An elongate head and tail of *N. alabamensis* distinguish this species from *N. beyeri* and likely represent adaptations of *N. alabamensis* to the rocky habitat of the Upper Black Warrior River of north-central Alabama. Similarly, Bonett et al. (2013) recovered body size as an important variable in the evolutionary divergence of *N. punctatus* (small size) from *N. lewisi* (large size) and for divergence of *N. maculosus* (large size) from *N. beyeri* and *N. alabamensis* (small size). Dubois and Raffaëlli (2012) placed *N. beyeri*, *N. lodingi*, and *N. punctatus* in a separate subgenus based largely on small body size. Additional examination of patterns of body size, especially for the Apalachicola and Escambia lineages relative to other lineages of *N. beyeri*, is important for understanding speciation within the group.

Here, we use larval body colour and adult body colour, size, and shape to demonstrate a dichotomy between the combined Mobile, Pearl, Pontchartrain, and Western lineages of Chabarria et al. (2017) and the Apalachicola and Escambia lineages. We use this dichotomy to re-describe *N. beyeri*. We then use accumulated evidence to evaluate the status of *N. lodingi* and to describe the Apalachicola and Escambia lineages as distinct species. We take these actions to stabilise taxonomy for a region where competing taxonomies currently yield confusion.

Materials and methods

We examined 862 specimens attributed to N. beyeri in the Auburn University Museum, Florida Museum of Natural History, Louisiana State Museum of Natural History, Mississippi Museum of Natural Science, Southeastern Louisiana University Vertebrate Museum, and US National Museum of Natural History herpetological collections (Appendix; all museum acronyms from Sabaj 2016). These specimens represented the Apalachicola, Escambia, and Mobile lineages and a composite group containing the Pearl, Pontchartrain, and Western lineages of Chabarria et al. (2017; Figure 1). The latter three lineages were pooled because genetic distances for pairs of specimens between lineages were short enough to cloud phylogenetic processes (e.g. Ballard and Whitlock 2004). We retained the Mobile lineage as a separate group in the analysis to examine the degree to which this lineage might be differentiated from other heavily spotted lineages west of Mobile Bay and to clarify the relationship of the Mobile lineage to N. lodingi and to relatively unspotted lineages east of Mobile Bay. For 232 larval specimens (31–186 mm total length), we recorded each individual as exhibiting one of the following patterns of colour (Figure 2): an unspotted form (0 = no light spots on sides and dorsum) or a spotted form (1 = numerous small light spots on sides and dorsum). For larger individuals, we used external morphological features to discover the smallest individual within each lineage that was a mature male (swollen cloaca; spur-like tip on each side of cloacal opening). Within each lineage, this value was used to create three age-sex categories: juvenile (< SVL of smallest known male), adult male (male features present and \geq smallest male), and adult female (\geq smallest male but lacking morphological features of a male).

For 179 adult individuals, we recorded snout-vent length (SVL; tip of snout to posterior edge of vent) measured with a ruler. We then used photographs of the dorsal, lateral, and ventral aspect of each specimen, along with a mm ruler for scale, to record the following



Figure 1. Geographic distribution and phylogeny of six mitochondrial lineages (boldface) of Gulf Coast waterdogs typically assigned to *Necturus beyeri*. Tree is rooted by *N. punctatus* and paraphyly of *N. beyeri* is shown by inclusion of *N. alabamensis* and two lineages of *N. maculosus*. Phylogenetic construction and lineage names are from Chabarria et al. (2017). Drainages for each lineage are Apalachicola lineage – Apalachicola, Chipola, Choctawhatchee, Econfina, Ochlockonee, and Pea Rivers; Escambia lineage – Blackwater, Escambia (Conecuh in Alabama), Perdido, and Yellow Rivers; Mobile lineage – Dog, Escatawpa, Fish, Mobile, Pascagoula, and Biloxi Rivers; Pearl lineage – Pearl and Wolf Rivers; Pontchartrain lineage – Amite, Bayou Bonfouca, Bayou Lacombe, Blind, Tangipahoa, Tchefuncte, and Tickfaw Rivers; Western lineage – Calcasieu, Cedar Bayou, Old, Neches, Sabine, San Jacinto, and Trinity Rivers.

eight linear measurements (Figure 3; distance measured in Photoshop 6.0): distance from anterior tip of snout to anterior corner of right eye (SRE), width of right eye as distance from anterior to posterior corners (REW), head width as distance between posterior corners of right and left eyes (HWE), snout length as shortest distance from line uniting anterior corner of right and left eyes to tip of snout (SL), postorbital length as distance from posterior corner of right eye to base of insertion of right anterior-most gill (POL), head width as distance between anterior insertion of anterior-most right and left gills (HWG), distance from anterior tip of snout to anterior-most point along gular fold (GL), and widest body width across contralateral costal folds (WBW). Five additional measurements were derived by calculating missing edges associated with right triangles defining



Figure 2. Dorsal colour pattern of *Necturus* juveniles showing unspotted (a) and spotted (numerous white spots; b) character states.

snout, eye, and gill regions or differences between head width measurements (Figure 3). These were snout width (SW; missing edge of right triangle associated with SL and SRE), right eye depth (ED; difference between HWE/2 and SW), right eye length (EL; missing edge of right triangle associated with WRE and ED), gill depth (GD; difference between HWG/2 and HWE/2), and length from posterior eye to level of gill insertion (GEL; missing edge of right triangle associated with POL and GD). Finally, we recorded a count of costal grooves, which were counted as recommended in Highton (1957). Our measurements were selected because they were associated with shape variables revealed by geometric morphometrics (Chabarria et al. 2017). We avoided use of other traditional measurements used to characterise salamander species because Hecht (1958) failed to find differences among recognised species with these measurements.

We also recorded colour patterns of adults from our images. Dorsally, adults were rated for dark spotting on the snout (0 = spotting absent or 1 = present), dark dorsal markings [0 = no dark markings, 1 = small dark spots (no larger than size of eye), or



Figure 3. Measurements taken of adult specimens from dorsal (a; AUM 35138) and ventral (b; AUM 18533) views. Solid lines of panel A are measurements taken from photographs of specimens (HWE, HWG, POL, SL, SRE, WRE; see text for description); dotted lines of panel A are measurements derived from solid lines (ED, EL, GD, GEL, SW; see text for description). Solid lines of panel B are measurements taken from photographs of specimens (GL, WBW; see text for description).

2 = large dark spots (larger than size of eye)], and dark lateral markings (0 = no dark markings, 1 = small dark spots, or 2 = large dark spots; Figure 4). Ventrally, individuals were rated for spotting on the chin (0 = dark spots absent, 1 = spots present along the mandibles, or 2 = dark spots across chin to level of gular fold) and dark ventral markings (0 = belly immaculate, 1 = some dark spots ventrolaterally, or 2 = dark spots across entire belly; Figure 5).

First, we examined patterns of larval colour by generating a contingency table of the four groups (Apalachicola, Escambia, and Mobile lineages plus the combined remaining lineages of *N. beyeri*) crossed with the two categories of colouration (Figure 2). A chi-square test was used to examine differences in colour frequencies among groups. Next, we used canonical discriminant analysis (PROC CANDISC of SAS; SAS Institute Inc. 2008) on the dataset of adult individuals to generate multivariate axes maximising separation of the three lineages from each other and from the combined group of remaining lineages. To remove the effect of differences in overall body size among individuals, each linear measurement was divided by the geometric mean of all linear measurements taken on that specimen (Mosimann and James 1979). Values from the total canonical structure were used to recover the variables that most strongly separated the four groups. For linear variables that loaded heavily on significant canonical axes, we used two-way (sex and group), non-parametric ANOVA (ARTool program in R; R Core Team 2016) of untransformed variables to reveal patterns that might be useful in identifying the four groups in field or museum settings. Nonparametric tests were used because the untransformed variables displayed extensive heteroscedasticity. Sex was included as a fixed effect to reveal patterns of sexual dimorphism within groups, a common feature of size and shape in salamanders. For colour variables that loaded heavily on significant canonical axes, contingency tables (groups crossed with colour categories of Figures 4 or 5) were generated. Proportional colour differences among groups were tested with chi-square analyses, using exact probabilities to account for low observed and expected cell counts (PROC FREQ of SAS).



Figure 4. Dorsal and lateral colour patterns of *Necturus* showing unspotted snout, dorsum, and sides (a), unspotted snout with small dark dorsal and lateral spots (b), and spotted snout with large dark dorsal and lateral spots (c).



Figure 5. Ventral colour patterns of *Necturus* showing immaculate chin and belly (a), limited chin spotting and narrow white mid-venter (b), and spotted chin and belly (c).

Because the multivariate analyses documented that adult colour patterns played a strong role in separation among lineages, we performed a final pair of canonical discriminant function analyses. These tests used the adult colour variables to determine the degree to which these characters can be used to distinguish the Apalachicola lineage from the Escambia lineage; we then repeated the analysis to determine the degree to which colour variables can distinguish the Mobile lineage from the pool of all other remaining lineages of *N. beyeri*. Values of misclassification based on cross-validation (PROC DISCRIM of SAS) were used to assess the utility of these variables in identifying lineages in field and museum settings.

Results

For larvae, we found significant differences in colour pattern among the Apalachicola, Escambia, and Mobile lineages and the pooled remaining lineages of *N. beyeri* (Figure 6; $\chi^2 = 204.2$, df = 3, *P* < 0.0001), with the Apalachicola and Escambia lineages having equivalent frequencies and a strong mode of unspotted juveniles ($\chi^2 = 1.9$, df = 1, *P* = 0.36). Larval colour patterns were similar for the Mobile lineage compared with a composite of all remaining lineages of *N. beyeri* ($\chi^2 = 3.4$, df = 1, *P* = 0.12), with both sharing a high proportion of larvae possessing numerous white dorsal and lateral spots.



Figure 6. Proportion of specimens of the Apalachicola, Escambia, and Mobile lineages, along with a composite group of the remaining western lineages of *Necturus 'beyeri'* conforming to two juvenile colour phenotypes (see text for explanation).

Within the Apalachicola and Escambia lineage, 8% and 2%, respectively, of larvae examined were judged to possess white dorsal spotting, but these spots were not as numerous as those characterising the other two groups. Within the Mobile lineage and the composite of all other lineages of *N. beyeri*, 18% and 7% of specimens, respectively, lacked white dorsal spotting.

Three significant canonical variables were recovered from our multivariate analysis of adult colour and morphology. Canonical variable 1 (F = 10.4; df = 57, 424; P < .0001; 70% explained variance) loaded heavily and positively on COLC, and COLV and heavily and negatively on SVL. This axis separated the Mobile lineage (negative scores; larger size associated with a lack of ventral spotting) from the Apalachicola and Escambia lineages and a group containing all remaining lineages of *N. beyeri* (positive scores; smaller size or large size associated with ventral spotting; Figure 7(a)). Canonical variable 2 (F = 5.7; df = 36, 286; P < .0001; 23% explained variance) loaded heavily and positively on COLL and COLD. This axis separated the Escambia and Apalachicola lineages (negative scores; at most, small spots on dorsum and sides) from the Mobile lineage and the pooled remaining lineages of *N. beyeri* (positive scores; large dark spots on dorsum and sides; Figure 7(a)). Canonical variable 3 (F = 3.0; df = 17, 144; P = .0002; 7% explained variance) loaded heavily and negatively on GL. This axis further separated the Escambia lineage (positive scores; longer size-free gular distance) from the other two lineages plus the pooled remaining lineages of *N. beyeri* (shorter size-free gular distances; Figure 7(b)).

Examination of body size of adults revealed significant differences among the three lineages and the pooled remaining lineages of *N. beyeri* (F = 11.7; df = 3, 142; P < 0.0001) but not between sexes (F = 0.7; df = 1, 142; P = 0.39). There was no significant interaction between sex and lineage (F = 1.6; df = 3, 142; P = 0.19) indicating no size dimorphism among groups (Figure 8). Nevertheless, the Mobile and pooled remaining lineages of *N. beyeri* were larger than the Apalachicola and Escambia lineages.



Figure 7. Plots of canonical variables 1 and 2 (a) and canonical variables 1 and 3 (b). Solid square (blue polygon) = Mobile lineage; solid circle (yellow polygon) = Escambia lineage; solid triangle (red polygon) = Apalachicola lineage; open circles = composite of all western lineages of *N. 'beyeri'*.

A composite score based on dorsal and lateral patterns of dark spotting of adults differed significantly among the three lineages and the pooled remaining lineages of *N. beyeri* ($\chi^2 = 86.4$, df = 12, *P* < .0001; Figure 9(a)). The Apalachicola and Escambia lineages did not differ from each other in adult dorsal pattern ($\chi^2 = 4.8$, df = 3, *P* = 0.16; modal category of small dark dorsal and lateral spots) but the Mobile lineage and the combined remaining lineages of *N. beyeri* differed in this colour pattern ($\chi^2 = 16.2$, df = 3, *P* = 0.0002; stronger mode of large dark dorsal and lateral spots in the remaining lineages of *N. beyeri* than in the Mobile lineage). A composite score for the chin and venter also differed significantly among the three lineages and the composite of remaining lineages of *N. beyeri* ($\chi^2 = 119.1$, df = 12, *P* < 0.0001). Here, the Escambia and Mobile lineages were not significantly different, both sharing venters that lacked dark spotting ($\chi^2 = 1.1$, df = 1, *P* = 0.88). However, the remaining two groups differed in modal colour categories ($\chi^2 = 21.3$, df = 4, *P* = 0.0001; Figure 9(b); mode of chin with mandibular spotting and

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Figure 8. Body sizes (snout-vent length) for the Apalachicola, Escambia, and Mobile lineages, along with a composite group of the remaining western lineages of *Necturus 'beyeri'*. Adult males (M) and females (F) are shown separately within groups. Large dark dots are means; small dashes are individual measurements; outline represents distribution of measurements.

ventrolateral spotting for Apalachicola lineage; mode of spotting across chin and venter in the combined remaining lineages of *N. beyeri*).

When examined across all five colour variables, a statistically significant discriminant function (F = 7.2; df = 6, 20; P = .0003) distinguished the Apalachicola lineage from the Escambia lineage. This function was weighed most heavily by COLC and COLV. Classification error rates based on cross-validation were 15% for the Apalachicola lineage and 21% for the Escambia lineage. Similarly, a statistically significant discriminant function (F = 21.0; df = 6, 130; P < .0001) distinguished the Mobile lineage from the composite of remaining lineages attributed to *N. beyeri*. This function was weighted most heavily by COLC and COLV. Classification error rates based on cross-validation were 11% for the Mobile lineage from the composite of remaining lineages attributed to *N. beyeri*. This function was weighted most heavily by COLC and COLV. Classification error rates based on cross-validation were 11% for the Mobile lineage and 27% for the composite of remaining lineages.

Discussion

Overall, we recovered a clear separation of the Escambia and Apalachicola lineages from all other lineages ascribed to *N. beyeri*. In particular, our data reveal two phenotypes of larvae previously categorised as unstriped. One lacks evidence of striping and lacks numerous small white spots. This phenotype characterises the Escambia and Apalachicola lineages as well as *N. punctatus*. The association of this phenotype with these two lineages is strong, with less than 10% of larvae from this region showing evidence of white spotting. Based on phylogenetic relationships inferred by Sessions and Wiley (1985) and Guttman et al. (1990), we assume this phenotype represents the ancestral condition for the genus. The second



Figure 9. Proportional representation of composite score for COLD and COLL (a; category 2 = no dark dorsal or lateral spots; category 3 = small dark dorsal spots with no dark lateral spots or small dark lateral spots with no dark dorsal spots; category 4 = small dark dorsal and lateral spots; category 5 = small dark dorsal spots with small dark lateral spots; category 6 = large dark dorsal and lateral spots) and composite score for COLC and COLV (b; category 2 = no dark spots on chin or venter; category 3 = dark chin spots on mandibles and no dark spots on venter or no dark spots on chin but small dark ventrolateral spots on venter; category 4 = dark chin spots on mandibles and small dark ventrolateral spots on venter; category 5 = dark chin spots on venter; category 4 = dark chin spots on wenter; category 4 = dark chin spots on mandibles and small dark spots across entire chin and small dark ventrolateral spots on venter; category 5 = dark chin spots across venter; category 6 = dark spots across chin and venter). Colour categories are shown for Apalachicola, Escambia, and Mobile lineages and a composite of all western lineages of *N. 'beyeri*'.

larval phenotype is characterised by a lack of striping and presence of numerous white spots, which we infer is a derived condition evolving first in the common ancestor of the Mobile, Pearl, Pontchartrain, and Western lineages of *N. beyeri* and then is retained by all those lineages. Again, the association of this phenotype with these lineages is strong, with less than 11% of larvae lacking evidence of white spotting. Given the utility of larval colour

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patterns in diagnosing other species of *Necturus*, we propose the white-spotted larva as an autapomorphy diagnosing *N. beyeri*. Our analysis of adult body size indicates that this revised concept of *N. beyeri* has larger average adult body sizes than the Apalachicola and Escambia lineages. Additionally, adult body patterns indicate that the revised concept of *N. beyeri* contains lineages that differ statistically in the size and distribution of spotting relative to the Apalachicola and Escambia lineages.

Genetic distances between the Mobile, Pearl, Pontchartrain, and Western lineages are short (Chabarria et al. 2017), corresponding to differentiation among populations that may not reflect phylogenetic history (Ballard and Whitlock 2004). For this reason, additional study is needed to determine whether these mitochondrial lineages are populations within a single metapopulation lineage or are multiple cryptic species. Genetic distances between these lineages and N. alabamensis and N. maculosus also are short. However, N. alabamensis and N. maculosus share a derived striped larva, a feature that we use to exclude these two species from the Mobile, Pearl, Pontchartrain, and Western lineages of N. beyeri. This interpretation is bolstered by the observation that N. alabamensis and the Mobile lineage of N. beyeri occupy the same major drainage but show no evidence of hybridisation [narrow zone of sympatry with no evidence of intermediate larval colouration (Bart et al. 1997); apparent morphological adaptation of N. alabamensis to swift water with abundant slab-rock crevices (Bart et al. 1997); reciprocal monophyly of the mitochondrial genome of specimens from above and below the zone of contact (Chabarria et al. 2017)]. We interpret this evidence to support the hypothesis that N. alabamensis and the Mobile lineage of N. beyeri are two biological species rather than representing metapopulation structure within a single species. For these reasons, we retain N. alabamensis and N. maculosus as separate species. This interpretation suggests that the molecular clock within Necturus is ticking at a slower rate than is observed for other salamander species of the Gulf Coastal Plain (e.g. Wray et al. 2017) and, therefore, that the short divergence distances are not necessarily indicative of differentiation among populations within a single species. Our analysis of the Mobile lineage of N. beyeri demonstrates it to have lower modal size, a tendency to possess smaller dark spotting of the dorsum, and a tendency to have unspotted chin and belly regions, compared with aggregated data from the Pearl, Pontchartrain, and Western lineages. Thus, our concept of N. beyeri may mask further speciation within the group, but we refrain from recognising additional taxa within our concept of N. beyeri until more precise measures of gene flow among lineages are available and clearer evidence of diagnostic features emerge.

Our data for waterdogs from the region occupied by the Apalachicola and Escambia lineages reveal that these salamanders are similar to *N. punctatus* in small body size, limited number and size of spotting in adults, and lacking numerous white spots as larvae. Based on our use of larval spotting to diagnose *N. beyeri*, the Apalachicola and Escambia lineages must be excluded from that species. Similarly, the Apalachicola and Escambia lineages are excluded from *N. punctatus* by derived numbers of telocentric chromosomes, derived degree of heterochromatism of the Y chromosome, derived allelomorphs of blood enzymes, and similarities of the mitochondrial genome that unite these two lineages with the rest of the Gulf coastal waterdog species to the exclusion of *N. punctatus* (Sessions and Wiley 1985; Guttman et al. 1990; Chabarria et al. 2017). This evidence supports placement of the Apalachicola and Escambia lineages in some other species than *N. punctatus* or our revised concept of *N. beyeri*, such as *N. lodingi*, or in newly

erected species. We note that adult *Necturus* are not known to cross overland barriers and are intolerant of saltwater, isolating lineages within river basins that have limited opportunities to experience dispersal from other drainages, except for rare events such as stream capture. These natural history observations provide further support for the hypothesis that the Apalachicola and Escambia lineages are species with short sequence divergence distances rather than representing divergent populations within a single species.

Viosca (1937) described N. lodingi for unspotted waterdogs collected from Eslava and Hall's Mill Creeks, major components of the Dog River, a small (230 km²) drainage in Mobile County, AL. From the same locality, Viosca (1937) included specimens of a phenotype with large dark spots in the type series of N. beyeri (Mobile lineage). Dubois and Raffaëlli (2012) broadened the concept of *N. lodingi* to include the entire range occupied by the Apalachicola and Escambia lineages (lower Mobile drainage eastward to the Ochlockonee River). However, this concept of N. lodingi is rendered paraphyletic by relationships of the Escambia and Apalachicola lineages to each other and to other waterdog species of the Gulf Coastal Plain (Figure 1). Additionally, despite efforts to confirm the presence of two sympatric species in Eslava and Halls Mill Creeks, only specimens known to be of the darkspotted Mobile lineage have emerged and all larval specimens taken from these sites display numerous white spots. Thus, no evidence confirms that an unspotted species with an unspotted larva is present in the Mobile drainage and that it is referable to either the Apalachicola or Escambia lineages. Therefore, we reject the concept of N. lodingi presented by Dubois and Raffaëlli (2012). Highton et al. (2017) consider N. lodingi to be a synonym of N. alabamensis, but this designation appears to conflict with Bart et al. (1997) who restricted N. alabamensis to dorsoventrally flattened, elongate waterdogs possessing striped larvae in the Upper Black Warrior drainage. Thus, we reject the concept of N. lodingi presented in Highton et al. (2017). Instead, we conclude that the Apalachicola and Escambia lineages represent unnamed species that have phylogenetic affinities with the heavily spotted waterdogs to the west rather than with the phenotypically similar N. punctatus to the east. The two lineages differ statistically in ventral colour pattern such that specimens from the Apalachicola, Choctawhatchee, Pea, and Ochlockonee Rivers (Apalachicola lineage) tend to have dull bellies created by invasion of dark spotting ventrolaterally. Specimens from the Perdido, Escambia, and Yellow Rivers (Escambia lineage) have brilliant white bellies lacking ventrolateral spotting. Our data indicate that, based on these colour features, Apalachicola lineage specimens are misidentified approximately 15% of the time while those of the Escambia lineage are misidentified approximately 21% of the time. Thus, the frustration expressed by Mount (1975) in use of colour characters for diagnosing species from the region occupied by the Apalachicola and Escambia lineages likely remains but is perhaps tolerable given the challenge of eliminating uncertainty in the identity of specimens from this region. We view the challenge of distinguishing the Apalachicola and Escambia lineages to be no greater than that involved in distinguishing Desmognathus apalachicolae from D. conanti or in distinguishing Eurycea hillisi and E. sphagnicola from these same drainages (Means and Karlin 1989; Wray et al. 2017).

If the Apalachicola and Escambia lineages are distinct species, then we are still left with clarifying the status of *N. lodingi*. We see four hypotheses to explain our inability to document this species from the type locality. One is that *N. lodingi* is a distinct, but rare species. We find this hypothesis to be implausible because it requires sympatry with

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N. beyeri of the Mobile lineage, a feature that is rare in waterdogs, and requires restriction of the species to the type locality. The second hypothesis is that N. lodingi represents migrants of the Escambia lineage. Mount (1975) hypothesised movements of musk turtles (Sternotherus) between the Escambia and Mobile drainages, and this process might be invoked to explain rare occurrences of unspotted Necturus in drainages of Mobile Bay. However, colour patterns of the type specimen of *N. lodingi* are inconsistent with the colour patterns we have documented for the Escambia lineage. In particular, the chin of the type for N. lodingi shows extensive pigmentation along the mandibles and across the anterior portion of the chin (Viosca 1937), features found in no specimen of the Escambia lineage. Similarly, the type specimen of *N. lodingi* has ventrolateral pigmentation that nearly covers the venter, a feature observed in no specimen of the Escambia lineage. Therefore, we find it unlikely that N. lodingi represents dispersing members of the Escambia lineage. A third hypothesis posits that the type specimen of N. lodingi represents a hybrid caused by dispersal of the Escambia lineage into Mobile Bay. Although this hypothesis does characterise gene flow within Sternotherus of the Gulf Coast (Scott et al. 2018), we find no evidence of clinal variation within Necturus between the Escambia River and Mobile Bay, especially of the larval phenotype, a pattern expected of hybridisation. The final hypothesis, that unspotted specimens of *Necturus* from the Dog River are aberrant colour morphs of the Mobile lineage, is judged to be plausible because the type specimen of N. lodingi is most similar in colour pattern to MMNS 1261, a melanistic specimen of the Mobile lineage from Black Creek, Lamar County, MS (Figure 10). These specimens share an immaculate dark dorsum, extensive ventrolateral dark markings, and dark chin markings, leading us to conclude that the type of *N. lodingi* is a melanistic member of the Mobile lineage. Bart et al. (1997) also noted that the type of N. lodingi appears to be melanistic. Thus, we conclude N. lodingi is a junior subjective synonym of N. beyeri.

Based on the conclusions above, the taxonomy of *Necturus* of the Gulf Coastal Plain requires revision. Below, we re-describe *N. beyeri* and describe the Apalachicola and Escambia lineages as new species.

Species descriptions

Necturus beyeri Viosca (1937)(Figures 11–12) Necturus lodingi Viosca 1937 Necturus maculosus beyeri Schmidt, 1953, in part Necturus beyeri beyeri Hecht 1958 Necturus punctatus beyeri Brode, 1969, in part Necturus (Parvurus) beyeri Dubois and Raffaëlli, 2012

Holotype. USNM 102674 (Figure 11(a,b)), an adult female from the Upper Calcasieu River at Oakdale (no exact locality given), Allen Parish, LA. This locality is within the range of the Western lineage of Chabarria et al. (2017).

Paratypes. USNM 102676; MCZ 17732, 17733 are listed in the original species description (Viosca 1937). To these we add representatives from within the ranges of the Mobile (AUM



Figure 10. Type specimen of *N. lodingi* (USNM 61752; Mobile County, AL) dorsum (a) and venter (b; photos by Jenna L. Welch, Division of Amphibians and Reptiles, National Museum of Natural History, Smithsonian Institution; used with permission); melanistic Mobile lineage specimen (MMNS 1261, Lamar County, MS) dorsum (c) and venter (d; photos by Craig Guyer).

40150), Pearl (MMNS 1288, LSUMZ 62933, SLU 744), and Pontchartrain (LSUMZ 99424, SLU 6211, 6213) lineages (Figure 11(c-h))

Diagnosis. Membership of this species in the genus *Necturus* is demonstrated by retention of external gills in adults, presence of pigmented skin, and reduction of digits on hind limbs to four toes. Our concept of *N. beyeri* contains specimens of the Gulf Coastal Plain possessing a larva with numerous small white spots and lacking dark stripes (Figure 2(b)).

Comparisons. Juveniles of *N. beyeri* have small white punctations, an apparent autapomorphy that distinguishes this species from *N. alabamensis* and *N. maculosus* (larvae with two light dorsolateral stripes), *N. lewisi* (larvae with light middorsal stripe), and *N. punctatus* and the Apalachicola and Escambia lineages (larvae lacking stripes and white spots). Adults of *N. beyeri* typically possess large, bold, dark dorsal and lateral spots and retain the white punctations of the juveniles, creating a particularly colourful phenotype that distinguishes *N. beyeri* from *N. punctatus* and the Escambia and Apalachicola lineages. Along with *N. maculosus*, the Western lineage of *N. beyeri* has 6 pairs of telocentric chromosomes and an increased level of heterochromatin in the Y chromosome of males that differentiate these taxa from *N. lewisi* and *N. punctatus* (reduced heterochromatin, no telocentric chromosomes), and from the Apalachicola



Figure 11. Type specimen of *N. beyeri* (USNM 102674; Allen Parish, LA) dorsum (a) and venter (b; photos by Jenna L. Welch, Division of Amphibians and Reptiles, National Museum of Natural History, Smithsonian Institution; used with permission); paratype of Mobile lineage (AUM 40150, Covington County, AL) dorsum (c) and venter (d; photos by Craig Guyer); paratype of Pearl lineage (MMNS 1288, Saint Tammany Parish, LA) dorsum (e) and venter (f; photos by Craig Guyer); paratype of Pontchartrain lineage (LSUMZ 99424, Tangipahoa Parish, LA) dorsum (g) and venter (h; photos by Craig Guyer).

lineage (4 pairs of telocentric chromosomes, intermediate levels of heterochromatin in Y chromosome; Sessions and Wiley 1985). Additional sampling of the Escambia, Mobile, Pearl, and Pontchartrain lineages are needed to demonstrate the complete distribution of these character states.

Emended description of holotype. In preservation, the type specimen is 211 mm TOT, 144 mm SVL, and 67 mm TL; number of costal grooves is 16. The dorsal ground colour in preservation is slate brown with numerous white punctations across the entire body. The head has round dark-brown spots that are small anteriorly (about the same size as eye) and enlarged and bolder posteriorly (starting at insertion of gills). The head lacks a dark stripe from the nostril through the eye. The enlarged, bold, dark brown spots continue along the dorsum and sides of body to the tip of the tail. The ground colour of the venter is uniform tan, lacking the white punctations along the mid-venter but with such punctations gradually appearing ventrolaterally. The chin has small brown spots on skin covering the



Figure 12. Distribution of localities of specimens assigned to *N. beyeri*. Open circle is approximate location of type specimen.

mandibles; spots are absent from the midventral skin of the chin. The venter has small brown spots midventrally, becoming enlarged laterally. The holotype has the following values for measured morphological features described above – ED: 2.9 mm; EL: 1.8 mm; GD: 4.7 mm; GEL: 20.6 mm; GL: 26.1 mm; HWE: 18.8 mm; HWG: 28.2 mm; POL: 21.1 mm; REW: 3.4 mm; SL: 8.1 mm; SRE: 10.4 mm; SW: 6.5 mm; WBW: 21.8 mm.

Variation. Necturus beyeri achieves relatively large adult sizes, with a maximum male size of 184.0 mm SVL (+68 mm TL) and a maximum female size of 177.0 mm SVL (+66 mm TL). Mean male size is 134.2 mm SVL (n = 53) and mean female size is 120.8 mm SVL (n = 94). In dorsal aspect, the modal adult lacks snout spotting (73% of specimens), has large dorsal (66% of specimens) and lateral (74% of specimens) spotting, and lacks chin (61% of specimens) and ventral (45% of specimens) spotting. The Pearl lineage diverges from this modal pattern in having a high percentage of snout (63%), chin (54%) and ventral (56%) spotting, and the Mobile lineage diverges from the mode in having a high percentage of small dorsal (54%) spotting and no chin (93%) or ventral (84%) spotting. The modal value for costal grooves is 17 (n = 147), with 35% of specimens possessing 16 grooves, and 2% possessing 18.

Adult males (n = 53) have the following mean (and range) values for measured morphological features – ED: 1.2 mm (0.1–5.3); EL: 1.8 mm (0.1–3.8); GD: 3.3 mm (0.9–6.2); GEL: 16.1 mm (10.4–2.2); GL: 21.1 mm (13.1–34.1); HWE: 12.6 (8.0–23.7); HWG: 19.5 (12.-8–36.1); POL: 16.5 mm (10.6–26.6); REW: 2.4 mm (1.5–4.2); SL: 6.9 mm (3.2–12.0); SRE: 8.7 mm (4.9–14.7); SW: 5.2 mm (2.0–9.7); WBW: 19.0 (12.0–37.4).

Adult females (n = 94) have the following mean (and range) values for measured morphological features – ED: 0.8 mm (0.1–4.3); EL: 1.9 mm (0.1–4.3); GD: 2.9 mm (1.4–6.8);

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GEL: 14.3 mm (7.1–27.9); GL: 19.1 mm (10.8–34.4); HWE: 11.2 mm (11.2–20.9); HWG: 17.0 mm (8.9–34.5); POL: 14.6 mm (7.3–28.5); REW: 2.3 mm (1.2–4.3); SL: 5.7 mm (2.7–12.4); SRE: 7.6 mm (3.5–14.6); SW: 4.9 mm (2.0–10.2); WBW: 16.7 mm (8.0–36.8).

Etymology. The specific epithet is a patronym for George E. Beyer, a naturalist at Tulane University early in the 1900s who provided summaries of the herpetofauna of the state of Louisiana. The recommended English common name is Western Waterdog.

Distribution and natural history. Our re-description of *Necturus beyeri* restricts each lineage to the following drainages: Mobile lineage – Mobile (AL) to Biloxi (MS); Pearl lineage – Wolf (MS) to Pearl (LA); Pontchartrain lineage – Bayou Bonfouca to Blind River (LA); Western lineage – Calcasieu (LA) to West Fork of the San Jacinto River (TX; Figure 12).

No life history study has been performed on the Mobile lineage. Specimens are known from drainages entering Mobile Bay, where these salamanders can be found in dipnet samples of leaf beds. Farther north, we have sampled them from rocky reaches of the Tallapoosa River and from leaf packs under bridges of the Coosa River. The species is found in the same drainage as *N. alabamensis*, but with the Mobile lineage occupying slower-moving waters of the Coastal Plain, and N. alabamensis occupying swifter waters of the terminus of the Sand Mountain formation. The two species are both present in the Black Warrior River just east of Northport Lock and Dam, Yellow Creek at Lake Nichol Road crossing, and the North River near Samantha, all in Tuscaloosa County, AL (Bart et al. 1997). These represent the only cases of sympatry involving Gulf Coast waterdogs. We examined no specimen that appeared to represent a morphological hybrid. Gunter and Brode (1964) collected specimens from the Biloxi and Pascagoula drainages, mostly on hooks baited with earthworms. Undercut banks or overhanging stumps and tree trunks associated with deep pools are key habitat features associated with this lineage (Gunter and Brode 1964). Use of litter bags might increase detection of the Mobile lineage in occupied streams. From this sampling technique, Eurycea cirrigera, E. quadridigitata, and Desmognathus conanti are known associates (Lamb and Qualls 2013).

Within the Pearl lineage, males have swollen cloacal openings and motile sperm in December and January, and detection of gravid females increases during November and December, when mating likely occurs (Shoop 1965). Undercut banks or overhanging stumps and tree trunks associated with deep pools are key habitat features associated with detection of females and nests. Clutch size averages 57 eggs. Eggs are retained by females and clutches are deposited in April or May in deep water under rocks, logs or other sunken objects. Females attend the nests and can store sperm for at least six months after mating (Sever and Bart 1996). Juveniles and subadults eat isopods, midges, and mayflies while adults feed on mayflies and caddisflies; prey consumption decreases in warm months when leaf litter used by detritivorous prey is decreased (Bart and Holzenthal 1985). Specimens from coastal areas may occasionally be eaten by blue crabs (Gunter and Brode 1964). The Pearl lineage often is infested with acanthocephalan parasites, for which waterdogs are a definitive host. These salamander hosts likely become infected via consumption of isopods, the intermediate host (Bart and Holzenthal 1985).

No life history study has been performed on the Pontchartrain lineage. However, recent study of a dense population in Bayou Lacombe has documented the presence of

Batrachochytrium dendrobatidis and *B. salamandrivorans* in populations of this lineage (Glorioso et al. 2017).

Brenes and Ford (2006) provide the only published field study of the Western lineage of *N. beyeri*. They examined the species in Gilley and Hill Creeks of Smith County, TX where minnow traps placed along stream banks were used to capture waterdogs. The lineage was detected more frequently during November through January; none was detected during May, June, July, and August. This pattern was associated with a negative relationship between captures and water temperature, with no captures occurring when water temperatures exceeded 18°C. Reduction of activity at this temperature is similar to that reported for *N. lewisi* by Braswell and Ashton (1985), suggesting similar thermal tolerance within the entire genus. Recaptured individuals typically were found within 20 m of the initial capture, but distances of up to 230 m were observed between captures. Males retained in aquaria showed behaviours consistent with defence of underwater refugia during January and February, the presumed breeding period for free-ranging individuals. Animals captured in the field were associated with sandy- or gravel-bottom regions, typically with logjams.

Remarks. The collection locality of the holotype likely is where LA highway 10 crosses the Calcasieu River west of Oakdale (30.822529 -92.684595; WGS84).

Necturus mounti sp. nov.(Figures 13–14) Necturus punctatus lodingi Hecht, 1958, in part Necturus beyeri alabamensis Hecht, 1958, in part Necturus alabamensis Neill 1963, in part and misidentification Necturus beyeri Mount 1975, in part Necturus cf beyeri Bart et al. 1997, in part Necturus (Parvurus) lodingi, Dubois and Raffaëlli, 2012, in part Necturus cf lodingi Escambia lineage, Chabarria et al. 2017

Holotype. AUM 40698 (Figure 13), a female collected 14 February 2014 at Camp Creek where it crosses Conecuh National Forest Road 332 (31.16427–86.53378; WGS84), Covington County, AL, by David Laurencio.

Paratypes. AUM 37483 (a female), FLMNH 68839 (a male), and MMNS 1248 (a male).

Diagnosis. Membership of this species in the genus *Necturus* is demonstrated by retention of external gills in adults, presence of pigmented skin, presence of four welldeveloped limbs, and reduction of digits on hind limbs to four toes. This species is unique in possessing a larval stage lacking numerous small white spots and an adult stage that is small in size, possesses dark dorsal and lateral spotting that is no larger than the size of the eye, and lacks evidence of spotting on the chin or belly. Ten sequence autapomorphies diagnose *N. mounti*, one of which is unambiguous (Chabarria 2008). The species is sister to all other *Necturus* of the Gulf Coastal Plain (Figure 1).

Comparisons. Larvae of *N. mounti* are uniform pinkish grey in life, typically lacking any small white spots, features that distinguish this species from the Mobile, Pearl, Pontchartrain and Western lineages of *N. beyeri* (numerous small white spots);



Figure 13. Photo of dorsum (a) and venter (b) of type specimen of *N. mounti* (AUM 40698; Covington County, AL; photos by Craig Guyer).



Figure 14. Distribution of localities of specimens assigned to *N. mounti*. Open circle is approximate location of type specimen.

N. alabamensis (light dorsolateral stripes); *N. lewisi* (middorsal light stripe); and *N. maculosus* (light dorsolateral stripes). Larvae of *N. mounti* are indistinguishable from those of the Apalachicola lineage and *N. punctatus*. Adults of *N. mounti* typically possess

small (no larger than size of eye) dark dorsal and lateral spots, features that distinguish this lineage from the Pearl, Pontchartrain and Western lineages of *N. beyeri* and from *N. lewisi* (large dark dorsal spots); adults of North Carolina populations of *N. punctatus* are similar in adult dorsal colour pattern to *N. mounti* while South Carolina and Georgia populations typically lack dark spots. Adults of *N. alabamensis* and *N. maculosus* frequently have large dark spotting on the dorsal and lateral surfaces (rarely seen in *N. mounti*) but may retain faded evidence of the dorsolateral light stripe of the larval stage (never seen in *N. mounti*). The belly of *N. mounti*, in life, is white, changing abruptly to the dark lateral colouration, and the chin also is immaculate. These features differ in the Apalachicola, Pearl, Pontchartrain, and Western lineages of *N. beyeri* (ventrolateral dark spots; spotting on mandibles of chin, occasionally to level of gular fold).

Description of holotype. In preservation, the type specimen is 143 mm TOT, 103.0 mm SVL, and 40 mm TL (but tail cut for tissue sample); number of costal grooves is 16. The dorsal ground colour in preservation is uniform dark grey brown and the dorsum has distinct round dark spots that are approximately the size of the eye. This colour pattern also characterises the dorsal surface of the tail. The head is uniformly dark slate brown, lacking a dark stripe from the nostril through the eye. The dark dorsal colouration extends onto the lateral surface of the venter; changing abruptly to immaculate white at midventer; the edge of this transition zone is scalloped. The chin is immaculate white. The holotype has the following values for measured morphological features – ED: 1.3 mm; EL: 2.0 mm; GD: 2.3 mm; GEL: 15.7 mm; GL: 17.2 mm; HWE: 11.4 mm; HWG: 16.1 mm; POL: 15.9 mm; REW: 2.4 mm; SL: 4.6 mm; SRE: 6.4 mm; SW: 4.4 mm; WBW: 18.1 mm.

Variation. Necturus mounti achieves relatively small adult sizes, with a maximum male size of 116 mm SVL (+49 mm TL) and a maximum female size of 110 mm SVL (+53 mm TL). Mean male size is 108.4 mm SVL (n = 9) and mean female size is 94.8 mm SVL (n = 11). Approximately 80% of specimens conform to the dorsal colour categories described above. About 10% of specimens lack dark dorsal and lateral spots and about 10% have large dark dorsal spots. Ventrally, about 5% of specimens have spots along the mandibles with all others being spotless. Similarly, about 15% of specimens have ventrolateral spots on the belly with the rest being spotless. Modal value for costal grooves is 17 (n = 17), with 47% of specimens having 16.

Adult males (*n* = 7) have the following mean (and range) values for measured morphological features – ED: 1.4 mm (0.1–2.9); EL: 1.9 mm (0.7–2.9); GD: 3.2 mm (2.2–3.9); GEL: 15.6 mm (12.6–18.4); GL: 18.2 mm (15.2–20.9); HWE: 11.9 mm (9.4–14.3); HWG: 18.4 mm (13.9–21.5); POL: 16.9 mm (11.0–21.8); REW: 2.7 mm (2.3–3.0); SL: 6.5 mm (5.3–7.8); SRE: 8.0 mm (5.9–9.2); SW: 4.6 mm (2.4–5.8); WBW: 16.9 (12.8–18.9).

Adult females (*n* = 10) have the following mean (and range) values for measured morphological features – ED: 0.7 mm (0.02–2.4); EL: 1.9 mm (0.3–2.6); GD: 3.0 mm (2.3–3.9); GEL: 14.8 mm (8.4–18.8); GL: 16.6 mm (12.1–21.2); HWE: 10.8 mm (9.2–13.3); HWG: 16.8 mm (14.7–19.3); POL: 14.4 mm (8.0–20.9); REW: 2.2 mm (1.5–2.6); SL: 5.2 mm (4.4–6.4); SRE: 7.0 mm (5.6–8.9); SW: 4.7 mm (3.3–6.3); WBW: 15.9 mm (12.6–18.1).

Etymology. The specific epithet is a noun in the genitive case honouring Robert H. Mount, the curator of Herpetology at Auburn University for many productive years

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and the person who pointed out the taxonomic challenge that was represented by the waterdogs of Alabama. The recommended English common name is Escambia Waterdog.

Distribution and natural history. Necturus mounti occurs in the Blackwater, Escambia (Conecuh in Alabama), Perdido, and Yellow River drainages of Alabama and the western Panhandle of Florida (Figure 14). No published study has documented the life history of N. mounti. But this taxon is common at the type locality where, for the past 30 years, we have made haphazard samples because the site contains a large, permanent leaf pack from which we have rarely failed to detect N. mounti in dipnet samples of those leaves. Juveniles are detected year-round, with detections of adults being concentrated in January and February. Siren intermedia, Desmognathus conanti, Ichthyomyzon gagei, dragonfly naiads, and small crayfish are frequent associates of N. mounti when they occupy these leaf packs. Samples from the surface of the leaf pack rarely yield specimens. Instead, samples from greater than two feet below the water surface, which are associated with cooler waters, yield these salamanders. Heavy silt appears to reduce the chances of detection as does heavily decayed leaves. We infer from these observations that adults migrate to leaf packs to mate. We suspect that nesting takes place in the leaf packs. However, nesting might take place elsewhere, with juveniles and adults migrating to leaf packs, as has been observed for the Pearl lineage of N. beyeri (Shoop 1965; Sever and Bart 1996). Nevertheless, juveniles of *N. mounti* appear to remain in leaf packs as they grow to adulthood.

> Necturus moleri sp. nov.(Figures 15–16) Necturus punctatus lodingi Hecht, 1958, in part Necturus beyeri alabamensis Hecht, 1958, in part Necturus alabamensis Neill 1963, in part and misidentification Necturus beyeri Neill 1963, in part Necturus cf beyeri Bart et al. 1997, in part Necturus (Parvurus) lodingi Dubois and Raffaëlli, 2012, in part Necturus cf beyeri Apalachicola lineage Chabarria et al. 2017

Holotype. AUM 35617 (Figure 15), a female collected 1 March 2002 in Big Creek near Cottonwood (31.01916 – 85.35017; WGS84), Houston County, AL, by Michael Buntin.

Paratypes. FLMNH 164246 (female); FLMNH 177187 (male).

Diagnosis. Membership of this species in the genus *Necturus* is demonstrated by retention of external gills in adults, presence of pigmented skin, presence of four well-developed limbs, and reduction of digits on hind limbs to four toes. This species is unique in possessing a larval stage lacking numerous small white spots and an adult stage that is small in size, possesses dark dorsal and lateral spotting that is no larger than the size of the eye, and evidence of spotting on the mandible of the chin and lateral portion of the belly. Five sequence autapomorphies distinguish this species, all of which are ambiguous (Chabarria 2008). This species is sister to the Mobile lineage + N. *alabamensis* + Pearl lineage + N. *beyeri* + N. *maculosus* (Figure 1).



Figure 15. Photo of dorsum (a) and venter (b) of type specimen of *N. moleri* (AUM 35617; Houston County, AL; photos by Craig Guyer).



Figure 16. Distribution of localities of specimens assigned to *N. moleri*. Open circle is approximate location of type specimen.

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Comparisons. Necturus moleri has a juvenile stage that is pinkish grey and lacks white spotting, features that distinguish this species from the Mobile, Pearl, Pontchartrain and Western lineages of N. beyeri (numerous small white spots); N. alabamensis (light dorsolateral stripes); N. lewisi (middorsal light stripe); and N. maculosus (light dorsolateral stripes). Larvae of N. moleri are indistinguishable from those of N. mounti and N. punctatus. As adults, N. moleri typically possesses small (no larger than size of eye) dark spots, but might lack spots altogether, features that distinguish this species from the Pearl, Pontchartrain and Western lineages of N. beyeri and from N. lewisi (large dark dorsal spots). Adults of North Carolina populations of N. punctatus are similar in adult dorsal colour pattern to N. moleri while South Carolina and Georgia populations typically lack dark spots. Adults of N. alabamensis and N. maculosus frequently have large dark spotting on the dorsal and lateral surfaces (rarely seen in N. moleri) but may retain faded evidence of the dorsolateral light stripe of the larval stage (never seen in *N. moleri*). In life, the chin of *N. moleri* typically has small dark spots on the mandibles and the belly is dull white with a gradual change along the border between the immaculate white mid-venter and the light brown sides of the body. These features distinguish this species from *N. mounti* (typically with bright white chin and belly with sharp border between venter and sides of body).

Necturus moleri may possess a karyotype that includes 3 (Econfina Creek, Jackson Co FL, Choctawhatchee drainage; Guttman et al. 1990), 4 (Black Creek near Bruce, Walton Co FL, Choctawhatchee drainage; Sessions and Wiley 1985), or 6 (Juniper Creek, Marion Co GA, Chattahoochee drainage; Sessions and Wiley 1985) pairs of telocentric chromosomes. These characteristics of the karyotype represent a transition from an ancestral state lacking telocentric chromosomes (*N. punctatus* and *N. lewisi*) to a derived state of 6 pairs of telocentric chromosomes (*N. maculosus* and the Western lineage of *N. beyeri*; Sessions and Wiley 1985). Similarly, the Y chromosome changes from an ancestral condition with reduced heterochromatism (*N. lewisi* and *N. punctatus*) to a derived condition possessing extensive heterochromatism (*N. maculosus* and the Western lineage of *N. beyeri*), with *N. moleri* displaying intermediate levels of heterochromatism (Sessions and Wiley 1985). Additional karyotypic data are needed from *N. mounti* and the Mobile, Pearl and Pontchartrain lineages to determine whether the above modifications of the presumed primitive *Necturus* karyotype are autapomorphies of *N. moleri* or are synapomorphies of all Gulf Coast *Necturus*.

Description of holotype. The type specimen measures 197 mm TOT, 134 mm SVL, and 63 mm TL; number of costal grooves is 16. The dorsum is a uniform dark greyish brown. The top of the head has numerous small dark brown punctations. The sides of the body are off-white, shading gradually to the dark dorsal colouration. The chin is light brown with a few dark brown punctations, especially along the mandibles. The gular fold is immaculate white, and the venter of the body is light brown with a few dark spots anteriorly, becoming immaculate shiny white at mid-venter, and shading gradually to a light smoky brown along the sides of the body. The holotype has the following values for measured morphological features – ED: 2.0 mm; EL: 0.9 mm; GD: 5.8 mm; GEL: 22.5 mm; GL: 24.9 mm; HWE: 17.2 mm; HWG: 28.9 mm; POL: 23.2 mm; REW: 2.2 mm; SL: 6.8 mm; SRE: 9.5 mm; SW: 6.6 mm; WBW: 27.5 mm.

Variation. Necturus moleri is of small size, with a largest male of 173 mm SVL (+73 mm TL) and a largest female of 134 mm SVL (+63 mm TL). Adult males average 126.4 mm SVL (n = 11) and females average 102.3 mm SVL (n = 15). Unlike the type specimen, most individuals of *N. moleri* are light brown to tan, often with a pinkish cast, and possess numerous small dark dorsal (75% of specimens) and lateral spots (71% of specimens). However, the species might lack spots (11% of specimens) or have bold dark spots (4% of specimens). In ventral colouration, most specimens (71%) have dark spots along the mandibles of the chin and the ventrolateral aspect of the belly but might have spots across the entire chin and belly (25% of specimens) or might be immaculate white (4% of specimens). Thus, the colouration of *N. moleri* is more variable than *N. punctatus* or *N. mounti*, the two species with the most similar size and colour pattern. Juveniles of *N. moleri* are uniform in colour at the smallest sizes but develop dark dorsal and lateral spotting at sizes above ca. 45 mm SVL. The modal value for costal grooves is 16 (n = 15), with 33% of specimens possessing 17 grooves.

Adult males (n = 6) have the following mean (and range) values for measured morphological features – ED: 1.4 mm (0.3–2.9); EL: 1.8 mm (0.1–3.8); GD: 3.8 mm (2.0–6.4); GEL: 19.1 mm (14.2–24.4); GL: 24.7 mm (18.9–32.1); HWE: 14.5 mm (10.5–17.6); HWG: 22.2 mm (17.5–29.7); POL: 18.7 mm (14.1–23.5); REW: 2.6 mm (1.9–3.8); SL: 7.2 mm (4.7–8.2); SRE: 9.3 mm (6.0–11.2); SW: 5.8 mm (3.6–8.5); WBW: 23.7 mm (17.0–35.5).

Adult females (n = 9) have the following mean (and range) values for measured morphological features – ED: 1.2 mm (0.1–2.9); EL: 1.4 mm (0.1–2.2); GD: 3.2 mm (2.2–5.8); GEL: 14.4 mm (7.9–23.2); GL: 19.4 mm (10.8–24.9); HWE: 11.3 mm (7.1–17.2); HWG: 17.6 mm (11.4–28.9); POL: 14.0 mm (7.4–22.5); REW: 2.1 mm (1.6–2.7); SL: 5.3 mm (3.1–6.8); SRE: 7.1 mm (3.7–9.5); SW: 4.6 mm (2.0–6.6); WBW: 16.7 mm (10.6–27.5).

Etymology. The specific epithet is a noun in the genitive case honouring Paul E. Moler for his tireless devotion to field herpetology of the southeastern United States and for recognising the need to improve our understanding of species richness within *Necturus* of the Gulf Coastal Plain. The recommended English common name is Apalachicola Waterdog.

Distribution and natural history. This species is restricted to the Apalachicola, Chipola, Choctawhatchee/Pea, Econfina, and Ochlockonee drainages of Alabama, Florida, and Georgia (Figure 16). No life history study has been performed on this taxon, but features are likely to be similar to those of *N. mounti*.

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Disclosure statement

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