FINAL REPORT

Conservation status of Chamberlain's Dwarf Salamander *Eurycea chamberlaini*, and One-toed Amphiuma, *Amphiuma pholeter* in Georgia.

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EXECUTIVE SUMMARY

Concern for the plight of amphibian populations worldwide requires detailed population assessments for species of special conservation concern. Two such species found within Georgia (Chamberlain's Dwarf Salamander, *Eurycea chamberlaini*, and the One-toed Amphiuma (*Amphiuma pholeter*) were recently petitioned for listing under the U.S. Endangered Species Act due to their apparently small population sizes, limited distributions, possible threats, and a general lack of information about their status. Systematic surveys were conducted in likely habitats within Georgia to determine the status of both species and in an attempt to identify new localities where they might be present. In addition, attempts were made to resolve the taxonomic status of *E. chamberlaini*, which was recently determined to belong to a species complex whose status in Georgia is uncertain. We conducted 282 surveys across 42 Georgia counties, representing 409 person hours of search effort looking for both species. We were unable to locate *A. pholeter* at any new localities, although we were able to confirm its persistence within the state at one of its historic localities. We located 115 salamanders tentatively identified as *E. chamberlaini* at several new sites, including many that bridge substantial distribution gaps for this species in Georgia. However, our genetic analysis confirms these specimens do not form a monophyletic clade with *E. chamberlaini* collected from the Carolinas. No specimens attributable to *E. chamberlaini* (in the strict sense) were located in Georgia, indicating this species probably has a distribution restricted to South and North Carolina and can be removed from Georgia's list of species. Instead, we confirm Georgia specimens referable to *E. chamberlaini* correspond to the "central clade" identified previously by Lamb and Beamer (2012) which will be described as a new species as a result of this study.

INTRODUCTION

Declining amphibian populations are cause for conservation concern worldwide (Stuart et al., 2004). Although the most frequent cause of these declines in North America is habitat loss, in many cases no clear factor associated with the declines can be unidentified (Stuart et al., 2004; Wake and Vredenburg, 2008). In addition, a lack of basic information about the biology of some species has hampered our efforts to determine if certain species have declined or if they are in need of conservation attention (Lannoo, 2005). For example, a recent petition to list the Seepage Salamander (*Desmognathus aeneus*) under the Endangered Species Act (Center for Biological Conservation, 2010) was deemed unwarranted when a survey found that the species was generally secure throughout its known range (Graham et al., 2013). In addition, taxonomic uncertainty can lead to confusion over the presence or absence of certain species in the area of interest, or ambiguity over species level identification (see Beamer and Lamb, 2008). An example of such a species is Chamberlain's Dwarf Salamander (*Eurycea chamberlaini*), a tiny, semi-aquatic plethodontid salamander whose known range includes Alabama, Georgia, South and North Carolina. A recent study showed dwarf salamanders represent a species complex (Lamb and Beamer 2012), complicating efforts to determine the conservation status and distribution of *Eurycea chamberlaini*. Finally, species whose distributions are peripheral to certain regions may hamper conservation efforts that would otherwise occur if the species has a more centralized distribution. An example within Georgia is the One-toed Amphiuma (*Amphiuma pholeter*), a small eel-like salamander found in only a handful of counties along the Gulf Coast of Florida, Georgia, and Alabama, whose distribution is peripheral in the state and represented by only two localities.

E. chamberlaini was only recently described as a new species from specimens collected in the Carolinas (Harrison and Guttman, 2003). Historically, all dwarf salamanders (e.g., *E. quadridigitata*) were thought to be a single, common, and morphologically variable species found in the Coastal Plain from North Carolina to Texas (Petranka, 1998). Two color forms were thought to make up this polymorphism: yellow-bellied and grey-bellied forms. The yellow-bellied form was eventually described as a separate species: *E. chamberlaini* (Harrison and Guttman, 2003), while the grey-bellied form was relegated to the original nominate species *E. quadridigitata*. Additional populations attributed to *E. chamberlaini* were since discovered in the Fall Line region of Georgia (Graham et al., 2007; Means, in Jensen et al., 2008) and Alabama (Graham et al. 2008). In addition, general information about the macro- and microhabitat associations of this species in Georgia and Alabama has been documented (Graham and Jensen, 2011a).

However, this species remains one of the most poorly known salamanders in North America, due in part to its recent taxonomic recognition and since most information known about the species is based upon a composite of information attributable to either *E. chamberlaini* or *E. quadridigitata* (e.g., Petranka, 1998). In fact, the only full-length articles about this species are its taxonomic description and a recent phylogenetic analysis of the *E. quadridigitata* complex (Harrison and Guttman, 2003; Lamb and Beamer, 2012). Its basic taxonomy is not fully resolved, and additional sampling and taxonomic attention is needed. For example, Lamb and Beamer (2012) suggest that two divergent lineages with species-level distinctiveness may occur in

Georgia and the Florida Panhandle (e.g., the "central" and "panhandle" clades of Lamb and Beamer [2012]). However, there is a very large sampling gap between central Georgia and South Carolina, which leaves open two possibilities: 1) with more thorough sampling, the "central clade" will be found to be conspecific with *E. chamberlaini*, or, if not, 2) populations of "good" *E. chamberlaini* may be discovered in eastern Georgia.

Due to our lack of knowledge about the basic distribution of *E. chamberlaini*, most states consider it a species of special concern. And, due to its small known range (21 populations in the Carolinas, with a handful of additional populations in Georgia and Alabama) and a number of possible threats to its continued existence (habitat loss, disease, endocrine disruptors, non-native predators), this species was recently petitioned for listing under the Endangered Species Act (Center for Biological Conservation, 2010).

The One-toed Amphiuma (*Amphiuma pholeter*) is the smallest and rarest of three amphiumid salamander species. It is found in mucky bottomlands and mucky seepage habitats in less than 15 counties from the Gulf Coast of Florida to Mississippi. It has previously been collected from only two sites in two Georgia counties (Stevenson, 2003), and very little is known about the biology of this species. Due to its limited distribution and the threat of habitat destruction, *A. pholeter* was also petitioned for listing under the ESA (Center for Biological Conservation, 2010).

The purpose of this study was to determine the conservation status of the Chamberlain's Dwarf Salamander and One-toed Amphiuma in Georgia.

METHODS

Surveys: Preliminary information suggests that *Eurycea chamberlaini* is found in bay swamps, vernal pools, and isolated wetlands in the Fall Line region of Georgia (Graham and Jensen 2011a). Surveys for *E. chamberlaini* concentrated on this region and these habitats. Methods followed Graham et al. (2010) and Graham et al. (2012); the overall strategy was to locate this species in as many new counties as possible to determine its county-level distribution in Georgia and inform the Georgia Department of Natural Resources' conservation status designation (based on the number and prevalence of populations throughout the state). Surveys consisted of 1-5 observers walking through available habitat and turning cover objects; all surveys were timed to determine relative search effort (salamanders/person h). We quickly determined that the best strategy for locating *E. chamberlaini* is to walk along swamp or pond edges to locate appropriate microhabitat: deep beds of sphagnum moss. Surveys evolved during the course of the study and eventually the bulk of surveys were simply attempts to quickly locate sphagnum moss beds; once they were located, *E. chamberlaini* were often also quickly found. To search sphagnum beds we got on our hands and knees and sorted through the sphagnum with my hands by digging back thick layers and rolling them like rolling back a section of carpet. On a few occasions weather permitted road cruising surveys: driving along paved roads on rainy nights looking for salamanders crossing the road. We previously used this technique effectively for locating new populations of *E. chamberlaini* in Georgia and Alabama, and were able to locate one new population by road cruising during the course of this study.

Similarly, searches for *Amphiuma pholeter* largely consisted of searching for appropriate muck beds and digging through them with our hands. After identifying four high-quality habitats for *A. pholeter* in 2014, we employed minnow and crayfish traps (crayfish traps were modified and were lined with small sized mesh) at these localities in 2015. All amphibians (including numbers of individuals) were counted during surveys to estimate the relative abundance of this salamander among sites compared to other species. Multiple surveys were conducted across months to determine seasonal trends and detectability of *E. chamberlaini* and *A. pholeter*. Several apparently suitable sites were re-surveyed in an attempt to locate the salamanders, including collecting localities for *E. quadridigitata* determined by consulting museum records in Williamson and Moulis (1994) and locality data available in Jensen (2008; J.B. Jensen, unpubl. data). Two large distribution gaps for *E. chamberlaini* were immediately identified and attempts were made to close these with thorough sampling: 1) a region of southwestern Georgia between the nearest localities in Stewart County (noted in Graham et al., 2010) and Early County (identified by Means, in Jensen et al., 2008); 2) a large region of central Georgia between localities in Crawford County (Jensen et al., 2008), Baldwin County (Graham and Jensen 2011b), and Emmanuel County (Lamb and Beamer 2012) and the nearest known populations of definitively identified *E. chamberlaini* in South Carolina (Barnwell and Orangeburg Counties; Harrison and Guttman, 2003). General habitat parameters were recorded at all sites in which *E. chamberlaini* and *A. pholeter* were found (e.g., microhabitat parameters, dominant vegetation).

Voucher specimens were collected for *E. chamberlaini* when they were located in new counties, and tissue samples (tail or liver) were collected and stored in 95% EtOH for genetic analyses. Tissue samples (tail clips) were taken from two specimens of *A. pholeter*. Specimens were deposited in the Auburn University Herpetological Collections (AUM) and the James Scudday Vertebrate Collections at Sul Ross State University (SRSU).

DNA extraction, PCR and Sequencing: Samples (n = 75) of *Eurycea quadridigitata* and salamanders tentatively identified as *E. chamberlaini* collected in Georgia included tail tips (≈1.5 cm), liver samples, and complete recently metamorphosed salamanders. These were placed into a cell lysis tube in 180 ATL buffer with two large 4 mm beads and many small 0.5 mm beads then homogenized in a FastPrep fp120 for 90 sec on setting 6. DNA was extracted using the Qiagen DNeasy blood and tissue kit according to the manufacturer's instructions and eluted in 200 µL AE Buffer. DNA extracts were then quantified on a Qubit 3.0 Fluorometer and diluted to an appropriate working solution for PCR. Two mitochondrial markers including the NADH dehydrogenase subunit 2 (*Nd2*, ≈1000 bp) and cytochrome b (*Cytb,* ≈1000 bp) were PCR amplified in 25 µL reactions including 12.5 µL Qiagen Hotstart Taq Mastermix, 1 µL (10 µM) forward primer, 1 µL (10 µM) reverse primer, 9.5 µL sterile H_2O, and 1 µL DNA quantified at 15 ng/µL for *Nd2* and 35 ng/µL for *Cytb*. The PCR primers used to amplify *Nd2* were H5934 (5' – AGRGTGCCAATGTCTTTGTGRTT – 3'; Macey et al. 1997) and L447 (5' – AAGCTTTCGGGCCCATACC – 3'; Macey et al. 1997) and *Cytb* primers MVZ15 (5' – GAACTAATGGCCCACACWWTACGNAA – 3'; Moritz et al. 1992) and Quad b r (5' – TGGTCCAATCTCAATAAATGGGGGTTC – 3'; Lamb and Beamer 2012). Touchdown PCR reaction conditions for *Nd2* were as follows, 95 C initial denaturation for 15 mins, 10 cycles of 94 C for 30 sec, 59 C for 30 sec (decreasing 0.8 C per cycle – touchdown annealing temp. 51 C), 72 C for 120 sec, followed by 22 cycles of 94 C for 30 sec, 51 C for 30 sec, 72 C for 120 sec, and a final elongation step of 72 C for 10 mins. Touchdown PCR reaction conditions for *Cytb* were as follows, 95 C initial denaturation for 15 mins, 10 cycles of 94 C for 30 sec, 58 C for 30 sec (decreasing 0.8 C per cycle – touchdown annealing temp. 50 C), 72 C for 120 sec, followed by 28 cycles of 94 C for 30 sec, 50 C for 30 sec, 72 C for 120 sec, and a final elongation step of 72 C for 10 mins. PCR reactions were visualized on a 1% agarose gel for the correct fragment size then enzymatically purified by adding 2 µL ExoSAP-IT to each 25 µL PCR reaction under the following conditions, 37 C for 30 mins then 80 C for 15 mins. Sequencing was completed at MCLAB on an ABI 3730XL sequencer under standard conditions using primers H5934, L447, EuCo r (5' - CTTTRTGGTTTGTTGARAATAGTCATCG – 3'; Lamb and Beamer 2012), L4882 (5' – GACAAAAACTAGCACC - 3'; Macey et al. 1997), H5617a (5' – AAAATRTCTGRGTTGCATTCAG - 3'; Macey et al. 1997) for *Nd2* and primers MZ15, Quad b r, Quad b int. (5' – TCAGTAGAYAAAGCAACACT – 3'; Lamb and Beamer 2012) for *Cytb.*

Phylogenetic Analyses: Sequences were trimmed and contigs made in ChromasPro. PopSets 385051493 (*Nd2*) and 385051125 (*Cytb*) were downloaded from GenBank (Lamb and Beamer 2012). Each PopSet contained 106 sequences - 102 ingroup sequences of *Eurycea* spp. and one sequence each of *Urspelerpes brucei, Pseudotriton ruber, Gyrinophilus porphyriticus,* and *Stereochilus marginatus* as outgroup species. We contributed ## *Nd2* and # *Cytb* sequences to each of these datasets representing *Eurycea chamberlaini* specimens sampled from ## localities in Georgia. Sequences were aligned using the ## strategy in Mafft v. 7 and model testing for molecular evolution completed in FindModel (http://hiv.lanl.gov/content/sequence/findmodel/findmodel.html) which implements methodology from MODELTEST (Posada and Crandall 1998). Maximum likelihood (ML) phylogenetic analyses were performed by the GARLI web service hosted at molecularevolution.org (Bazinet, Zwickl, and Cummings 2014) using GARLI 2.1 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006) with 1000 bootstrap replicates.

RESULTS

Surveys: Surveys were conducted from October 2013 to November 2015. For *Eurycea chamberlaini* we conducted 236 surveys at 218 localities across 37 Georgia counties (see Fig. 1; Appendix 1). This survey effort consisted of 355.6 person hours of survey effort (excluding travel time to and from study areas). In addition, 44 person hours were spent road cruising on four occasions. We conducted 48 surveys of 40 sites for *Amphiuma pholeter* across three Georgia counties (Appendix 2). This effort consisted of 53.7 person hours of surveys. In addition, 93 minnow traps nights and 24 crayfish trap nights were deployed at four high-quality sites in hopes of capturing *A. pholeter*.

115 salamanders tentatively identified as *E. chamberlaini* were found at 43 sites in 25 counties. This included the discovery of *E.* cf. *chamberlaini* for the first time in 14 Georgia counties (e.g., new county records). These localities completely filled the distribution gap between Stewart County and Early County (Quitman and Clay Counties) and Crawford and Baldwin Counties (Bibb, Twiggs, and Jones Counties; Fig. 1). New records between Baldwin County and Emmanuel County also bridged a substantial

distribution gap (Wilkinson, Washington, Hancock, and Jefferson Counties; Fig. 1). This suggests a continuous distribution for this species across the Fall Line Hills of Alabama (Monroe County, Alabama; Graham et al., 2008) to Jefferson County, Georgia (Fig. 2). However, we failed to locate *E.* cf. *chamberlaini* in Richmond, Glascock, or Burke County, despite aggressive efforts to bridge this last gap between the eastern-most populations of *E.* cf. *chamberlaini* in Georgia and the nearest localities for *E. chamberlaini* sensu stricto in South Carolina (Fig. 1). Therefore, a small distribution gap exists between populations identified as the "central clade" in Lamb and Beamer (2012) and definitively-identified *E. chamberlaini* from the Carolinas (Fig. 2). In this region habitats seemingly ideal for *E.* cf. *chamberlaini* are instead occupied by *E. quadridigitata* or *E. cirrigera*.

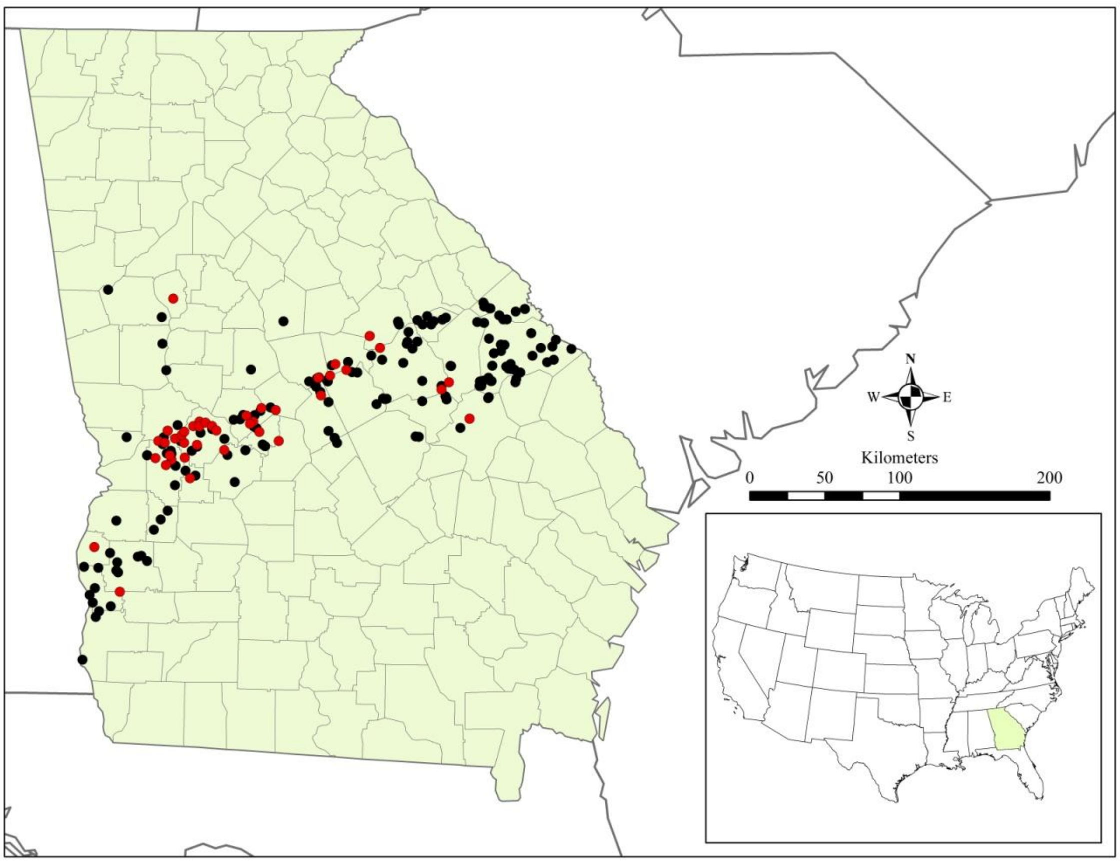

Fig. 1 Search coverage for *Eurycea chamberlaini* in Georgia, 2013-2015. Black dots represent sites surveyed where *E.* cf. *chamberlaini* was not located. Red dots indicate sites with confirmed presence of *E.* cf. *chamberlaini*. Several sites were re-surveyed multiple times.

The surveys also suggest the apparent boundaries between the closely related *E. quadridigitata* and *E.* cf. *chamberlaini* (Fig. 2). We located *E. quadridigitata* in several localities fairly close to locations where we located *E.* cf. *chamberlaini* (Bleckley, Richmond, Laurens, and Burke Counties), and in some cases we found both species in the same county (e.g., Peach County, Emmanuel, and Jefferson County). However, we never located the two species in sympatry (e.g., within the same collection site). *E. quadridigitata* apparently occupies most of the outer Coastal Plain of Georgia, which is drained either by large alluvial rivers, small to large blackwater creeks, and cypress ponds, all of which commonly support populations of *E. quadridigitata* but typically not *E. chamberlaini*. By contrast, *E.* cf. *chamberlaini* was mostly found in bay branches and swamps, gum ponds, beaver ponds, or depressional wetlands, which are more commonly found in the Fall Line sandhills region of Georgia (see below). Surprisingly, *E.* cf. *chamberlaini* populations are also found well into the Georgia Piedmont region, where populations of any member of the *E. quadridigitata* complex evaded detection until recently (Graham et al., 2007).

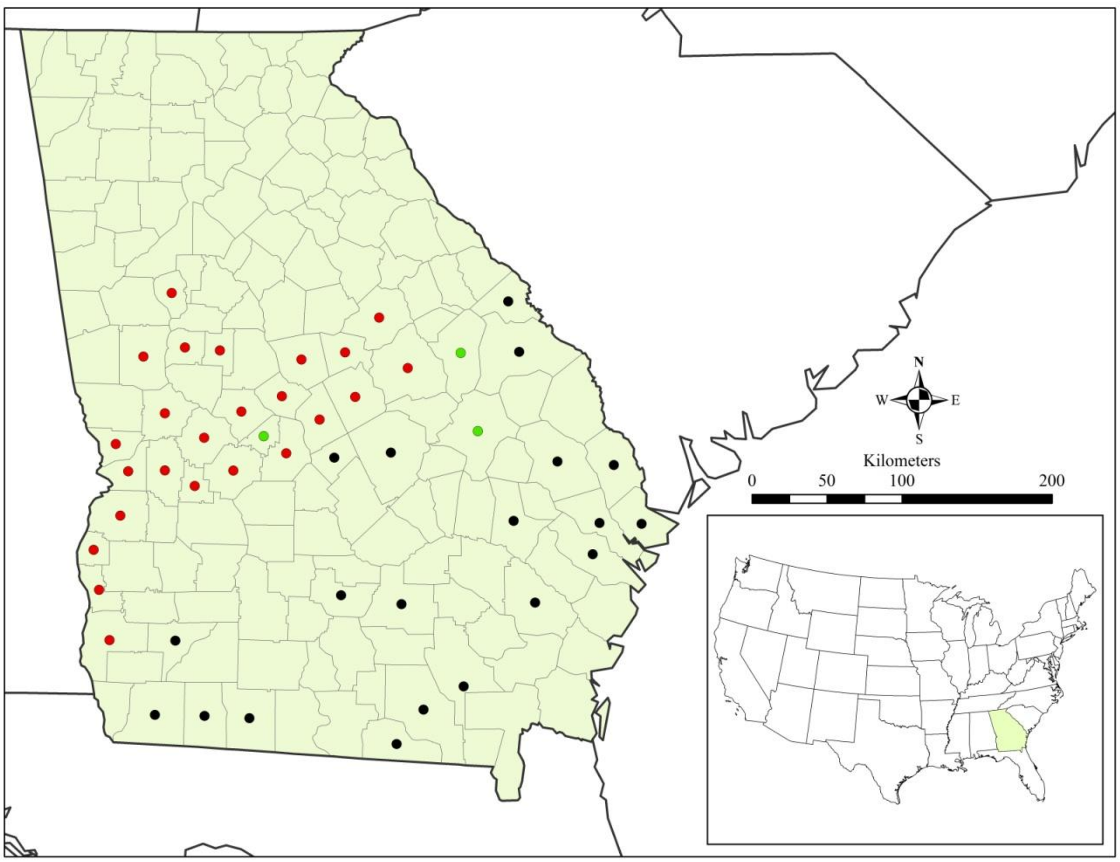

Fig. 2 Tentative county-level range map for *E.* cf. *chamberlaini* ("central clade") in Georgia. Red dots indicate known presence of the central clade (see phylogenetic results) in Georgia based on this study and recent range extensions (West and Hartman, 2014; Strickland et al., 2015). Black dots indicate locations where the lead author has found *E. qudridigitata* sensu stricto during this research and during previous studies (Graham et al., 2010). Green dots indicate counties in Georgia where both species have been located. The central clade is mostly found along the Fall Line as well as a few locations in the Piedmont and Red Hills region in western Georgia. *E. quadridigitata* is found mostly in the outer coastal plain. There is a well-surveyed hiatus in the easternmost Fall Line hills of Georgia that is occupied only by *E. quadridigitata* (Richmond and Burke Counties).

Eurycea cf. *chamberlaini* were found most often (65% of occupied sites) in bay swamps or bay branches (sensu Wharton 1978; Edwards et al., 2013)—narrow floodplains on either side of sluggish first or second order creeks (Fig. 3). These habitats usually include an overstory of sweetbay (*Magnolia virginiana*) swamp black gum (*Nyssa biflora*), and have an extensive and often nearly impenetrable shrub layer composed of rusty blackhaw (*Viburnum rufidulum*), dog hobble (*Leucothoe axillaris*), titi (*Cyrilla recemiflora*) big gallberry (*Ilex coriacea*), and bamboo vine (*Smilax laurifolia*). Ferns are often present (*Woodwardia virginica*), along with some characteristic herbaceous plants (a swamp violet, neverwet, *Orontium aquaticum*, and sphagnum moss; Fig. 4). Other habitats occupied, in decreasing order of abundance, were: beaver marshes (three sites; usually impoundments of streams that would have otherwise supported bay swamps and shared many of the same plant associations) gum ponds (three sites), alluvial floodplains of third or fourth order creeks (two sites), isolated depressional wetlands (two sites), a first order creek flowing through a hardwood forest (one site), an old abandoned farm pond (one site), and a ravine seepage (one site). Only 6% of microsites where salamanders were located had seepage flow; most sites were along the margins of slow moving floodplains or lentic floodplain pools where saturated leaf litter and boggy areas were present, but subsurface seepage flow was not. 37% of sites occupied by *E.* cf. *chamberlaini* also supported other plethodontid salamanders (most frequently *E. cirrigera*). Birds known to breed in mesic hardwood forests (hooded warblers, *Setophaga citrina*, and wood thrushes, *Hylocichla mustelina*) were noted at many localities supporting *E.* cf. *chamberlaini*.

Fig. 3 Example of a bay swamp likely to harbor *E.* cf. c*hamberlaini*: an overstory of sweetbay and black gum with a thick bed of sphagnum moss.

Fig. 4 Sid Riddle demonstrating the best technique for finding *E.* cf. *chamberlaini*; rolling back thick beds of sphagnum moss. This figure also illustrates the thick shrub layer often present at suitable localities.

By contrast, sites unoccupied by *E. chamberlaini* were more frequently found to have seepage flow (18%) and included a variety of habitats other than sluggish bay swamps and branches. 41% of these sites were occupied by other plethodontid salamanders (*E. cirrigera, E. guttolineata, E. quadridigitata, Pseudotriton ruber, Desmognathus* sp., *Pseudotriton ruber*).

We searched for *E.* cf. *chamberlaini* during all months except January-February, April, and September. Our best search month was June, when we located 53 of 115 *E.* cf. *chamberlaini* individuals. Our success at this time was most likely due to a temporary rise in abundance due to the emergence of recently metamorphosed juveniles. Although more difficult to detect due to their small size (~ 1cm total length), their presence at this time greatly increased our success. Gravid females were found in March, indicating late March or April is likely the nesting season. Unfortunately, no attempts were made to locate nests during this time.

In general, population densities of *E.* cf. *chamberlaini* in Georgia are low. Usually only one to two indiviudals were located, often after searching for up to an hour. Typically we would search long enough to establish the presence of the species, and then move on to find them at a new site. This resulted in an average search time of 45 person min./survey. The highest abundance we detected was 18 individuals at one site in Taylor County, Georgia (7.2 salamanders/person h.); the maximum detactability was at a site in Marion County, Georgia (10.3 salamanders/person h). However, the average relative abundance across all occupied sites was only 2.54 salamanders/person h. The average overall abundance across all sites surveyed (occupied and unoccupied) was 0.49 salamanders/person h.

40 of the sites located during this survey represent new localities for this species, bringing the total number of populations known from 15 before this study to 55. In addition, other researchers located 3 new localities during my project (one in Pike County, one in Lamar County, and one in Bibb County; West and Hartman, 2014;

Strickland et al., 2015), bringing the total known number of *E. chamberlaini* populations up to 58 (in 27 counties). This represents a near-quadrupling of the total number of populations known in the state. Precise locations of *E.* cf. *chamberlaini* sites are presented in appendix 1.

Despite efforts we did not locate new populations of *A. pholeter* in Georgia. However we did confirm the persistence of this species at the site located by D. Stevenson in 2003 (Fig 5-6). Locations of localities searched for *Amphiuma pholeter* are presented in appendix 2.

Fig. 5 This small (25 cm deep; < $1m^2$) muck hole was the only location within Georgia where we were able to locate *Amphiuma pholeter*. Two were caught, photographed, and released here.

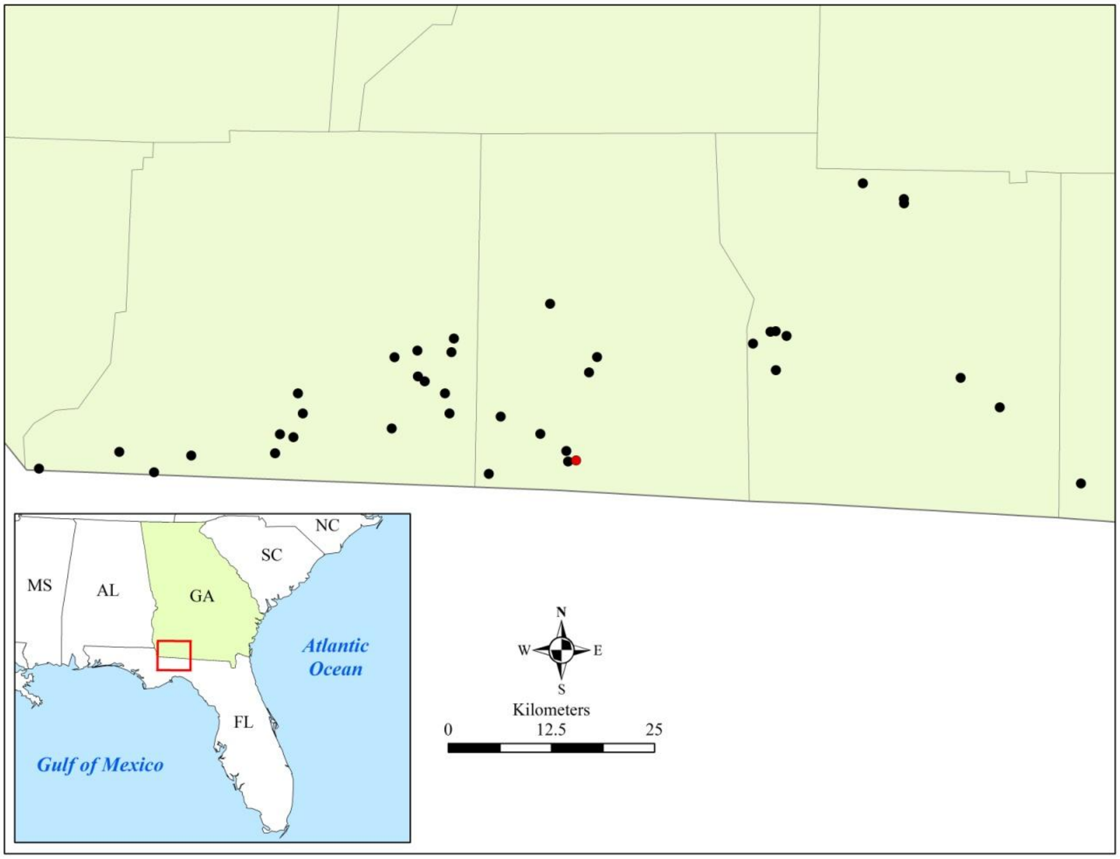

Fig. 6 Search effort coverage for *Amphiuma pholeter* in Georgia. Black dots represent sites surveyed where no one-toed amphiumas were found. The red dot indicates the location where the persistence of *A. pholeter* in Georgia was confirmed. Some locations were surveyed multiple times.

Phylogenetic results: We combined data from Lamb and Beamer (2012) with 75 new samples collected 2013-2015 in Georgia to test the hypothesis that *Eurycea* cf. *chamberlaini* populations from Georgia are conspecific with populations of *E. chamberlaini* from the Carolinas. Although the overall topology of our phylogenetic trees (for cyt b and ND2 genes) differ, they both show *E.* cf. *chamberlaini* populations in Georgia form a separate clade from *E. chamberlaini* sensu stricto (Fig. 7-8). If *E.* cf. *chamberlaini* in Georgia was conspecific with *E. chamberlaini*, we would have expected them to form a clade together. Furthermore, both phylograms show that the central clade present in Georgia is sister to a combined *E. chamberlini* + *E. quadridigitata clade*. These results support the results of Lamb and Beamer (2012) and demonstrate the pattern they observed was likely not a sampling artifact due to a paucity of samples from central Georgia. None of the samples collected in Georgia grouped with *E. chamberlaini*, indicating *E. chamberlaini* sensu stricto likely does not occur west of the Savannah River.

After addition of many new samples across a broad geographic area central to the previous study, we found continued support for all five *E. quadridigitata* complex clades identified by Lamb and Beamer (2012), including the "western clade" found from Louisiana to Texas, the "panhandle clade" found along the Florida and Alabama Gulf Coast, the "central clade" found from Washington County, Florida, through the Fall Line region of Alabama to eastern Georgia, *Eurycea chamberlaini* sensu stricto found in the Carolinas, and *E. quadridigitata* sensu stricto found in the outer Coastal Plain from North Carolina to Louisiana. Bootstrap support for these five clades ranged from 92 to 100% (mean 97%; cyt b) and 66 to 100% (mean 93%; ND 2), indicating strong support for monophyly for each of the five clades. Only two of our samples tentatively identified as *E. chamberlaini* did not group with the previously identified "central clade" using the cyt b gene: one from Crawford County, Georgia, and one from Washington County, Georgia. Instead, these grouped with *E. quadridigitata* sensu stricto collected from Georgia. Both of these were small metamorphic individuals, which are more difficult to identify and do not exhibit the belly coloration typical of adults. However, the sample from Crawford County, Georgia did group with the central clade using the ND2 gene. The individual from Washington County, Georgia was not analyzed using ND2 so we cannot determine whether this may be an artifact of the study or a misidentification. Similarly, only one individual identified as *E. chamberlaini* did not group with the central clade using the ND2 gene. Instead, according to ND2, this specimen grouped with *E. quadridigitata* sensu stricto. This was a specimen collected from Taylor County, Georgia, which was also analyzed using the cytb gene. The cytb gene showed it grouped with the central clade, indicating that this mismatch was possibly due to a study artifact, or possibly due to limited hybridization between the species.

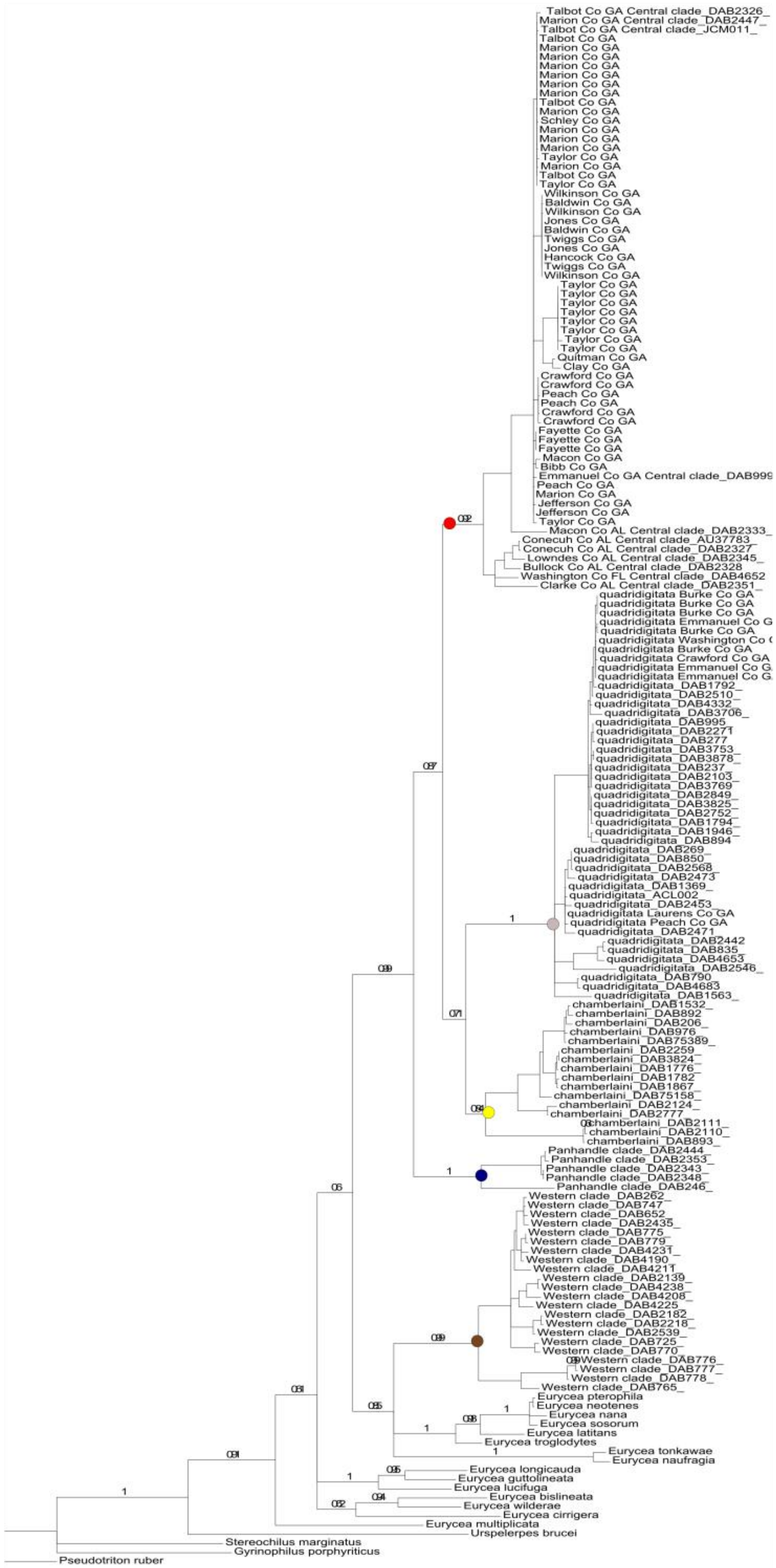

Fig. 7 Phylogram depicting relationships of *Eurycea quadridigitata* complex members and several outgroups, based upon similarity of the cyt b mitochondrial gene. Branch lengths are proportional to evolutionary distance, and major bootstrap values are listed. Samples listed with identification numbers (DAB #) are from Lamb and Beamer (2012) and were derived from genbank. Other samples are from the current study. Nomenclature of the five complex clades are from Lamb and Beamer (2012) and color coded for comparison to fig. 5. The red dot indicates the central clade, which all yellow-bellied dwarf salamanders collected in Georgia group with.

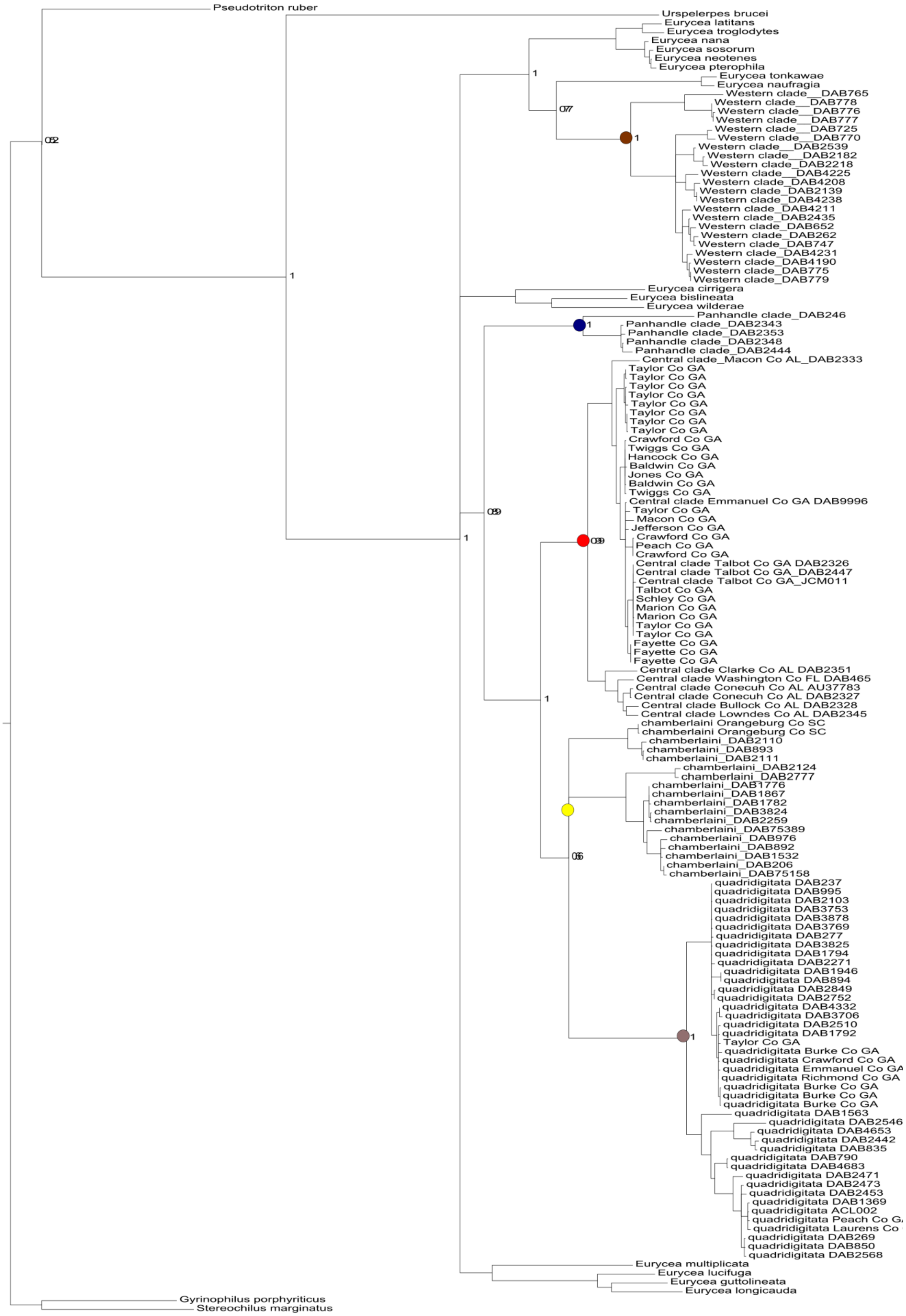

Fig. 8 Phylogram depicting relationships of *Eurycea quadridigitata* complex members and several outgroups, based upon similarity of the ND2 nuclear gene. Branch lengths are proportional to evolutionary distance, and major bootstrap values are listed. Samples listed with identification numbers ("DAB #") are from Lamb and Beamer (2012) and were derived from genbank. Other samples are from the current study, and their collection county is labelled. Nomenclature of the five complex clades are from Lamb and Beamer (2012) and color coded for comparison to figure 4. The red dot indicates the central clade, which all yellow-bellied dwarf salamanders collected in Georgia group with.

DISCUSSION

Given the similarity between specimens collected in Georgia during 2013-2015 to the type description of *Eurycea chamberlaini*, we were surprised to discover that salamanders tentatively identified as *Eurycea chamberlaini* (henceforth referred to as the "central clade") do not form a monophyletic clade with definitively-identified, topotypic *E. chamberlaini* from the Carolinas. Furthermore, despite thorough sampling, no specimens attributable to *E. chamberlaini* were located in the eastern Coastal Plain of Georgia near the South Carolina border. In fact, there appears to be a gap in the distribution of yellow-bellied *Eurycea quadridigitata* complex members in the vicinity of Augusta, Georgia (Fig. 2). The furthest east in Georgia we were able to procure specimens of the central clade was Jefferson County, Georgia. *E. chamberlaini* reaches as far west as the Savannah River Site in Barnwell County, South Carolina (Harrison and Guttman, 2003). We were only able to find *E. quadridigitata* sensu stricto in the intervening region, despite exhaustive attempts to find yellow-bellied complex members in Richmond and Burke Counties, Georgia (Fig 1). The Savannah River apparently represents a boundary in the distribution between these morphologically similar, yet genetically distinct clades. This river is a biogeographic boundary for other species as well; the distribution of *Ambystoma mabeei* apparently does not cross the river to the Georgia side, and the distribution of *Pseudacris brimleyi* crosses into Georgia but does not extend much further beyond the Savannah River (Jensen et al., 2008).

Preliminary morphological data (Graham et al., in prep) show the central clade is diagnosable from *E. chamberlaini*. For example, the modal number of costal grooves for *E. chamberlaini* is 16 (Harrison and Guttman, 2003), while the number for the central clade is slightly higher (17; Graham et al., in prep). In addition, the limbs of central clade specimens are slightly longer; the mean number of costal grooves between adpressed limbs in the central clade = 3.5 (Graham et al., in prep), while the mean number for *E. chamberlaini* = 4.2 (Harrison and Guttman 2003). These features, their allopatric distribution, and our genetic analyses strongly support recognition of the central clade as a separate species, which we intend to describe in the coming months (Graham et al., in prep).

This finding has conservation implications for *E. chamberlaini*. It was our goal to determine the conservation and genetic status of salamanders similar to *E. chamberlaini* in Georgia, in hopes of establishing that this species is more widespread and common than previously supposed. Instead, we failed to positively identify *E. chamberlaini* in Georgia, indicating that the handful of populations assignable to *E. chamberlaini* in North and South Carolina (~ 21) are indeed the only ones currently known (Center for Biological Diversity, 2010). Previous attempts to extend the known range of this species in Georgia (Graham et al., 2007; Means, in Jensen et al., 2008) and Alabama (Graham et al., 2010) were based on morphology alone and these populations are instead assignable to the central clade. It seems unlikely *E. chamberlaini* will be found in eastern Georgia. However, additional searches in counties bordering South Carolina may eventually prove fruitful.

However, there is reason for guarded optimism regarding *E. chamberlaini*'s global conservation status. Although our genetic results demonstrate we admittedly have very little experience with this salamander, a survey within the known range of *E.*

chamberlaini similar to the one we report here may succeed in finding many new populations in North and South Carolina. We were able to nearly quadruple the known populations of the central clade in Georgia and fill all of its previous distribution gaps. It is likely that similar, dedicated searches in the Carolinas would contribute greatly to our understanding of *E. chamberlaini*'s range, and would likely fill important distribution gaps. In addition, we can report anecdotally that *E. chamberlaini* may be far more abundant locally than populations of the central clade, which may make such searches even more fruitful. During this research SPG visited a locality in western South Carolina known to harbor *E. chamberlaini* (a property of Whit Gibbons in Orangeburg County, South Carolina). These were the only specimens collected that grouped genetically with *E. chamberlaini* (identified as "chamberlaini Orangeburg County" in Fig. 8). Until this visit only a single *E. chamberlaini* had been found there. After using our search technique (digging through sphagnum moss), we (Whit Gibbons and his grandson Parker) were able to quickly find dozens of *E. chamberlaini*. The population densities of *E. chamberlaini* at this site exceeded the densest locality for the central clade I have found (in Taylor County, Georgia). Although this observation is speculative and needs additional surveys and confirmation, in our limited experience *E. chamberlaini* appears to occur at much higher local densities than populations assignable to the central clade.

Given the large number of occupied sites for the central clade found from the Panhandle of Florida to Mississippi, across southern Alabama east to Jefferson County, Georgia, it does not appear that the central clade requires immediate conservation attention. We and others have located 58 localities within Georgia that harbor this undescribed species, and it is likely many more unidentified sites contain them in Georgia and elsewhere. It is possible that Georgia alone contains over 100 populations; had our surveys concentrated solely on finding new populations, rather than locating new populations in new counties, we would likely have found many more locations harboring this species in the western part of its state distribution. In addition, many of the localities supporting the species are nothing special from a natural area standpoint; we found them along the edge of an old farm pond, along narrow, eroded first order streams overgrown with invasive shrubs, crossing roads between pastures, and other such habitats. The single most important habitat requirement appears to be bay swamps with relatively intact hardwood overstories and thick accumulations of sphagnum moss. First, second, and third order streams, as well as gum ponds completely surrounded by agricultural and suburban development, support such habitats and harbor populations. Given Georgia's current system for conservation listings, once described as a new species, the central clade should be listed as "G4, S3"—indicating a comparatively secure global status and a "vulnerable" state status (between 21-100 occurrences). Furthermore, the high local concentrations of high density populations in the western Fall Line region of Georgia likely represent the greatest number of populations and individuals for this species within its four state distribution.

On the other hand, *Amphiuma pholeter*'s current global and state status is certainly warranted. Despite intense efforts to locate new populations, we were only able to determine that this species still occurs within the state at one of its two historical collection localities. Search effort between this study and Stevenson's (2003) survey for *A. pholeter* were similar; e.g., we surveyed a comparable number of sites (this study, N = 40 vs. N = 42 in Stevenson, 2003). However, we also located four high quality mucky seepage habitats (two each in Thomas and Decatur Counties) and resurveyed these sites multiple times (using traditional muck raking as well as crayfish and minnow traps), but were still unable to locate any new populations. It is likely that a few additional *A. pholeter* localities remain to be discovered in Georgia, however, they likely occur on private land that is difficult to access. Our surveys were mostly restricted to bridge crossings where access is good (as well as a few sites within large, privately-owned tracts where permission for access was granted), and therefore remote sites far from bridges on private land will be very difficult to locate and survey. This species should retain its "G3, S1" rank within Georgia, and attempts should be made to place the only remaining site where it persists within a conservation easement agreement. The landowners (Caroline Murphy) were exceedingly polite and receptive to the conservation of the salamanders, and it is likely they would be easy to work with to develop a conservation plan.

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Appendix 1 Localities surveyed for presence of *Eurycea* cf. *chamberlaini* in Georgia. Other amphibian and reptile species encountered are listed, however, they are presented as codes; the first two letters of the genus and first two letters of species name.

county	lat	long	site description	mo.	day	yr.	effort (min.)	# found	other species
Wilkinson	32.78531	82.97257	first order stream in hardwood forest; good sphagnum	10	19	2013	225	0	ANTE, HYFE
Washington	32.78343	82.94798	cypress swamp; dry at time of survey	10	19	2013	50	0	
Washington	32.99782	82.82824	first order stream and beaver marsh	10	19	2013	100	0	PSRU
Washington	33.11202	82.80171	beaver marsh	10	19	2013	100	0	
Washington	33.12502	82.79677	first order bay branch with good patches of sphagnum	10	20	2013	150	0	EUCI, ACCR
Glascock	33.22798	82.62694	small roadside park; bay swamp, eroded muck with little sphagnum	10	20	2013	100	0	
Jefferson	33.23971	82.29681	bay swamp associated with Reedy Creek; good patches of sphagnum	10	20	2013	150	0	HYAV
Richmond	33.31372	81.95414	David Selph's property; seepages and bay swamp associated with Spirit Creek; some sphagnum	10	20	2013	150	0	EUCI
Richmond	33.31456	81.95514	Cypress and lowland hardwood swamp	10	20	2013	150	0	EUCI
Meriwether	33.1111	84.54726	seepages and first order branch in hardwood forest	12	19	2013	100	0	PLSER
Talbot	32.62255	84.4345	3rd order creek with crystalline rock shoals; seepy areas	12	19	2013	60	0	ACCR, EUGL
Talbot	32.58824	84.50449	depressional wetland in sandhill	12	20	2013	20	0	
Marion	32.52624	84.56994	bay branch tributary of Juniper creek; good leaf and stick packs and some sphagnum	12	20	2013	70	12	
Marion	32.54138	84.45091	depressional wetland in sandhill	12	20	2013	80	0	RACL

Muscogee	32.54661	84.79526	first order bay branch and gum pond	12	20	2013	60	0	AMOP, PSFE, PSCR, RACL
Stewart	32.04415	84.86067	second order creek and beaver marsh	12	20	2013	30	0	
Randolph	31.82998	84.70596	bay swamp and seepage downstream from millpond	12	21	2013	70	0	PSRU, PLGL, EUCI, HYCI
Randolph	31.83479	84.68189	beaver marsh with seepy margins and some slope seeps	12	21	2013	50	0	
Macon	32.28253	84.02594	McKenzie Millpond; some seepy areas downstream of dam and sphagnum along edge of pond near buttonbush zone	12	21	2013	120	0	DEAP, RASP, HYSQ, PLGL
Wilkinson	32.75119	83.01981	Hardwood forest and bottomlands associated with Little Sandy Creek; some seeps, vernal pool, little sphagnum	12	22	2013	60	0	AMOP
Wilkinson	32.78531	82.97257	first order stream in hardwood forest; good sphagnum	12	22	2013	110	0	PLGL, DIPU, ACCR
Wilkinson	32.78404	82.967	cypress swamp	12	22	2013	50	0	ACCR, PSCR, PSFE
Jefferson	33.22798	82.62694	bay swamp associated with Reedy Creek; good patches of sphagnum	12	23	2013	60	0	
Burke	33.24158	81.94878	bay swamp seepages on slope near McBean Creek	12	23	2013	40	0	DECO
Monroe	32.95956	83.914	Floodplain of Tobesofkee Cr.; young beaver pond, vernal pools, and first order stream	3	3	2014	60	0	PSCR, PSFE, RASP
Jasper	33.24981	83.68328	Monticello Glades; low wet woods, first order branch, good leaf packs little sphagnum	3	4	2014	90	0	ACCR, HESC, PSFE
Baldwin	33.00646	83.22118	Edge of beaver marsh	3	4	2014	100	0	ACCR, PSCR

Wilkinson	33.04288	83.05585	Beaver pond, first order creek; some good sphagnum	3	4	2014	100	0	RASP, AGPI
Wilkinson	32.78531	82.97257	first order stream in hardwood forest; good sphagnum	3	4	2014	40	0	ELOB, PSCR, PSFE, SCLA
Wilkinson	32.78176	82.96182	cypress swamp	3	5	2014	120	0	
Jefferson	32.97777	82.49044	beaver pond with good seepy margins; good sphagnum beds	3	5	2014	140	0	EUGU, EUCI, PLGL, DECO, PSRU
Jefferson	32.97669	82.4868	beaver pond with good seepy margins; good sphagnum beds	3	5	2014	70	0	DECO, PSRU
Jefferson	33.23971	82.29681	bay swamp associated with Reedy Creek; good patches of sphagnum	3	5	2014	40	0	DIPU
Jefferson	33.23399	82.24772	Pewee Jordan's property; bay swamp floodplain of Reedy Creek; great habitat with sphagnum and all other common plant associates	3	5	2014	120	0	DECO, PSRU, ACGR
Burke	33.03307	81.90487	Beaver pond and stream	3	5	2014	56	0	PLGL
Burke	32.99223	81.80118	Bottomland hardwoods and stream downstream from millpond	3	5	2014	44	0	EUCI, EUGU, HYSQ, PLGL
Burke	33.00618	81.75353	Cypress swamp	3	5	2014	20	0	
Burke	33.16838	81.91093	Mesic slope forest and seepages, first order stream	3	5	2014	60	0	DECO, EUCI, PLGL
Richmond	33.31372	81.95414	David Selph's property; seepages and bay swamp associated with Spirit Creek; some sphagnum	3	5	2014	70	0	
Jefferson	32.87984	82.50334	Hardwood forest and headwaters of bay branch. Abundant sphagnum spotted from the road; also a vernal pool ringed with sphagnum (where the	3	7	2014	130	2	HESC, PLGL, EUCI, EUCH

			salamanders were found)						
Johnson	32.77667	82.52177	Second growth bay swamp; abundant sphagnum	3	7	2014	110	0	HYCI
Laurens	32.55318	82.72117	bay branch at edge of well-managed longleaf pine forest; some sphagnum	3	7	2014	44	0	STOC
Laurens	32.5554	82.74353	beaver pond and gum pond/swamp	3	7	2014	60	0	EUQU
Muscogee	32.439	84.64867	first order bay branch and gum pond	3	8	2014	120	0	AMOP, PLGL
Marion	32.439	84.64867	bay branch associated with Pine Knot Creek; abundant sphagnum and seepages	3	8	2014	195	0	EUCI
Marion	32.52624	84.56994	bay branch tributary of Juniper creek; good leaf and stick packs and some sphagnum	3	8	2014	150	6	RACL
Talbot	32.58824	84.50449	depressional wetland in sandhill	3	8	2014	30	0	
Marion	32.54138	84.45091	depressional wetland in sandhill	3	8	2014	114	0	NOVI, TRSC, PLGL
Talbot	32.54873	84.53285	Bay swamp floodplain of Black Creek; little sphagnum	3	8	2014	99	0	EUCI
Coweta	33.27026	84.55464	Mucky seepage at margin of beaver marsh	5	1	2014	60	0	PSRU, DIPU, TECA, RASP, SCLA
Talbot	32.58824	84.50449	depressional wetland in sandhill	5	1	2014	20	0	ACGR, RASP
Marion	32.52624	84.56994	bay branch tributary of Juniper creek; good leaf and stick packs and some sphagnum	5	1	2014	70	2	EUCI
Marion	32.50687	84.53903	Bay swamp with good leaf packs and sphagnum	5	1	2014	60	0	

Marion	32.51626	84.52467	Tiffany Spike's property; Bay swamp with Atlantic white cedar and abundant sphagnum	5	1	2014	106	0	EUCI, PLGL
Marion	32.44075	84.48743	Bay swamp with Atlantic white cedar and sphagnum	5	1	2014	60	0	EUCI
Taylor	32.64482	84.27921	Timm's Creek; bay swamp floodplain with sphagnum & seepage	5	2	2014	80	2	EUCI, PLGL, ACCR
Macon	32.47476	84.1019	Jimmy McDaniel's property; bay swamp floodplain of horse creek; some sphagnum	5	2	2014	100	1	EUCI, RASP
Schley	32.32002	84.30528	hardwood forest seepage	5	2	2014	30	0	PLGL
Schley	32.34862	84.37558	first order stream	5	2	2014	50	0	NESI
Schley	32.3042	84.34256	floodplain forest; found under leaf pack	5	2	2014	50	2	RASP, RACA
Quitman	31.88438	85.0129	ravine forest and seepage; some clumps of sphagnum	5	3	2014	140	1	DEAP, DIPU, EUCI
Randolph	31.84933	84.90254	Seepage and floodplain forest	5	3	2014	120	0	EUGU, AMOP, PLGL
Randolph	31.79404	84.85134	swamp; old beaver pond	5	3	2014	40	0	
Randolph	31.83479	84.68189	beaver marsh with seepy margins and some slope seeps	5	3	2014	140	0	EUCI, DEAP, PLRU, PLGL
Randolph	31.80402	84.64015	bottomland swamp with some bay swamp margins; little sphagnum	5	3	2014	88	0	PLGL, NEER
Randolph	31.6368	85.00547	first order bay branch; seepy margins	5	3	2014	120	0	PSRU, DEAP, DIPU, PLGL, TECA
Randolph	31.74519	84.85656	first order stream with steep eroded banks	5	3	2014	10	0	
Clay	31.6368	85.00547	bay branch with pools; good sphagnum	5	3	2014	126	0	EUCI
Clay	31.76487	85.08366	stream with no sphagnum	5	14	2014	20	0	
Clay	31.59541	85.04153	ravine forest and streams	5	14	2014	90	0	EUGU, PLGL,

									PSRU
Clay	31.54995	85.01876	first order stream with steep eroded banks	5	14	2014	40	0	ACCR
Early	31.46401	84.9971	first and second order stream with bay swamp; good seepages and sphagnum	5	14	2014	130	0	DEAP, PLGL, EUCI, PSRU
Clay	31.49881	84.97361	bay swamp with little sphagnum	5	14	2014	40	0	ACCR, DIPU
Clay	31.52921	84.89197	first order branch and cypress swamp; no sphagnum	5	14	2014	32	0	EUGU, EUCI, PLGL, GACA, SCLA
Marion	32.52624	84.56994	bay branch tributary of Juniper creek; good leaf and stick packs and some sphagnum	6	3	2014	140	7	EUCI
Marion	32.58824	84.50449	depressional wetland in sandhill	6	3	2014	60	2	COCO, ACGR, RACL
Marion	32.54138	84.45091	depressional wetland in sandhill	6	3	2014	132	2	NOV, HYCI, DIPU
Taylor	32.52623	84.41014	bay branch with seepage and abundant sphagnum	6	3	2014	120	0	PSMO, EUCI, DIPU, RASP
Taylor	32.61675	84.32227	floodplain of Whitewater Creek; bay swamp with Atlantic white cedar	6	3	2014	120	0	PSRU
Taylor	32.61675	84.32227	Gum bay swamp floodplain of Patsiliga Creek; good sphagnum beds along dry margin of floodplain	6	4	2014	120	1	EUCI, ACCR, AGPI
Taylor	32.61498	84.28037	Clarence Bickley's property; bay swamp along Reynold's Creek; good sphagnum along margin of floodplain	6	4	2014	220	1	EUCI, AGPI, DEAP
Taylor	32.63817	84.23534	Bay swamp/gum pond along Timm's Creek; sphagnum beds along margin of floodplain	6	4	2014	80	5	

Taylor	32.61929	84.18969	Beaver marsh; small clumps of sphagnum along margin	6	4	2014	20	1	AGPI
Taylor	32.59996	84.18868	Bays within alluvial swamp; no sphagnum	6	4	2014	88	0	
Taylor	32.59138	84.15795	first order creek downstream from beaver marsh; second growth bay branch with pine and young bays; good sphagnum	6	4	2014	80	2	
Marion	32.51626	84.52467	Tiffany Spike's property; Bay swamp with Atlantic white cedar and abundant sphagnum	6	5	2014	120	1	EUCI, PSRU
Marion	32.41417	84.47469	Shoal Creek floodplain; bottomland hardwoods and bay swamps with little sphagnum; SW side of creek w/ extensive sphagnum beds	6	5	2014	180	1	
Marion	32.37782	84.4458	Buck Creek floodplain; bay-gum swamp with little sphagnum	6	5	2014	180	0	EUCI
Taylor	32.51683	84.38733	Whitewater creek floodplain; bay-gum swamp with thick beds of sphagnum; "new honey hole"	6	3	2014	150	18	EUCI, AMME, ACCR
Marion	32.44054	84.64809	bay branch associated with Pine Knot Creek; abundant sphagnum and seepages	6	5	2014	150	0	EUCI, PSRU, RASP
Bibb	32.7318	83.77393	beaver marsh and slope seepages	6	12	2014	99	0	DECO, ACCR, RACL, HYCI
Bibb	32.71667	83.73713	1st order branch and gum-bay swamp; seepages and dry beaver pond	6	12	2014	75	1	
Twiggs	32.76488	83.36159	small swampy bay branch	6	12	2014	15	0	
Twiggs	32.59115	83.36143	stream and gum	6	12	2014	54	0	

			swamp						
Twiggs	32.54979	83.31876	small swampy bay branch	6	12	2014	15	0	
Taylor	32.57751	84.27065	depressional wetland in sandhill	6	12	2014	180	0	
Webster	32.10796	84.4985	alluvial swamp; no sphagnum	6	13	2014	45	0	EUGU, PLGL
Webster	32.05482	84.54713	alluvial swamp; first order stream with one patch of sphagnum	6	13	2014	60	0	EUCI, RASP, PLGL
Webster	31.9921	84.59551	beaver pond and seepage; no sphagnum	6	13	2014	45	0	DEAP, RASP
Randolph	31.73377	84.84326	first order tributary of Hog Creek; little seepage no sphagnum	6	13	2014	90	0	EUGU
Clay	31.6368	85.00547	bay branch with pools; pools dry; good sphagnum	6	13	2014	60	0	
Clay	31.75892	84.98348	beaver pond and second growth pine jungle	6	13	2014	5	0	
Washington	33.12502	82.79677	first order bay branch with good patches of sphagnum	6	20	2014	200	0	EUCI, DECO, PSRU, RASP, RACL, AGPI, PLGL
Glascock	33.22882	82.68713	bay swamp grown over old millpond; seepy margins with some sphagnum	6	20	2014	180	0	COCO, DECO, RASP, RACL, ACCR
Glascock	33.25627	82.54572	bay swamp downstream from Blankenship Millpond; bay swamp with seepy margins and thick beds of sphagnum	6	20	2014	180	0	DECO, EUCI, HYAV, ACCR, RASP
Jefferson	33.23399	82.24772	Pewee Jordan's property; bay swamp floodplain of Reedy Creek; great habitat with sphagnum and all other common plant associates	6	20	2014	200	0	EUQU, DECO, PSRU, EUCI, RASP, RACL, HYCH, DIPU
Jones	32.91114	83.43585	Slash creek; beaver pond, bottomland hardwoods with some good patches	6	21	2014	120	0	

			of sphagnum						
Jones	32.90611	83.44391	Slash creek floodplain; gum swamp	6	21	2014	10	0	
Twiggs	32.87076	83.45453	beaver marsh w/ gums edged with sphagnum; south side bay swamp	6	21	2014	105	0	
Wilkinson	32.78531	82.97257	first order stream in hardwood forest; good sphagnum	6	21	2014	90	0	RASP, GACA, SCLA
Johnson	32.77667	82.52177	Second growth bay swamp; abundant sphagnum	6	21	2014	60	0	EUGU
Burke	32.93333	81.99442	Mill's Creek floodplain; gum swamp with good sphagnum	6	22	2014	75	0	EUQU
Jenkins	32.89503	82.02386	Blackwater creek and cypress swamp	6	22	2014	75	0	EUCI, RASP, RACL
Jenkins	32.87477	82.03018	Duke's Pond WMA; gum pond along entrance road	6	22	2014	60	0	
Burke	33.07881	81.84488	Bay swamp with seepy margins; little sphagnum	6	22	2014	120	0	DECO, SIIN, RASP
Twiggs	32.90664	83.44345	gum-beaver pond; good sphagnum beds and seepy margins	7	5	2014	60	0	EUCI
Twiggs	32.87076	83.45453	beaver marsh w/ gums edged with sphagnum; south side bay swamp	7	5	2014	45	0	SCUN, COCO, RASP
Twiggs	32.86945	83.46022	Griswoldville Battlefield Historic site; first order branch and impoundments	7	5	2014	15	0	
Twiggs	32.85732	83.4497	Little Sandy Creek; beaver swamp and gum-bay swamp; some sphagnum	7	5	2014	30	0	DECO, AGPI, RASP
Wilkinson	32.89611	83.37231	beaver ponds	7	6	2014	20	0	
Wilkinson	32.8883	83.36662	wide, thick bay gum swamp grown over with gallberry	7	6	2014	26	0	
Wilkinson	32.92301	83.35059	hardwood forest and first order creek; leads to beaver pond; small	7	6	2014	60	2	DECO

			patch of sphagnum						
Wilkinson	32.95732	83.23399	Black Creek headwaters; spring and bay swamp	7	6	2014	30	1	DIPU, AGPI
Wilkinson	32.94403	83.19388	gum bay swamp with mucky bottom and exposed roots	7	6	2014	30	0	AGPI, EUCI
Wilkinson	32.94178	83.15573	Gum bay swamp little sphagnum	7	6	2014	30	0	EUGU, PSRU
Jones	32.88813	83.49973	bay swamp, little sphagnum	7	7	2014	10	0	
Macon	32.4739	83.95143	alluvial swamp with some bays and gum; good seepages and sphagnum much hog damage	7	7	2014	60	0	EUCI, PSRU
Macon	32.44548	84.07922	bay swamp; entire floodplain worked over by hogs	7	7	2014	10	0	
Crawford	32.68608	83.96752	large beaver pond; alder thickets and little sphagnum	7	8	2014	30	0	
Crawford	32.68169	83.9451	north side beaver pond, S side immature gum swamp w/ seepage	7	8	2014	21	1	EUCI
Crawford	32.68513	83.88709	first order stream; ravine seepages and thick sphagnum; seepage salamanders!!!	7	8	2014	210	0	DEAE, PLGL, EUCI, PSRU, RASP
Crawford	32.64632	83.89732	Mossy Creek; bay swamp, little sphagnum, found at edge of old drying beaver pond	7	8	2014	144	1	PSCR, GACA, PLGL
Crawford	32.61839	83.89476	beaver ponds, bay swamp	7	8	2014	72	0	
Crawford	32.71056	83.84645	first order bay branch w/ some sphagnum	7	8	2014	48	0	
Crawford	32.72719	83.83866	Deep Creek; blackwater bay swamp & decent sphagnum	7	8	2014	75	1	
Marion	32.4229	84.58973	bay swamp with sphagnum beds	7	10	2014	36	1	
Marion	32.38152	84.51472	along beaver pond; good sphagnum patch	7	10	2014	35	1	

Taylor	32.45209	84.50883	Shoal Creek tributary; bay swamp at edge of beaver pond; gallberry jungle	7	10	2014	45	0	
Taylor	32.44075	84.48743	Bay swamp with Atlantic white cedar and sphagnum	7	10	2014	23	1	
Marion	32.45061	84.47507	gum pond and first order branch; no sphagnum	7	11	2014	5	0	
Marion	32.46748	84.48067	Tributary of Shoal Creek; bay-gum swamp; good sphagnum	7	11	2014	30	0	
Marion	32.26151	84.44901	bay swamp w/ good sphagnum beds	7	11	2014	60	0	
Marion	32.4276	84.3806	Cedar Creek; bay swamp w/ Atlantic white cedar; extensive sphagnum	7	11	2014	30	2	
Marion	32.46847	84.33256	Ambulette Creek; deeply eroded, little sphagnum	7	11	2014	12	0	
Taylor	32.49383	84.29458	Large floodplain; little sphagnum	7	11	2014	30	0	
Clay	31.61711	84.82995	First order stream with floodplain forest; found in stick pack	7	12	2014	5	1	
Early	31.46401	84.9971	first and second order stream with bay swamp; good seepages and sphagnum	7	12	2014	45	0	PSRU, DEAP, EUGU
Early	31.20599	85.08527	Williams Bluff Preserve; ravine forest and seepages	7	12	2014	170	0	PSRU, PLGL, RACL, RASP
Washington	30.03636	82.99098	first order tributary of Deepstep Creek; second growth gum-bay swamp; good sphagnum beds	7	21	2014	15	0	EUCI, RASP
Washington	33.08919	82.99332	First order tributaries of Tiger Creek; bay swamp and first order stream	7	21	2014	90	1	
Washington	32.88968	82.69346	Little Ohoopee River headwaters; alluvial swamp with no sphagnum	7	21	2014	5	0	

Washington	32.85765	82.55632	Salter Branch; alluvial, dry	7	21	2014	5	0	
Washington	32.83639	82.5562	Smith Creek headwaters; gum swamp with good sphagnum beds	7	21	2014	75	2	
Washington	33.01823	82.97812	Deepstep Creek floodplain; bay swamp edged with seepages and sphagnum	7	22	2014	90	0	EUGU, AGPI, RASP, DIPU
Washington	33.08341	82.7615	Anderson Swamp Creek below Mays Millpond; bay swamp	7	22	2014	75	0	COCO, DECO
Washington	33.12588	82.72604	first order headwaters and gum pond	7	22	2014	10	0	DECO, AGPI
Glascock	33.26822	82.52045	Deep Creek tributary; beaver pond and bayswamp; seeps and sphagnum	7	22	2014	80	0	EUCI, TECA, AGPI, RACL, RASP
Burke	32.85632	82.28549	hardwood forest with second to third order creek, small seepage and little sphagnum	7	23	2014	37	0	GACA
Burke	32.85521	82.27339	alluvial floodplain, little sphagnum	7	23	2014	15	0	
Burke	32.78788	82.21982	alluvial floodplain, little sphagnum	7	23	2014	10	0	
Emmanuel	32.78088	82.22538	gum ponds	7	23	2014	15	0	
Emmanuel	33.12588	82.72604	gum-cypress pond	7	23	2014	30	0	EUQU
Emmanuel	32.65938	82.3592	first order branch, dry gum-hardwood pond; extensive sphagnum	7	23	2014	45	0	
Burke	32.8844	82.19893	first order stream	7	23	2014	20	0	
Burke	32.9704	82.0932	Di-Lane Plantation, small grassy pond edged with good sphagnum	7	23	2014	50	0	EUQU
Burke	32.88267	82.28225	first order creek; second growth hardwoods	7	25	2014	25	0	
Burke	32.8837	82.27079	bottomland hardwoods, first order stream; some sphagnum along margin of floodplain	7	25	2014	25	0	
Burke	32.8868	82.25829	alluvial gum-bay-hardwood swamp	7	24	2014	28	0	
Burke	32.90408	82.22163	first order stream	7	24	2014	5	0	

Burke	32.88443	82.20427	hardwood forest, second order stream & small seepage; no sphagnum	7	24	2014	10	0	
Burke	32.97615	82.19306	third order alluvial creek	7	24	2014	15	0	
Burke	32.95684	82.07771	Di-Lane Plantation, gum swamp headwaters and slough; good sphagnum, looks like Hemidactylium habitat	7	24	2014	55	0	
Burke	32.95684	82.07771	Di-Lane Plantation; gum pond edged with sphagnum	7	24	2014	45	0	
Burke	33.08666	81.76192	Steephead; followed stream to newly flooded segment & young beaver pond	7	24	2014	60	0	
Burke	32.95684	82.07771	Di-Lane Plantation, gum swamp headwaters and slough; good sphagnum, looks like Hemidactylium habitat	11	24	2014	120	0	PSMO, AMOP, GACA, ACCR, EUQU
Burke	32.98584	82.06143	Di-Lane Plantation; first order stream and seepages	11	24	2014	120	0	PSRU, PLGL, SCLA, EUQU
Upson	32.95156	84.51829	hardwood ravines and seepages; good sphagnum	11	26	2014	120	0	DEAP, PSRU, EUCI, DIPU, RACA, RACA
Jasper	33.24981	83.68328	Monticello Glades; low wet woods, first order branch, good leaf packs little sphagnum	5	20	2015	40	0	
Jones	32.91261	83.42875	bay swamp	5	20	2015	30	1	
Jones	32.90946	83.43584	first order bay branch; sphagnum at edge of power line right of way	5	20	2015	20	1	
Twiggs	32.87076	83.45453	beaver marsh w/ gums edged with sphagnum; south side bay swamp	5	20	2015	40	0	EUCI
Twiggs	32.82601	83.41942	young red maple swamp and beaver pond	5	20	2015	20	0	

Twiggs	32.80483	83.41461	Big Sandy Creek floodplain; bay swamp; good sphagnum beds and hog sign	5	20	2015	35	2	
Richmond	33.29974	82.02009	gum swamp, some good sphagnum	5	22	2015	75	0	
Richmond	33.25326	82.08501	hardwood ravines and seepages; no sphagnum	5	22	2015	40	0	DECO, EUCI, PSRU, AMME, ACCR, PLFA
Burke	33.27858	82.13884	gum swamp with thick sphagnum beds; was able to work sphagnum beds for one hour	5	22	2015	60	0	EUCI
Burke	33.12583	81.73609	first order bay branch; seepages little sphagnum	5	22	2015	30	0	EUCI, PSRU
Baldwin	32.98505	83.3373	Jimmy Brown's first order bay branch	5	23	2015	15	0	LAGU
Baldwin	32.99214	83.31277	Gum pond with some sphagnum; found under wet leaf litter at pond edge	5	23	2015	90	3	
Hancock	33.16127	83.06646	first order creek and drying pools with thick leaf litter	5	23	2015	60	0	AMME, EUCI, DECO, PSRU, HESC
Hancock	33.16127	83.06646	gum pond; headwaters with good sphagnum	5	23	2015	40	1	
Houston	32.53001	83.71556	beaver pond edged with bay swamp; some good sphagnum	5	25	2015	60	1	EUCI
Bleckley	32.51875	83.30103	gum pond with first order branch and some good sphagnum	5	25	2015	30	0	EUQU
Hancock	33.24679	82.86308	gum pond with first order branch	5	26	2015	60	0	PSMO, EUCI
Hancock	33.23731	82.8613	beaver pond; no sphagnum	5	26	2015	60	0	
Hancock	33.22936	82.85604	gum swamp and seepages	5	26	2015	60	0	DECO, EUCI
Hancock	33.18336	82.78973	first order stream; abundant sphagnum at confluence with impoundment	5	26	2015	60	0	
Glascock	33.25511	82.724383	gum pond	5	26	2015	20	0	AGPI

Glascock	33.27888	82.65477	gum bay swamp downstream from millpond; good sphagnum beds	5	26	2015	45	0	DECO, EUCI
Glascock	33.24724	82.60927	bay gum branch, little sphagnum	5	26	2015	15	0	
Johnson	32.76693	82.6947	gum swamp, one good sphagnum bed	5	27	2015	30	0	STOC, AMOP
Johnson	32.79592	82.52893	gum swamp; good sphagnum beds	5	27	2015	45	0	
Johnson	32.77667	82.52177	second growth gum swamp; good sphagnum	5	27	2015	40	0	
Emmanuel	32.6046	82.42499	bay branch overgrown with shrubs; striped newt pond	5	27	2015	30	0	EUQU
Emmanuel	32.65917	82.35888	first oder branch, dry gum-hardwood pond; extensive sphagnum	5	27	2015	45	1	
Richmond	33.33282	82.24504	Atlantic white cedar pond; edged with sphagnum	5	28	2015	60	0	EUQU
Richmond	33.33282	82.24504	Atlantic white cedar pond; bay swamp feeding pond	5	28	2015	60	0	DECO, EUCI
Richmond	33.32116	82.20135	gum pond; open canopy	5	28	2015	60	0	ANTE
Richmond	33.32177	82.20923	small depressional wetland; ring of dry, thin aquatic sphagnum	5	28	2015	60	0	AMOP
Richmond	33.32069	82.20579	small depressional wetland	5	28	2015	60	0	ANQU, SCHO
Richmond	33.34229	82.24355	bay swamp along Sandy Run Creek	5	28	2015	120	0	EUCI
Richmond	33.35551	82.25158	bay branch; extensive, thick sphagnum	5	28	2015	120	0	
Peach	32.58493	83.85369	bay swamp downstream from old beaver dam; some good sphagnum	5	30	2015	30	2	EUCI, DIPU
Peach	32.50958	83.82893	beaver pond edged with seepy hardwoods	5	30	2015	10	0	EUCI
Peach	32.49909	83.81243	gum-bay swamp, some sphagnum	5	30	2015	20	0	EUQU
Peach	32.60665	83.87316	beaver swamps, no sphagnum	5	30	2015	20	0	
Crawford	32.63232	83.91895	bay-gum branch, good sphagnum beds	6	1	2015	10	2	

Crawford	32.65803	84.03531	alluvial gum swamp, no sphagnum	6	1	2015	30	0	EUGU
Crawford	32.65998	83.98981	gum-bay swamp; little sphagnum	6	1	2015	45	0	
Taylor	32.54173	84.10106	bay swamp with seepy margins; good sphagnum	6	1	2015	45	0	EUCI
Fayette	33.38287	84.47386	old farm pond grown over with young sweetgum and pine; some patches of sphagnum; found under leaf litter at edge of pond	6	10	2015	60	4	
Coweta	33.43129	84.94086	Noah Field's bay swamp; little sphagnum	6	8	2015	120	0	AMMA, HESC, LISP
Taylor	32.56247	84.40471	bay branch; good sphagnum	6	13	2015	120	3	EUCI
Taylor	32.58268	84.38638	bay branch; head of beaver pond with good sphagnum	6	13	2015	60	1	EUCI
Taylor	32.50468	84.29522	Bay swamp with Atlantic white cedar and sphagnum	6	14	2015	45	1	
Burke	33.05033	82.18049	hardwood forest and first order streams	6	14	2015	15	0	
Burke	33.06411	82.1296	cypress pond	6	14	2014	10	0	EUQU
Burke	32.95012	82.035	cypress-gum swamp, seepy and mucky, no sphagnum	6	16	2014	15	0	
Burke	32.95474	82.06081	Di-Lane WMA gum-cypress pond & blackwater creek floodplain	6	16	2014	60	0	
Burke	33.16845	81.91103	beaver ponds edged with bay swamp; good sphagnum	6	16	2014	60	0	EUCI, EUGU, DECO, DIPU
Burke	33.2541	82.10982	alluvial hardwood forest	6	16	2014	5	0	
Burke	33.27858	82.13884	edge of impoundment; alders and thick beds of sphagnum	6	16	2014	60	0	EUCI
Burke	33.06986	81.6266	cypress swamp with no sphagnum	6	17	2014	10	0	
Burke	33.10008	82.10381	gum pond	6	17	2014	60	0	RASP,
Burke	33.1031	82.13029	hardwood forest and first order stream	6	17	2014	10	0	

Burke	33.13895	82.2139	gum pond	6	17	2014	15	0	
Burke	32.95684	82.07771	Di-Lane Plantation, gum swamp headwaters and slough; good sphagnum, looks like Hemidactylium habitat	11	27	2015	90	0	PSMO, PLGL, ACCR, ANCA
Burke	32.95326	82.08239	Di-Lane Plantation, tiger salamander pond and gum pond	11	27	2015	45	0	NEFA
Burke	33.13747	82.02875	hardwood swamp and seepages	11	27	2015	45	0	PSRU, EUCI, ANCA
Screven	32.84068	81.85007	Carolina bay	11	27	2015	90	0	PSOR, PSOC, AMTA, AMOP, PSNI, LICL, PSCR

Appendix 2 Sites surveyed for presence of *Amphiuma pholeter*. The one site where the species' presence was detected is highlighted in grey. Species codes for other species detected at each site are the same as in appendix 1.

county	lat	long	site description	mo.	day	yr.	effort (min.)	other species
Grady	30.73286	84.27475	Joe Knight's property second order creek and bottomland hardwood, some sphagnum beds no deep mucks	5	15	2014	60	EUCI, ACCR
Thomas	31.00277	83.90648	James' property, blackwater creek with steep banks and privet	5	15	2014	30	EUQU
Thomas	31.00752	83.9067	Patricia Wilson's property, blackwater creek with floodplain forest; privet abundant; some muck found and searched	5	15	2014	80	EUGU, PLGL
Decatur	30.72781	84.68354	N Fork Mosquito Creek; slopes leading to narrow floodplain forest; some shallow, leafy, clay mucks	5	16	2014	30	EUGU, RACL
Decatur	30.70967	84.72405	Mosquito Creek floodplain; little black muck, some sphagnum	5	16	2014	110	EUGU, EUCI, SCL
Decatur	30.73186	84.76203	Mosquito Creek; alluvial swamp	5	16	2014	10	
Decatur	30.73039	84.59227	extensive seepage habitat & seepages; mucks about a foot deep with sphagnum beds	5	16	2014	240	EUCI, EUGU, PSRU, PSMO, DEAP, AMME
Decatur	30.74792	84.57213	Willacoochee Creek tributary; bay swamp with little sphagnum and shallow mud	5	16	2014	60	
Decatur	30.77384	84.40197	small creek; no muck	5	16	2014	10	
Decatur	30.75742	84.46508	Alluvial swamp	5	16	2014	10	
Liberty	30.5256	84.97014	cypress-bay swamp edged with seepage & sphagnum. Pholeter found within 5 min. in ~ 3 inch muck pools	5	16	2014	90	
Decatur	30.73039	84.59227	extensive seepage habitat & seepages; mucks about a foot deep with sphagnum beds	5	16	2014	120	
Decatur	30.85541	84.3972	Tributary of Attapulgus Creek; bay swamp with abundant sphagnum and good seepy muck	6	14	2014	255	SIIN, EUQU, DEAP, EUCI, PSMO
Decatur	30.80872	84.42899	small tributary of Attapulgus creek; leaf packs and lowland forest	6	14	2014	45	EUQU
Decatur	30.81415	84.43644	third order blackwater creek, small seepage with muck	6	14	2014	30	

Decatur	30.83501	84.4619	small creek; no seep, no muck	6	14	2014	30	
Decatur	30.71363	84.84965	Torreya ravines; no wetland habitat	6	15	2014	105	
Decatur	30.75107	84.58689	Willacoochee Creek; alluvial swamp; followed small branch to source; steephead at the edge of ag lands; some deep muck	6	15	2014	150	
Decatur	30.77383	84.56188	unnamed tributary of Willacoochee Creek; gum-bay swamp; little sphagnum, no seepage	6	15	2014	30	
Decatur	30.79563	84.56735	gum-bay swamp; no seepage or muck	6	15	2014	30	
Decatur	30.84221	84.43702	Small Attapulgus Creek tributary; second growth forest w/ no seep or sphagnum	6	15	2014	15	
Decatur	30.84049	84.40001	beaver pond/gum bay swamp	6	16	2014	30	
Thomas	30.84993	84.07116	Gum pond	6	16	2014	135	EUQU, RASP, RACL
Thomas	31.02464	83.95149	unnamed tributary of Ochlockonee River, good mucky area, blackwater sloughs	6	16	2014	105	AMME, SIIN, RASP
Thomas	30.82091	84.04617	Myrtlewood Plantation; first order branch feeding impoundment; good seepage and muck beds	6	17	2014	225	AMME, EUCI, EUQU, RACL, RASP, AGPI, PSMO
Thomas	30.82091	84.04617	Myrtlewood Plantation; first order branch feeding impoundment; good seepage and muck beds	6	17	2014	300	AMME, EUQU, AGPI, ACGR, RASP
Thomas	30.86279	84.0521	River Creek WMA; blackwater sloughs associated w/ Ochlockonee River	6	18	2014	30	RACL, RACA
Grady	30.72251	84.26396	Caroline Murphy property; hardwood forest and first order stream; *pholeter* found in tiny muck pool at base of tree (Fig. 5); source of stream is downstream from earthen impoundment which is now dry.	6	18	2014	90	EUCI, AGPI, ACGR
Grady	30.72141	84.27273	small bay branch and dry beaver marsh	6	18	2014	75	
Decatur	30.79575	84.407	third order blackwater creek, N side with beaver pond; no muck; hog sign	7	13	2014	20	
Decatur	30.77028	84.34627	Small seep w/ muck	7	13	2014	25	

Decatur	30.80872	84.42899	Second order stream in hardwood forest; explored 2 first order feeder streams	7	13	2014	50	
Decatur	30.83527	84.24115	mature bottomland hardwoods; Wolf Creek floodplain	7	13	2014	15	
Decatur	30.81852	84.24993	Mature bottomland hardwoods; Turkey Creek floodplain	7	13	2014	15	
Decatur	30.70789	84.35914	hardwood forest, first order stream with eroded bank	7	13	2014	5	
Decatur	30.75144	84.30313	dry first order bay branch	7	13	2014	5	
Thomas	30.85832	84.0347	Greenwood Plantation; first order branch with small patch of muck at headwaters	7	14	2014	150	
Thomas	30.86337	84.04642	Greenwood Plantation; bluffs along Ochlockonee River; small seeps; little muck along slough	7	14	2014	90	EUQU
Thomas	30.78052	83.80216	Aucilla River floodplain; alluvial, no muck	7	15	2014	5	
Thomas	30.81267	83.84489	hardwood cypress swamp; alluvial no muck	7	15	2015	5	
Thomas	30.6975	83.71378	Blackwater Creek; sand bottom	7	15	2014	5	
Thomas	30.8934	84.29239	Slope forest, bottomland hardwoods, sand bottom, no muck	7	15	2014	30	
Decatur	30.85541	84.3972	Tributary of Attapulgus Creek; bay swamp with abundant sphagnum and good seepy muck	7	15	2014	30	
Thomas	30.82091	84.04617	Myrtlewood Plantation; first order branch feeding impoundment; good seepage and muck beds	3	15	2015	60	EUGU, EUCI, AGPI, HYCH, ACCR, ANCA
Thomas	31.02464	83.95149	Myrtlewood Plantation; first order branch feeding impoundment; good seepage and muck beds	3	15	2015	60	
Decatur	30.85541	84.3972	Tributary of Attapulgus Creek; bay swamp with abundant sphagnum and good seepy muck	3	15	2015	60	PSMO, EUCI
Decatur	30.73039	84.59227	extensive seepage habitat & seepages; mucks about a foot deep with sphagnum beds	3	15	2015	60	