ELASSOMA GILBERTI, A NEW SPECIES OF PYGMY SUNFISH (ELASSOMATIDAE) FROM FLORIDA AND GEORGIA

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ABSTRACT

A new species of pygmy sunfish, *Elassoma gilberti* (Elassomatidae), is described from northwestern Florida and extreme southwestern Georgia. It previously has been confused with its sister species, *Elassoma okefenokee* Böhlke 1956. The two are very similar morphologically, but differ in the number of preopercular canal pores (four in *E. gilberti*, three in *E. okefenokee*), in average number of anal fin rays (usually seven in *E. gilberti,* usually eight in *E. okefenokee*), and in more subtle differences in coloration, body depth, and dorsal and anal fin size. The distinction of the two species is supported by eight fixed differences at the mitochondrial 16S rRNA locus and 12 fixed differences at the nuclear S7 locus. Phylogenetic analyses using these molecular characters supported monophyletic clades that contained haplotypes and alleles found uniquely in the two taxa. *Elassoma gilberti* is found in stream systems draining into the Gulf of Mexico from Choctawhatchee Bay in the Florida panhandle south to the Withlacoochee and Homosassa drainages in west-central Florida. Both species occur in the Suwannee River drainage, *E. gilberti* in the lower and middle sections and *E. okefenokee* in the middle and upper sections. They remain genetically distinct where sampled in this drainage but have not been found syntopically.

The history and nomenclatural status of the name *Elassoma evergladei orlandicum* Lönnberg 1894 is discussed and a lectotype is designated based on the earlier findings of R. M. Bailey and J. E. Böhlke. Lectotype designation relegates the name to the synonymy of *Elassoma evergladei* Jordan 1884.

Key Words: pygmy sunfish, *Elassoma*, Florida, Georgia, new species, nomenclature.

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INTRODUCTION

The pygmy sunfishes, family Elassomatidae, are endemic to the southeastern United States. The family consists of six described species, all in the genus *Elassoma*. The characters distinguishing the species are summarized by Mayden (1993). Although the phylogenetic affinities and classification of the family have been the subject of considerable controversy and speculation (see summary in Nelson 2006), the monophyly of the six *Elassoma* species is supported by genetic analysis (Jones & Quattro 1999; Quattro et al. 2001; Roe et al. 2002) and by a number of shared apomorphic morphological traits (Branson & Moore 1962; Johnson 1984). All species of *Elassoma* are small, averaging between 25-35 mm standard length as adults, and prefer springs, swamps, ditches, or slow moving streams with abundant submerged vegetation.

In this paper we describe a new species of pygmy sunfish that is sister and closely related, both morphologically and genetically, to *Elassoma okefenokee* Böhlke 1956. In the early 1990's, FFS noticed that *E. okefenokee* collected in central Florida usually had three pores in the preopercular (PO) branch of the cephalic lateral-line canal, whereas *Elassoma evergladei* Jordan and *Elassoma zonatum* Jordan collected in the same region usually had four PO pores. Further study of material housed at the Florida State Museum of Natural History revealed that all species of *Elassoma* normally have four PO pores, including populations of *E. okefenokee* from the Florida panhandle. Additional study, now supported by genetic analysis and distributional data, reveals that the four-pored form of "okefenokee" is specifically distinct from the true *E. okefenokee*, and that *E. okefenokee* is unique in the genus in having three PO pores throughout its range.

Herein we describe this new species and detail its distribution in relation to *Elassoma okefenokee*, especially in the Suwannee River system of Florida and Georgia, where both species occur but remain separated geographically. We also present analysis based on both mitochondrial and nuclear gene sequence data that is entirely consistent with the morphological analysis and supports reciprocal monophyly of the two sister taxa. Finally, we re-examine the nomenclatural status of "Elassoma evergladei orlandicum" Lönnberg 1894 and designate a lectotype based on earlier analysis by R. M. Bailey and J. E. Böhlke.

METHODS

MORPHOLOGY

Methods used in making counts and measurements follow Hubbs and Lagler (1974) and Rohde and Arndt (1987). All measurements were made to the nearest 0.1 mm with dial calipers under a dissecting microscope. Head length was measured to the fleshy end of the opercular flap and head depth was measured at the occiput. Body depth was measured vertically at the origin of the dorsal fin. Dorsal and anal fin lengths were measured from the base of the first spine to the tip of the longest ray. Paired fin measurements were made on the right side of the body if the left side was damaged or appeared abnormal. Pectoral fin length was measured from the structural base to the tip of the longest ray near the middle of the fin, with the fin pressed flat against the body. Pelvic fin length was measured from the base of the spine to the tip of the longest ray. The dorsal and anal fin ray counts include all elements separately, with the last two elements counted independently when they were separated to the body. Fin ray elements were counted under a dissecting microscope with transmitted light. Scale and fin ray counts were made under a dissecting microscope using a jet of compressed air to aid in scale definition. Scales are small and their patterns are often irregular, making it necessary to repeat some counts several times until a consensus count was reached.

The naming of the cephalic lateral line canals follows Branson and Moore (1962) except that their preoperculomandibular canal (POM) is called the preopercular canal (PO) since the mandibular portion of the canal is absent in *Elassoma*. Preopercular pore counts presented in the format 3-3 mean that there are three pores in the PO on the left side of the head and three on the right. Counts presented in the format 4-2+2 mean that there are four pores on the left side and, on the right side, the canal is divided into two independent segments, each containing two pores.

Life color descriptions were made with magnification from live specimens immobilized in ice water, from material preserved in 10% buffered formalin for less than 30 minutes, and from color photographs of live and fresh specimens.

MOLECULAR TECHNIQUES

Total genomic DNA was obtained from caudal fin clippings using the QIAGEN DNeasy® Blood and Tissue Kit following the manufacturers' protocol. Presence of total genomic DNA was confirmed visually by ethidium bromide-stained 1.5% agarose gel electrophoresis. A 553 base pair fragment of 16S rRNA (16S, mtDNA) and the entire 583 base pair S7 Intron 1 (S7, nuclear DNA) were amplified via the polymerase chain reaction (PCR). The 16S mtDNA locus was amplified using the 16Sa and 16Sb primer pair described in Palumbi (1996), while the nuclear S7 locus was amplified with

S7RPEX1F and S7RPEX2R primers described in Chow and Hazama (1998). Reaction conditions consisted of an initial 94 °C disassociation phase for 4 minutes, followed by 40 cycles of 94 °C for 1 minute, 48 °C for 1 minute and 72 °C for 1 minute. A final 7-minute 72 °C extension phase was added to the end of each 40 cycle reaction profile. Presence of amplicons was confirmed visually by ethidium bromide-stained 1.5% agarose gel electrophoresis.

PCR products were precipitated with a 20% polyethylene glycol/2.5 M NaCl mixture and the precipitates washed twice with 70% ethanol (Applied Biosystems 1994). The forward and reverse PCR primers were used as forward and reverse sequencing primers in separate reactions using the ABI BigDye® Terminator version 3.1 Cycle Sequencing Kit. Sequencing reactions were then read on an ABI 377 automated sequencer. Sequence files were exported into Sequencher™ (Gene Codes Corporation, Ann Arbor, Michigan) and contigs made of forward and reverse sequences from each individual. The accuracy of all base calls for all contigs was checked by eye. Contigs were exported from Sequencher™ as text files for further genetic analyses. Sequences were aligned using ClustalW (Thompson et al. 1994) using the default parameters. Minor modifications to the initial alignment were made manually. Both data matrices in nexus format are available from JMQ upon request. Since sequence variation at the S7 locus involved very few polymorphic sites within species, heterozygotes could be inferred unambiguously from chromatograms as overlapping, equally intense bands at single base positions on the trace files; these positions were consistent on both sequenced strands. Nucleotide sequences of unique mtDNA haplotypes and nuclear DNA alleles have been submitted to Genbank (accession numbers GQ477414-GQ477438).

PHYLOGENETIC ANALYSIS

Parsimony and maximum likelihood trees were constructed individually on unique 16S haplotypes and S7 alleles using PAUP* (Swofford 1998). Sequences obtained from *Elassoma zonatum* were used as an outgroups taxon (*sensu* Quattro et al. 2001), and two individuals representing the breadth of sequence variation in *E. evergladei* (TJK unpublished data) were included to assess the sister group relationship between the two "okefenokee" PO pore type groups. Phylogenetic analyses were viewed as independent tests of species-level divergence between the four-pored and three-pored populations of "okefenokee". Given minor variability in pore counts within populations, we considered whether individuals with four PO pores, sampled from populations where four pores predominate, form monophyletic groups in two independent gene trees to the exclusion of those haplotypes or alleles sampled from individuals with three pores taken from populations where three pores predominate. Similarly, we ask whether genetic distinctions between these groups are consistent across their respective geographic ranges, especially where they are found in proximity.

Bayesian analyses were performed on both data matrices separately, but in all cases the Bayesian results were entirely consistent with both the parsimony and likelihood analyses except for the placement of various terminal nodes that have no bearing on relationships between the three- and four-pored forms. For simplicity, only the parsimony and likelihood results are reported here. For likelihood trees, models of DNA evolution were selected using likelihood ratio tests as implemented in MODELTEST (Posada & Crandall 1998, Version 3.7). Bootstrapping (Felsenstein 1985; 1,000 pseudoreplicates) was used to gauge support for nodes of interest, in particular monophyly of the three- and four- pored forms in both gene trees and a sister group relationship between the two types of "okefenokee".

Elassoma gilberti n. sp.
(Fig. 1)

Diagnosis.– *Elassoma gilberti* is distinguished from its close relative *E. okefenokee* by possessing four pores in the preopercular (PO) canal on each side of the head and usually seven anal fin rays. *Elassoma okefenokee* has three PO pores and usually eight anal fin rays. *Elassoma gilberti* has slightly less deep body and slightly smaller dorsal and anal fins than *E. okefenokee*. Breeding females of *E. gilberti* often have blue dashes below and behind the eye, which are lacking in female *E. okefenokee*. Otherwise, the two species are almost identical or broadly overlapping in meristic, morphometric, and color features. The distinction of the two species is supported by molecular data. Eight fixed differences (of 553 bp assayed) at the mitochondrial 16S rRNA locus and 12 fixed differences (of 583 bp assayed) found at the nuclear S7 locus differentiated the two species. Phylogenetic analyses using these molecular characters supported monophyletic clades that contained haplotypes and alleles found uniquely in *E. gilberti* and *E. okefenokee*, respectively.

Type Material. – All type material is located at the Florida Museum of Natural History (UF). Holotype: UF 173591 (FFS 07-51, tag # 1M); adult male 25.2 mm SL; Econfina drainage, Florida, Taylor County, Econfina River at US Hwy. 27&19 bridge 12.0 road miles NW of jct. with US Hwy. 98W in Perry; 30° 15.09' N, 83° 42.06'
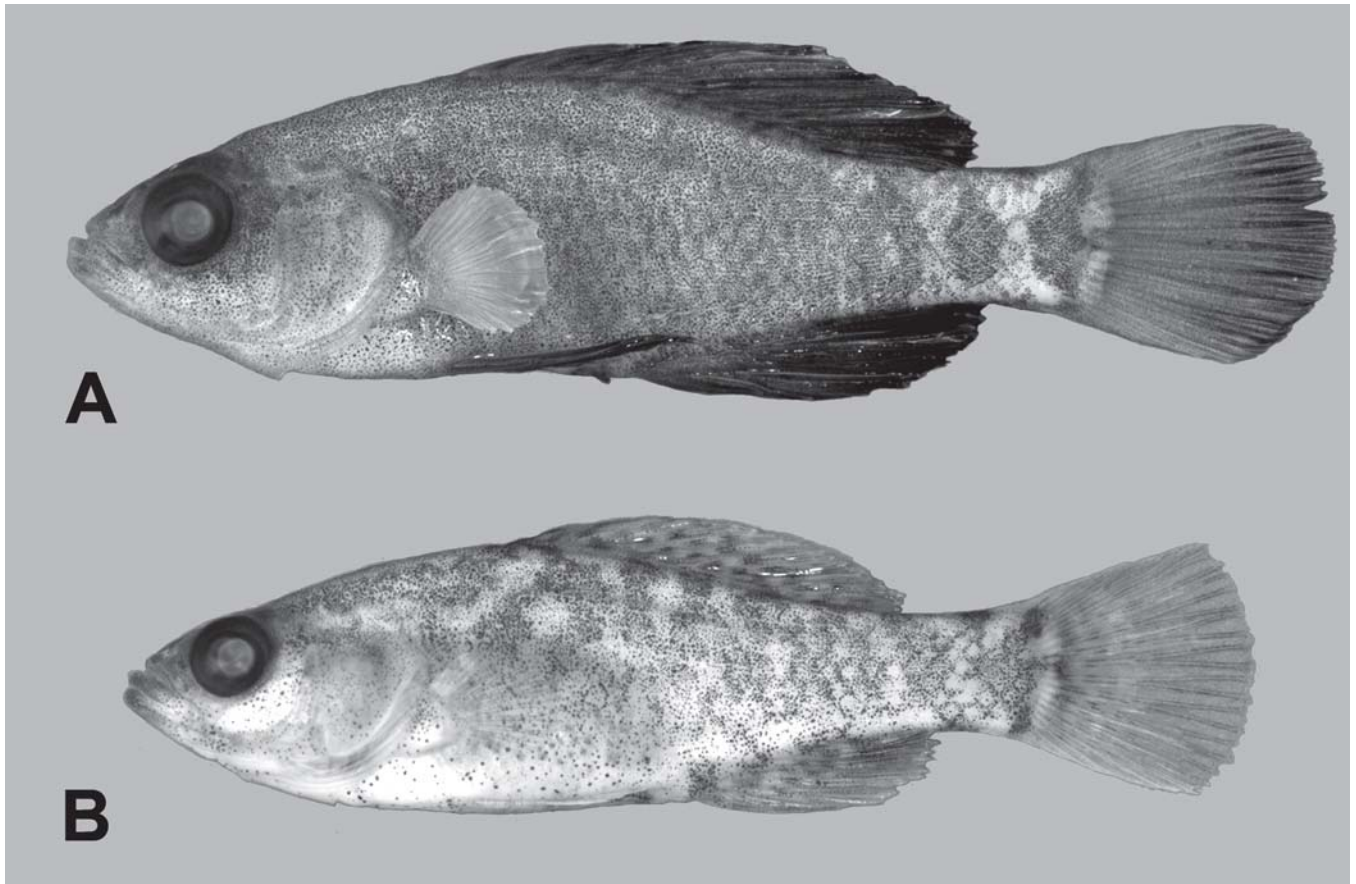
Figure 1. *Elassoma gilberti.* (A) Breeding male 25.2 mm SL. (B) Breeding female 22.7 mm SL. UF 173607. Suwannee River drainage, FL, Dixie County, backwater off Suwannee River at Fanning Springs; 25 April 2006.

W; 8 April 2007; F. F. Snelson, Jr. Allotype: UF 173592 (FFS 07-51, tag # 14F); adult female 23.1 mm SL; collected with the holotype.

Paratypes: UF 173593, 29 specimens collected with the holotype. UF 173595 (FFS 06-34); 17 specimens (3 of which were fixed and stored in 95% ETOH and were used for DNA tissue samples); collected at the type locality; 25 April 2006; F. F. Snelson, Jr. UF 173606 (FFS 05-25); 13 specimens (5 of which were fixed and stored in 95% ETOH and were used for DNA tissue samples); collected at the type locality; 24 March 2005; F. F. Snelson, Jr. UF 173594 (FFS 92-02); 15 specimens collected at the type locality; 8 April 1992; F. F. Snelson, Jr.

Etymology. – The new species in named in honor of Dr. Carter R. Gilbert, Curator of Fishes at the Florida Museum of Natural History from 1961-1998 and now Curator Emeritus. This name will stand in recognition of the many contributions Dr. Gilbert has made to the study of North American fishes and as special thanks from FFS for serving as a guide and mentor for many years. The suggested common name is Gulf Coast Pygmy Sunfish, in view of its distribution only in drainages that empty into the Gulf of Mexico.

Description. – Average adult body size 22.1 mm SL for males, 22.0 mm SL for females. The largest male measured was 25.5 mm SL, the largest female 26.3 mm SL. The general body shape and appearance are shown in Figure 1. Body laterally compressed, with greatest depth at dorsal fin origin. The head is moderately compressed. The anterior profile is narrowly rounded; the mouth is terminal, with the lips projecting slightly beyond the snout tip. All fins are broadly rounded in posterior profile except the pelvics, which are pointed. Proportional measurements are presented in Table 1.

Scale and fin-ray counts are presented in Tables 2 and 3. Lateral scale rows 27-32, usually 28-31. No pored lateral line scales. Transverse scale rows 13-19, usually 14-17. Caudal peduncle scale rows 14-20, usually 16-19. Body fully clad in thin, partially embedded cycloid scales. Top of head anterior to nape naked. Dorsal fin spines 3-5, the first spine often short and partially embedded. Dorsal fin rays 9-13, usually 10-12. Anal fin spines always 3. Anal fin rays 6-9, usually 7 or

Table 1. Proportional measurements of *Elassoma gilberti* and *E. okefenokee*. Standard length (SL) is in millimeters. All other measurements are thousandths of SL with the mean given above and the range below.

| | *Elassoma gilberti* | | | | *Elassoma okefenokee* | |
| | *Holotype* | *Allotype* | *Paratypes* | | | |
	Male UF173591	Female UF173592	Males n=11	Females n=10	Males n=10	Females n=10
Standard Length	25.2	23.1	21.0 19.7-23.2	21.2 18.0-26.3	22.5 20.2-23.8	21.5 19.6-23.6
Body Depth	298	277	293 269-310	294 261-325	318 312-327	314 306-327
Predorsal Length	444	441	441 427-461	442 422-455	428 412-447	429 407-453
Prepelvic Length	353	355	364 347-379	364 345-375	372 346-395	365 352-379
Preanal Length	579	597	577 564-599	605 589-626	565 547-580	594 576-608
Dorsal Fin Length	496	420	477 451-504	436 408-453	506 481-521	456 439-470
Anal Fin Length	329	273	329 304-357	277 266-298	348 335-376	288 276-304
Pectoral Fin Length	151	147	154 129-167	142 134-151	157 146-168	144 137-152
Pelvic Fin Length	266	225	263 234-287	233 217-249	270 254-282	240 225-260
Caudal Peduncle Length	258	251	260 244-276	245 222-277	256 243-282	242 208-262
Caudal Peduncle Depth	135	125	135 123-147	129 118-140	135 125-146	130 124-138
Head Length	333	351	341 326-364	341 327-362	350 325-371	346 332-364
Head Depth	214	190	216 209-226	198 185-214	214 200-229	202 186-217
Snout Length	95	95	87 78-96	84 78-95	85 74-98	83 78-88
Eye Diameter	99	87	98 91-106	100 94-109	99 94-104	101 93-105
Upper Jaw Length	99	95	100 91-110	93 89-100	105 98-112	102 97-111

Table 2. Scale counts of *Elassoma gilberti* and *E. okefenokee*. For *E. gilberti*, the count of the holotype is bolded, the count of the allotype is underlined. For *E. gilberti*, west is Choctawhatchee Bay east through the Wakulla drainage; south is the Econfina south through the lower Suwannee drainage.

	26	27	28	29	30	31	32	33	N	Mean
				Lateral Scale Rows						
E. gilberti										
West		3	7	14	16	12	4		56	29.7
South		3	_13_	23	**14**	5	3		61	29.2
E. okefenokee	1	3	15	11	14	9	3	4	60	29.6

	13	14	15	16	17	18	19	N	Mean
			Transverse Scale Rows						
E. gilberti									
West		10	17	14	13	2	1	57	15.7
South	3	_11_	24	**19**	6			63	15.2
E. okefenokee	2	10	31	19	7	1		70	15.3

	14	15	16	17	18	19	20	N	Mean
			Caudal Peduncle Scales						
E. gilberti									
West		1	13	15	19	5	3	56	17.4
South	1	1	10	_19_	**20**	9	3	63	17.5
E. okefenokee		2	20	20	16	9	3	70	17.3

8. Pectoral fin rays 14-18, usually 15 or 16. Pelvic fin always with 1 spine and 5 rays. Branched caudal fin rays 10-13, usually 11 or 12.

The canals and pores of the cephalic lateralis system are similar to the pattern described for *Elassoma zonatum* by Branson and Moore (1962). The anterior nasal pore of the supraorbital canal (SO) is just anterior and medial to the anterior nare opening. The posterior nasal pore is just medial and posterior to the posterior nare opening, positioned on the rim of or slightly inside the narial depression. Over the center of the eye, the SO canal gives off a short, medially directed canal that terminates in a single pore. This represents the supraorbital commissure of Branson and Moore (1962), but it is not a true commissure since it does not meet the same canal on the opposite side of the head. The SO canal then curves downward to meet the postocular commissure (POC) at a large pore just behind the upper quadrant of the eye. Posteriorly there is a pore at the point where the POC meets the posttemporal (PT) canal. At this point, the short supratemporal canal branches off dorsally and medially with a single pore at its terminus. Further posteriorly, the PT ends in a pore just anterior to the upper corner of the opercular opening. The preopercular (PO) canal (POM of Branson & Moore 1962) does not join the POC. It usually has four pores, one at the upper end of the preopercle, one at or just dorsal to the broad curvature of the preopercle where the opercle and subopercle bones meet, one just below the angle of the preopercle where the curvature begins to straighten, and one at the anterior terminus of the preopercle, almost directly below the middle of the eye (Fig. 2). Geographic variation in PO pore number is shown in Table 4 and is discussed later. The PO canal does not extend onto the mandible. The infraorbital canal is greatly reduced, limited to a short tube in the lachrymal region with two pores, one pore posterior and lateral to the anterior nare, the other just below and anterior to the anterior edge of the eye near the border of the upper lip.

Table 3. Fin ray counts of *Elassoma gilberti* and *E. okefenokee*. For *E. gilberti*, the count of the holotype is bolded, the count of the allotype is underlined. For *E. gilberti*, west is Choctawhatchee Bay east through the Wakulla drainage; south is the Econfina south through the lower Suwannee drainage.

	Dorsal Spines				
	3	4	5	N	Mean
E. gilberti					
West	4	48	5	57	4.0
South	8	<u>**52**</u>	8	68	4.0
E. okefenokee	1	54	15	70	4.2

	Dorsal Rays						
	9	10	11	12	13	N	Mean
E. gilberti							
West	1	4	41	9	1	56	11.1
South		10	<u>**38**</u>	18	1	67	11.1
E. okefenokee		1	32	32	4	69	11.6

	Anal Rays					
	6	7	8	9	N	Mean
E. gilberti						
West	3	36	17	1	57	7.3
South	3	<u>**44**</u>	20		67	7.3
E. okefenokee		22	42	6	70	7.8

	Pectoral Rays						
	14	15	16	17	18	N	Mean
E. gilberti							
West	5	31	17	3		56	15.3
South	<u>13</u>	30	**19**	4	1	67	15.3
E. okefenokee	5	35	22	3	1	66	15.4

	Branched Caudal Rays						
	9	10	11	12	13	N	Mean
E. gilberti							
West		7	22	25	3	54	11.3
South		3	<u>**28**</u>	31	1	63	11.5
E. okefenokee	1	6	29	32		68	11.4

Breeding Colors, Males. – In breeding males, the background color of the body ranges from dark gray to sooty black. The sides of the body are marked with 5-8 narrow iridescent blue bars. The first bar is anterior to the caudal base at about the midpoint of the caudal peduncle; bars extend forward a varying distance to midbody. The blue bars are about half the width of the intervening black spaces. The blue bars are best developed on the caudal peduncle, where they are slightly oblique and often extend nearly from the dorsal to the ventral midline. Anteriorly, the bars are less well developed, confined mostly to the flanks, and usually broken into separate blue spots or dashes. The head is marked by two bright iridescent blue dashes or crescents on the border of the orbit; one lies behind the eye, centered slightly below a horizontal through the middle of the eye; the other lies below the eye, centered slightly in front of a vertical through the center of the eye. There is a narrow gap between the two blue eye dashes. The light band or "racing stripe" (see below) across the snout tip and the lips is usually obliterated by the overall dark pigmentation of the head.

The distal third of the dorsal and anal fins have an iridescent powder-blue wash or band that is bordered by a narrower black band. The background color in the basal half of these fins is sooty black with 1-2 (anal fin) or 2-3 (dorsal fin) irregular rows of bright blue spots, which are best defined in the posterior part of the fins. The caudal fin is dark blue to black basally with bright iridescent blue wash or band distally. It is usually bordered terminally by a narrow black border, but this may be lacking in some specimens. Two unpigmented window-like or vertically elongated spots over the caudal fin base range from beige to pale blue. The spine and the first 2-3 rays and intervening membranes of the pelvic fins are bright iridescent blue; the remainder of the fin is sooty gray or black. The pectoral fins are clear. The blue colors on the body and fins fade rapidly after death.

Breeding Colors, Females. – The body colors of breeding females are much as described below for preserved material, being a combination of tans and browns. The overall intensity of the pigmentation can vary from pallid to dark depending on the color of the water and the nature of the habitat from which they are taken. There is no blue color on the body or fins. The dashes behind and below the eye, described for males, are often present in females, but are less striking than in males and are iridescent blue-green, rather than powder blue in color. Their presence/absence may be a function of readiness to spawn. A concentration of black pigment around the vent is usually conspicuous, and a light band across the snout tip and the dark spotting in the dorsal and anal fins is usually evident on close inspection.

Color in Preservation, Males. – After preservation, life colors fade quickly and reveal the general pattern shown in Figure 1A. Two unpigmented windows, vertically-elongated or semicircular in shape, are present at the caudal base, one above, one below the midline. A darker area separates the two windows at the horizontal midline and they are bordered posteriorly by black pigment. These windows range from very obvious to rather inconspicuous. Anterior to the basicaudal windows, there is usually a dark rectangular or crescent-shaped bar or blotch, bordered anteriorly by a lightly pigmented bar or blotch. The dark blotch may not extend to the dorsal and ventral midline. The light bar typically does extend to the dorsal and ventral midline, encircling the caudal peduncle. Often this light area expands back as far as the upper and lower procurrent
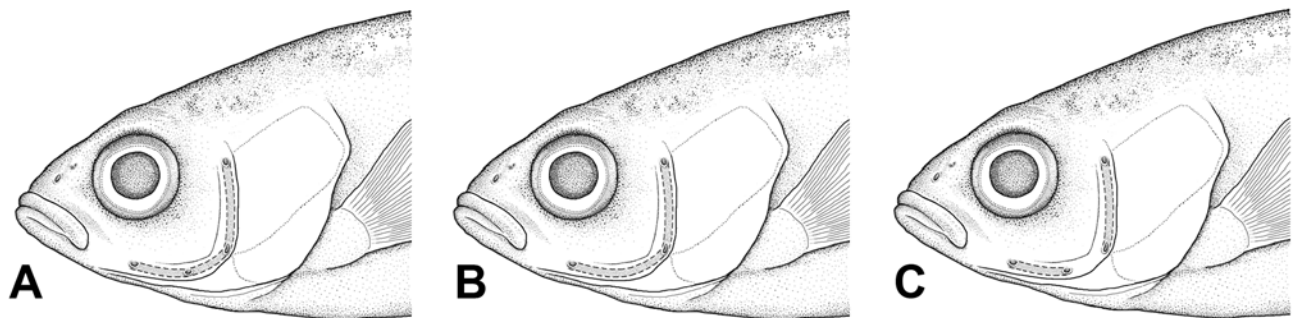

Figure 2. Patterns of preopercular (PO) pores in (A) *Elassoma gilberti* with four pores and (B) *E. okefenokee* with three pores. Pattern C, with 2+2 pores, is found is a small percentage of *E. okefenokee*, primarily in the St. Johns drainage.

Table 4. Preopercular (PO) pore counts by drainage system for *Elassoma gilberti* and *E. okefenokee.* The counts of the holotype and allotype are bolded.

	3-3	3-4	4-3	4-4	2+2-3	3-2+2	2+2-2+2	5-4	4-2	N
E. gilberti										
Choctawhatchee		2	2	140	-	-	-	1		145
Apalachicola	1	-	1	85	-	-	-	1		88
New				14						14
Ochlockonee	1	-	-	78						79
St. Marks		1	1	104						106
Aucilla	1	-	1	87						89
Econfina		6	4	**93**						103
Fenholloway				16						16
Spring Warrior				10						10
Steinhatchee		1	-	46						47
California Creek				3						3
Suwannee	1	4	3	208						216
Waccasassa			1	12	-	-	-	1		14
Homosassa				1						1
Subtotal	4	14	13	897	-	-	-	3		931
E. okefenokee										
Altamaha							1			1
Satilla	30	1								31
St. Marys	66	2								68
St. Johns	307	7	6	1	5	7	2	-	1	336
Suwannee	241	6	4	3	2					256
Withlacoochee	32	-	1	-	-	2	1			36
Hillsborough	9									9
Kissimmee	23	-	-	-	2					25
Subtotal	708	16	11	4	9	9	4	-	1	762
Grand Total										1693

caudal rays, resulting in a distinctly light spot in the area, especially ventrally. Anterior to the caudal peduncle, the alternating pattern of dark and light bars or blotches becomes variable from specimen to specimen. In some, only one additional band pair is evident. In others, banding extends as far forward as mid-body, with up to 5-6 additional band/blotch pairs. Banding becomes progressively more obscure anteriorly, often breaking up into a mottled pattern. Pigment is sparser on the venter, breast, and lower half of the head. There is a dark ring of pigment surrounding the vent, most conspicuous in specimens with overall reduced body pigmentation. The tip of the urogenital papilla is blackened.

The median and pelvic fins range from dusky to black, depending on the reproductive condition of specimens at the time of collection. In the dorsal fin, pigment is uniformly distributed on the membranes and the spines/ rays. In slightly faded specimens the distal portion of the last 2-4 dorsal rays are darker than the remainder of the fin, resulting in a dark blotch in this area. In faded specimens or in individuals with less intensely pigmented dorsal fins, a series of 2-3 rows of black spots is present on the basal third of the fin, the spots centered over the spines/rays. The tips of the dorsal spines are slightly depigmented compared to the reminder of the fin. The pectoral fin rays are outlined with melanophores but the membranes are clear. A few dark spots, as in the dorsal fin, may also be present in the basal half of the anal fin.

The preorbital region, including the area around the nares and the lateral aspects of the upper and lower lips, is more heavily pigmented than the midline of the head, snout, lips, and chin tip. This results in a broad, pale mid-sagittal "racing stripe" across the top of the snout and lips (see Boschung & Mayden 2004:613 for an illustration). This pigment feature may range from conspicuous to faint. It is generally more pronounced in specimens that were not in breeding condition at the time of capture. There may be a faintly defined postorbital stripe to the edge of the operculum. There is no suborbital bar.

Breeding Colors, Females. – Females have overall pigmentation much reduced compared to males (Fig. 1B). The unpigmented basicaudal windows are usually less conspicuous in females than in males owing to the less heavily pigmented caudal fin and paler body pigmentation. The alternating light and dark bars or blotches on the posterior trunk and caudal peduncle are usually more prominent in females than in males. From mid-body forward, the body pigmentation is usually a mottled or marbled pattern. Often the dark bars/blotches on the caudal third of the body are intensified ventrally, resulting in a series of dark spots or sub-rectangular blotches along the base of the anal fin. Less frequently, similar

spots may also be present along the base of the soft dorsal fin. The undersides of the head, the flanks, and the venter are less heavily pigmented than dorsal aspects of the body, but there is always an intensification of black pigment around the vent. The mid-sagittal "racing stripe" pattern across the snout tip is typically more conspicuous in females than in males. As in males, there may be a faintly defined post-orbital stripe but there is no suborbital bar.

The soft dorsal and anal fins are marked with 2-3 rows of black spots centered over the rays. The interradial membranes are lightly stippled with melanophores, sometimes becoming slightly darker distally. The caudal fin rays are outlined with melanophores but pigment is faint and scattered over the membranes; occasionally the basal third of the fin may be faintly spotted. The pelvic fins may be clear or may have a few scattered melanophores laterally in the basal third of the fin. The pectoral fins have the rays narrowly outlined with black, but the membranes are immaculate.

Sexual Dimorphism. – Sexual differences in coloration and pigmentation are described above. There are no sexual differences in meristic characters, but dimorphism in fin size is conspicuous. Males have much longer dorsal, anal, and pelvic fins than females, with little or no overlap in proportional size (Table 1). The differences in fin size are most pronounced during the breeding season (March through early May), but are evident in adults at all times of the year. Females also have proportionately greater preanal length than males (Table 1).

MORPHOLOGICAL COMPARISON OF *ELASSOMA GILBERTI* AND *E. OKEFENOKEE*

The characters distinguishing *Elassoma okefenokee* from all other species of *Elassoma* known at that time are presented by Mayden (1993). Those same features will also serve to distinguish *E. gilberti* from all other species in the genus except for *E. okefenokee*.

The colors of breeding males in these two species show no consistent differences. Variation within a species is evident depending on site-to-site habitat conditions such as water clarity and color. Individual variation within a collection seems to be related to reproductive readiness of individual males. The only color difference noted was in breeding females. Blue-green dashes behind and below the eye (see Description) were usually present in heavily gravid female *E. gilberti*. It appeared that specimens in which the dashes were indistinct or absent were not in peak reproductive condition. These dashes were never observed in breeding females of *E. okefenokee*.

To the naked eye, specimens of *E. okefenokee* appear to be slightly deeper bodied than *E. gilberti*. This appearance is confirmed by measurements showing that both males and females of *E. okefenokee* have greater body depth (Table 1). Further, both males and females of *E. okefenokee* have more expansive dorsal and anal fins (Table 1), which contribute to the appearance of greater body depth. Otherwise, the two species are remarkably similar in their general appearance.

Scale counts of the two species are broadly overlapping and there are no consistent geographic trends within *E. gilberti* (Table 2). Likewise, fin ray counts are very similar and broadly overlapping (Table 3). For dorsal fin rays, 64% (79 of 123) of the *E. gilberti* specimens examined had a count of 11, but *E. okefenokee* was equally likely to have 11 or 12. Anal fin ray counts showed the most differentiation: 65% (80 of 124) of *E. gilberti* counted had seven rays whereas 60% (42 of 70) of *E. okefenokee* had eight rays (Table 3).

The PO pore count is the only reliable morphological character that distinguishes *E. gilberti* from *E. okefenokee*. Because there is only a one pore difference between the two species, absolute identification of single specimens may not be possible on this character alone. However, if several specimens from a site are available, preferably five or more, positive identification of the series is always possible based on the average PO count for the series.

Excluding the Suwannee drainage, where both species occur, only 3 of 715 (0.4%) specimens of *E. gilberti* examined had a PO count of 3-3, which is the characteristic count of *E. okefenokee* (Table 4). In the Apalachicola drainage, 96.6% of the specimens examined had a count of 4-4 (Table 4). However, a single specimen in a lot of one (UF 105552) has a count of 3-3. In the Aucilla drainage material, a single specimen in a lot of one (UF 75283) had count of 3-3. Overall, 97.7% of specimens examined from the Aucilla drainage had a count of 4-4. Among the material from the Ochlockonee drainage, a single specimen in a lot of 34 (UF 50309) has the odd count of 3-3; overall, 98.7% of the specimens examined from the Ochlockonee had the typical count of 4-4.

Likewise, with the exclusion of the Suwannee material, only 1 of 506 specimens of *E. okefenokee* has a PO count of 4-4, characteristic of *E. gilberti* (Table 4). That specimen, from the St. Johns drainage, was in a lot of 13 (UF 40); the other specimens in the lot had PO counts of 4-3 (in 1) and 4-4 (in 11).

With the exclusion of the Suwannee drainage material, 37 of 1221 specimens (3.0%) examined had a bilaterally asymmetrical PO pore count of 4-3 or 3-4

(Table 4). By themselves, such specimens would be equivocal, but in most cases they were from lots with multiple specimens where the predominant count (>90%) was the typical count for the species. In only seven small series of *E. gilberti* did the number of specimens with asymmetrical PO counts exceed 10% of the counts for the series: UF 5851 (1 specimen in a lot of 1), WTLC BA150-97 (1 of 1), GMNH 349 (1 of 1), UF 91808 (1 of 2), UF 95958 (2 of 8), UF 95959 (1 of 5), and UF 173606 (4 of 13). Among the series of *E. okefenokee* examined, specimens with 4-3 or 3-4 PO pores were found to exceed 10% in only eight lots: GMNH 1474 (1 of 4 specimens), UF 56514 (1 of 5), GMNH 1149 (1 of 5), UF 2496 (1 of 5), UF 5852 (1 of 5), UF 5870 (1 of 7), UF 22905 (2 of 5), and UF 173638 (2 of 13). The Econfina drainage material had the highest percentage of 3-4 or 4-3 PO counts among all drainages (9.7%, Table 4).

Several other PO pore count patterns were exhibited by rare specimens of both species (Table 4). The only consistent pattern was a count of 2+2 on one or both sides of the head. In these cases, the PO canal was interrupted at the angle of the preopercle leaving short tubes in the vertical and horizontal limbs of the bone, each with a pore at either end (Fig. 2C). This pattern was never observed in *E. gilberti*, but was found in 20 of 506 (4.0%) specimens of *E. okefenokee* from outside the Suwannee basin, predominately in the St. Johns River system (Table 4). The 2+2 pattern was usually found on only one side of the head. It was bilaterally symmetrical in only 4 of 762 (0.5%) of *E. okefenokee* specimens examined.

Because both species occur in the Suwannee drainage, their PO pore count pattern is examined in more detail (Table 5). In the lower Suwannee basin, a single specimen in a lot of 23 (UF 173607) has a count of 3-3. Specimens with asymmetrical pore counts were found in two lots: UF 173608 (2 of 20 specimens) and UF 90972 (5 of 123). All remaining specimens from the lower Suwannee (194 of 202, 96.0%) had the PO count of 4-4 typical for *E. gilberti* elsewhere in its range. In contrast, the upper Suwannee River drainage in Georgia is occupied exclusively by *E. okefenokee*; 83 of 85 specimens examined have the typical PO pore count of 3-3. Only two specimens had asymmetrical counts (GMNH 1312a, 1 of 11 specimens; and GMNH 2049, 1 of 12).

The Santa Fe system in Florida, a tributary to the Suwannee River, is clearly occupied by *E. okefenokee* as indicated by the predominance of the PO count of 3-3 (Table 5). There are only five asymmetric 3-4 or 4-3 counts among the specimens examined (UF 7645, 3 of

28 specimens; UF 25535, 1 of 28; and UF 101509, 1 of 10). Out of 127 specimens examined from the Santa Fe, only two have a count of 4-4, which is the typical count for *E. gilberti* (UF 7645, 1 of 28 specimens, and UMMZ 210073, 1 of 1).

Only seven specimens from six lots are available from the Ichetucknee Spring and spring run. This small tributary is of interest because it is the most downstream population in the Santa Fe River system before the latter joins the Suwannee River proper. The majority of these specimens (4 of 7) have the count of 3-3, typical of *E. okefenokee* in the remainder of the Santa Fe system. Two specimens, each in singleton lots, have the asymmetric count of 3-4 (UF 123566 and UF 126467). One specimen in a singleton lot (UF 4984) has a count of 4-4, which is the typical count for *E. gilberti*.

Only three samples are available from the middle portion of the Suwannee drainage between the mouth of the Santa Fe and the state border to the north. The most downstream site is Allen Mill Pond Spring (UF 30238; Fig. 7, site 4). This series is identified as *E. gilberti* based on PO counts of 4-4 in nine specimens (Table 5). The next most upstream of the three samples is a small series from White Springs in Hamilton County (UF 4646; Fig. 7, site 5). All five specimens have PO counts of 4-4, consistent with *E. gilberti*. The third and

most upstream of the three middle Suwannee sites is Robinson Branch, a direct tributary to the Suwannee River (UF 173634; Fig. 7, site 6). This sample has PO counts (Table 5) and DNA sequences consistent with *E. okefenokee*. Only two specimens in the lot of 37 from Robinson Branch have an asymmetrical pore count.

We have assumed in all cases that specimens with an odd PO count from a large lot, especially in cases where there is a large sample size from the drainage, are simply atypical individuals of the "expected" species, not the alternate species. In the case of singletons or small lots, it would be impossible to confidently assign odd specimens without matching DNA sequence data. The situation in the Ichetucknee Spring system, with few specimens in small lots, is problematic in this regard. The co-occurrence there of both species could not be ruled out with the available data.

MOLECULAR ANALYSIS

The locations from which specimens were sampled for DNA sequence data are shown in Figure 3 and specific collection data are given in DNA Materials Examined.

RESULTS

16S. – Sixty-two individuals of "okefenokee" were sequenced for 16S. Thirty specimens had 4-4 PO pores

Table 5. Preopercular (PO) pore counts for *Elassoma gilberti* and *E. okefenokee* in the Suwannee River drainage of Florida and Georgia.

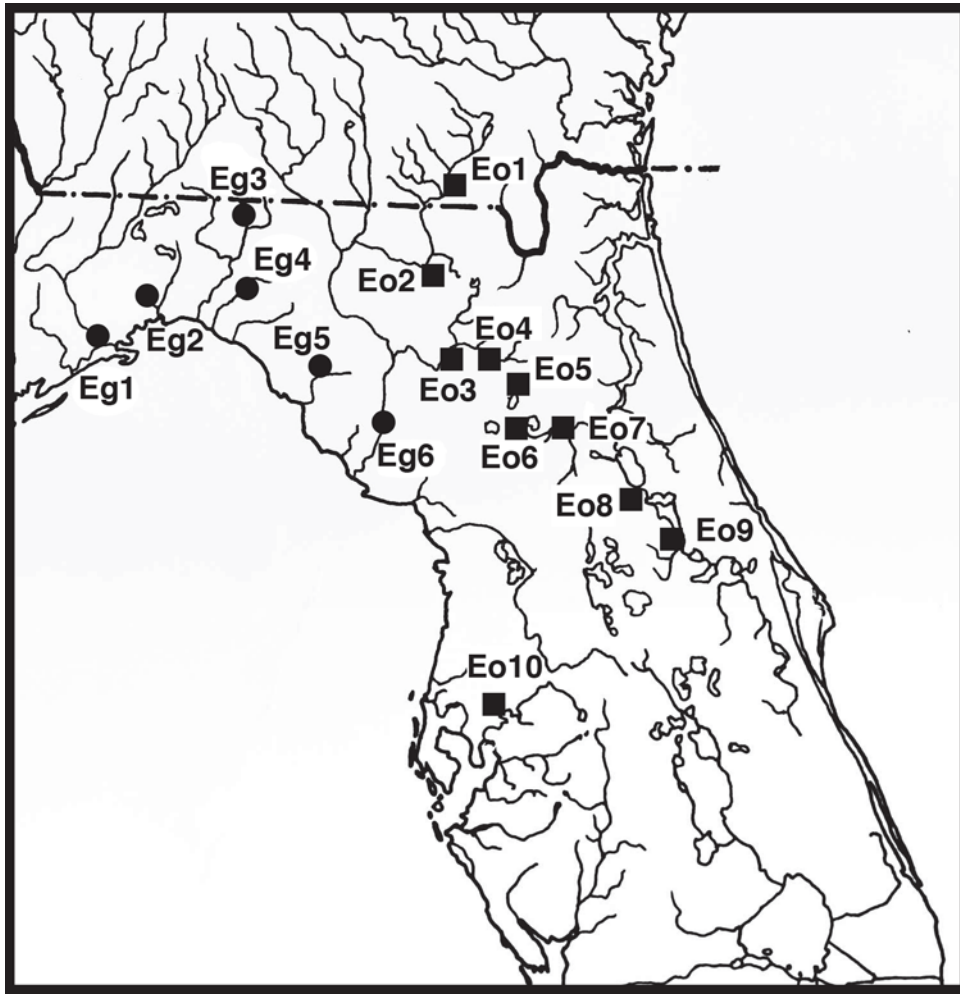|	PO Pore Count					N
	3-3	3-4	4-3	4-4	2+2-3	
Lower Suwannee						
E. gilberti	1	4	3	194		202
Santa Fe						
E. okefenokee	119	3	2	2	1	127
Ichnetucknee						
E. okefenokee	4	2	-	1		7
Middle Suwannee						
E. gilberti				14		14
E. okefenokee	35	-	1	-	1	37
Upper Suwannee						
E. okefenokee	83	1	1			85

Figure 3. The distribution of samples used in the DNA analysis of *Elassoma gilberti* (Eg1 – Eg6) and *E. okefenokee* (Eo1 – Eo10). Specific collection information can be found in the DNA Material Examined section.

and were from four-pore populations (*E. gilberti)* and 32 had 3-3 PO pores and were from three-pore populations (*E. okefenokee)* (Table 6). In addition, we sequenced two *E. evergladei* from divergent locations (western and eastern Florida) and one *E. zonatum* from Florida. Twenty polymorphic sites (3.75% of 553 assayed bases) were found within *E. gilberti* and *E. okefenokee*, and these variable positions defined 14 16S haplotypes (Table 7). Diversity was evenly distributed between these two taxa; six polymorphic sites (five transitions, one transversion) defined seven haplotypes in *E. okefenokee*, while six polymorphic sites (all transitions) defined seven haplotypes in *E. gilberti*. Eight sites (six transitions, two transversions; 1.50% uncorrected sequence divergence) exhibited fixed differences between the two species.

S7. – Forty-three individuals of "okefenokee" were sequenced for the nuclear S7 locus, 20 specimens with 4-4 PO pores from four-pore populations (*E. gilberti)*

and 23 with 3-3 PO pores from three-pore populations (*E. okefenokee)* (Table 6), plus two *E. evergladei* from two divergent locations and one *E. zonatum*. This resulted in 86 sampled alleles excluding the outgroup (Table 8). Of 583 base positions surveyed, 15 polymorphic sites defined five S7 alleles in *E. gilberti* and *E. okefenokee*. One polymorphic site (one transversion) defined two S7 alleles in *E. okefenokee*, while two polymorphic sites (one transition, one transversion) defined three haplotypes in *E. gilberti*. Twelve sites (three transitions, nine transversions; 2.06% uncorrected sequence divergence) exhibited fixed differences between the two species.

Heterozygotes were evident in four populations, one population of *E. okefenokee* (Santa Fe) and three populations of *E. gilberti* (Econfina, St. Marks, Ochlockonee). Small sample sizes preclude reasonably powerful tests of Hardy-Weinberg equilibrium, although the distribution of variation within individual populations did not suggest any systematic bias in the frequency of

Table 6. Number of individuals of *Elassoma gilberti* and *E. okefenokee* sampled for 16S and S7.

Drainage	Abbrev.	16S	S7
St. Johns	StJ	13	8
Hillsborough	Hil	4	2
Santa Fe	SaF	9	7
Suwannee	Suw	9	9
Steinhatchee	Ste	3	1
Econfina	Eco	6	4
Aucilla	Auc	3	1
St. Marks	StM	9	5
Ochlockonee	Och	6	6
Total		62	43

genotypes within any individual population. Importantly, although two divergent alleles were sampled, no heterozygotes composed of these two divergent alleles were detected at the S7 locus in the Suwannee River.

PHYLOGENETIC ANALYSIS

16S. – Parsimony analyses on the 14 haplotypes surveyed from *E. gilberti* and *E. okefenokee*, two haplotypes from *E. evergladei* and the outgroup *E. zonatum* recovered a single tree (length = 66, consistency index (CI) = 0.939, retention index (RI) = 0.956) containing three reciprocally monophyletic clades. One of these clades included haplotypes sampled in *E. evergladei*, while the remaining two clades contained seven haplotypes surveyed from *E. gilberti* and seven from *E. okefenokee* (Fig. 4). A sister group relationship between *E. gilberti* and *E. okefenokee* was supported strongly by bootstrap analysis, as was a sister group relationship between the *E. gilberti* + *E. okefenokee* clade and haplotypes surveyed in *E. evergladei*. Maximum likelihood analyses (best-fit model = K80 from MODELTEST) on these same data recovered a single topology (-Ln likelihood = 1174.646) that was entirely consistent with the shortest tree recovered from the parsimony analysis. Bootstrap sup-

port for these relationships was likewise very strong.

S7. – Parsimony analysis on the eight (including *E. evergladei* and *E. zonatum*) unique S7 alleles recovered a single tree (length = 85, CI = 0.988, RI = 0.976) that contained three reciprocally monophyletic clades comprising two alleles sampled in *E. gilberti*, three alleles sampled in *E. okefenokee*, and two alleles in *E. evergladei* (Fig. 5). As in the 16S trees, *E. evergladei* is recovered as the sister group to the *E. gilberti* + *E. okefenokee* clade. Maximum likelihood analyses (best-fit model = K81 from MODELTEST) on these same data recovered a single topology (-Ln likelihood = 1254.102) that was entirely consistent with the shortest tree recovered from the parsimony analysis. Monophyly of the three clades was strongly supported by bootstrap analysis, as was the sister group relationship between alleles sampled from *E. gilberti* and *E. okefenokee*.

DISTRIBUTION

Elassoma gilberti is found in stream systems draining into the Gulf of Mexico from the panhandle of Florida and extreme southwestern Georgia south through the western portion of the north-central Florida peninsula (Fig. 6). The western-most drainages occupied in the panhandle are the Choctawhatchee and several smaller stream systems that empty into Choctawhatchee Bay. The species is common in the Florida portion of the Choctawhatchee system but is not yet known from Alabama (Boschung and Mayden 2004). In the Apalachicola drainage, the species is found in tributaries to both the Chipola and Apalachicola systems, with a few records from extreme southwestern Georgia. The species is found in the small Whiskey Creek and New drainages to the east of the Apalachicola and is common in the Florida portions of the Ochlockonee drainage further east. Böhlke and Rohde (1980) plotted a record from the Georgia portion of the Ochlockonee drainage, but supporting specimens have not been located. The species was not reported from the Georgia portion of the Ochlockonee drainage by Swift et al. (1977). East and south of the Ochlockonee, the species is found in all major and some minor drainages south through the Suwannee (discussed in more detail below). The next Gulf drainage south of the Suwannee is the Waccasassa; although material is very limited from this drainage, the PO count of 4-4 in 12 of 14 specimens is consistent with *E. gilberti* (Table 4). The next Gulf drainage south of the Waccasassa is the Withlacoochee; PO counts from this drainage clearly identify this population as *E. okefenokee* (Table 4). A single specimen is known from Homosassa Springs Run in Citrus County, south

Table 7. Nucleotide diversity at the 16S mtDNA locus uncovered in *Elassoma okefenokee (Eo)* and *E. gilberti (Eg)*. Twenty variable positions define 14 unique haplotypes. Counts are the number of haplotypes (number of individuals) surveyed per population. Periods within the sequence matrix indicate identity to the first haplotype. Numbers refer to specific nucleotide positions in the alignment. Drainage abbreviations can be found in the Table 6.

Nucleotide Position

```
          1 1 2 2 2 2 2 2   2 2 2 3 3 3 3 3 4 4
          8 9 1 6 0 1 3 3 5 6   6 6 7 4 5 7 7 9 1 9
          8 2 5 7 2 8 4 8 6 2   6 9 1 9 2 7 8 8 5 2
```

Haplotype	Sequence	StJ	Hil	SaF	Suw	Ste	Eco	Auc	StM	Och
*Eo*16S1	G A G G A T C C A G G T T T C C G A T A	3		7	6					
*Eo*16S2	. . . A			1						
*Eo*16S3	. . . . A			1						
*Eo*16S4	. . . . A A A	2								
*Eo*16S5	. . . . A . A	3	4							
*Eo*16S6	. . A . . . . . . A . A	1								
*Eo*16S7	. . . . T . A . A	4								
*Eg*16S1	. . . . G C A . . A . . A . T . A G C G				3	2	3	3	7	
*Eg*16S2	A . . . G C A . . A . . A . T . A G C G					1				
*Eg*16S3	. . . . G C A . . A . . A . T . A G C .									6
*Eg*16S4	. . . . G C A . . A . . A . T T A G C G								1	
*Eg*16S5	. . . . G C A . G A . . A . T . A G C G								1	
*Eg*16S6	. G . . G C A . . A . . A . T . A G C G						2			
*Eg*16S7	. G . . G C A . . A . . A C T . A G C G						1			

Table 8. Nucleotide diversity at the S7 nuclear locus uncovered in *Elassoma okefenokee (Eo)* and *E. gilberti* (*Eg*). Fourteen variable positions define five unique haplotypes. Counts are the number of alleles (twice the number of individuals surveyed when added across columns) surveyed per population. Periods within the sequence matrix indicate identity to the first haplotype. Numbers refer to specific nucleotide positions in the alignment. Drainage abbreviations are given in Table 6.

Nucleotide Position

```
          1 3 3 3 3 3 4   4 4 4 5 5
          4 5 9 6 0 1 4 7 9 3   4 7 7 1 2
          6 4 1 2 0 9 9 4 8 0   2 5 9 1 1
```

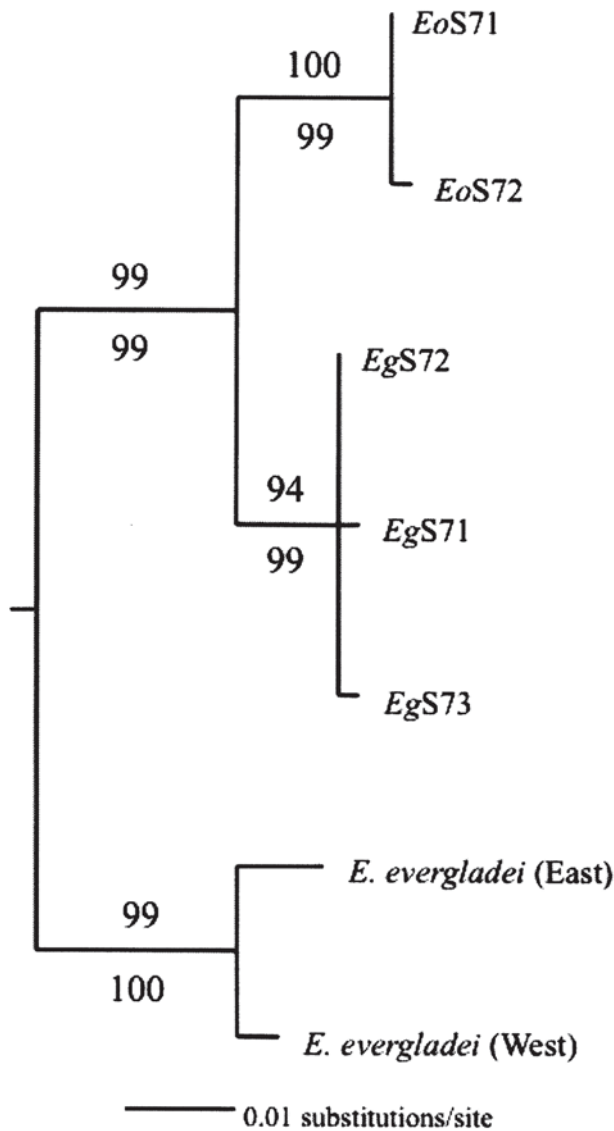| Haplotype	Sequence	StJ	Hil	SaF	Suw	Ste	Eco	Auc	StM	Och
*Eo*S71	C A A C A A G T A T G C A A G	16	4	13	12					
*Eo*S72	A				1					
*Eg*S71	. C G . C T T A C G A A C G T					6	6	2	7	
*Eg*S72	. C G . C T T A C G A . C G T					2	2		3	2
*Eg*S73	. C G T C T T A C G A . C G T									10

Figure 4. Phylogenetic relationships among 16S mtDNA haplotypes observed in *Elassoma gilberti*, *E. okefenokee*, and *E. evergladei*. Shown is the shortest topology recovered under the parsimony criterion. Bootstrap support (parsimony on top, maximum likelihood on bottom) is indicated for individual branches. For clarity, only values greater than 50% are shown. Although rooted with a sequence from *E. zonatum*, this taxon is not shown, but its position is implied from the root as drawn.

Figure 5. Phylogenetic relationships among S7 nuclear alleles observed in *Elassoma gilberti*, *E. okefenokee*, and *E. evergladei*. Shown is the shortest topology recovered under the parsimony criterion. Bootstrap support (parsimony on top, maximum likelihood on bottom, 1000 pseudoreplicates) is indicated for individual branches. For clarity, only values greater than 50% are shown. Although rooted with a sequence from *E. zonatum*, this taxon is not shown, but its position is implied from the root as drawn.

of the Withlacoochee drainage. The PO count is 4-4, consistent with *E. gilberti*. Further south, the last Gulf drainage occupied by either species is the Hillsborough, and it is occupied by *E. okefenokee* (Table 4).

Elassoma okefenokee is distributed from southeastern Georgia south through much of the Florida peninsula (Fig. 6). The occurrence of the species in the Altamaha drainage in Georgia is based on a single specimen (GMNH 1073). The only other record of *E. okefenokee* from the Altamaha drainage, mapped by Böhlke and Rohde (1980), is based on a misidentified series of 19 *E. evergladei* (GMNH 1023). South of the Altamaha basin, the species is found in the Satilla drainage in Georgia and the St. Marys and Nassau drainages in southeastern Georgia and northeastern Florida. The species also occupies the upper portions of the Suwannee drainage in Georgia and its major tributary, the Santa Fe system, in Florida (details below). *Elassoma okefenokee* occupies the interior lake basins in north-central Florida and populations are scattered in tributaries of the St. Johns River as far south as Orlando. There are a few recent records from the Kissimmee River basin in south-central Florida, extending from near Kissimmee to the north shore of Lake Okeechobee. These records suggest that *E. okefenokee* once may have been more widespread in the southern peninsula. As noted above, the species is also found in three drainages, the Suwannee, Withlacoochee, and Hillsborough, that empty into the Gulf of Mexico.

The distribution of the two species in the Suwannee drainage is shown in Figure 7 and the supporting PO count data are given in Table 5. All material from the lower portion of the Suwannee drainage south of the confluence with the Santa Fe (Fig. 7, from site 2 south to site 1) is clearly consistent with *E. gilberti* both in PO counts and in the DNA analysis (Tables 7, 8). The Ichetucknee Spring system (Fig. 7, site 3) is the lowermost tributary to the Santa Fe River before the latter joins the Suwannee River. As noted earlier, only a few specimens are available from this small tributary. The spread of pore count data (Table 5) suggests there may be, or may have been, co-occurrence or hybridization of the two species in this system. However, based on the predominant PO count of 3-3, we assign this population to *E. okefenokee*. The remainder of the Santa Fe system upstream from Ichetucknee Springs is occupied by *E. okefenokee*, as indicated both by PO counts and the DNA analysis. The upper Suwannee River drainage basin above the Florida-Georgia border is occupied exclusively by *E. okefenokee*, a conclusion based on both PO counts and DNA sequence analysis. In the
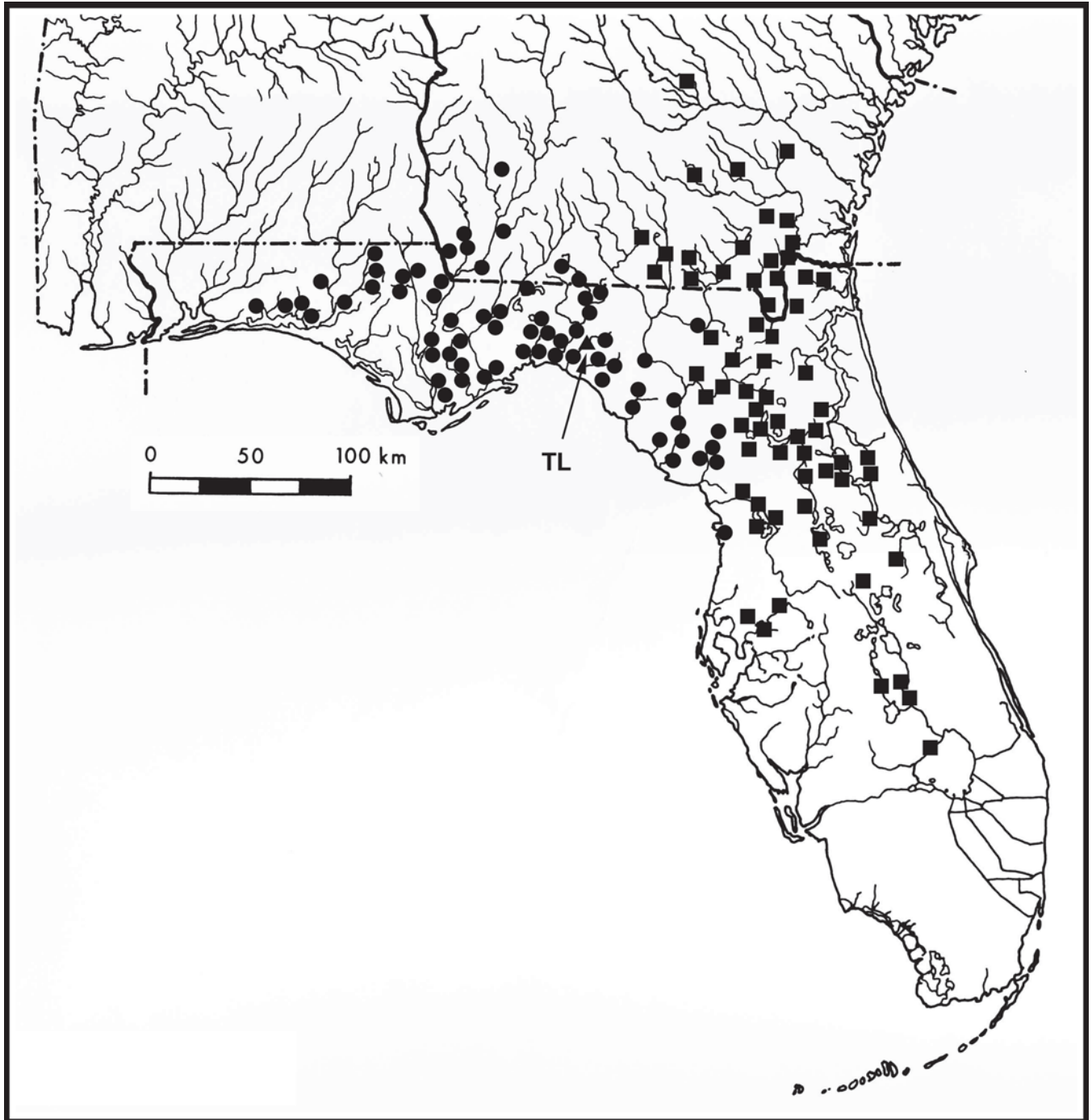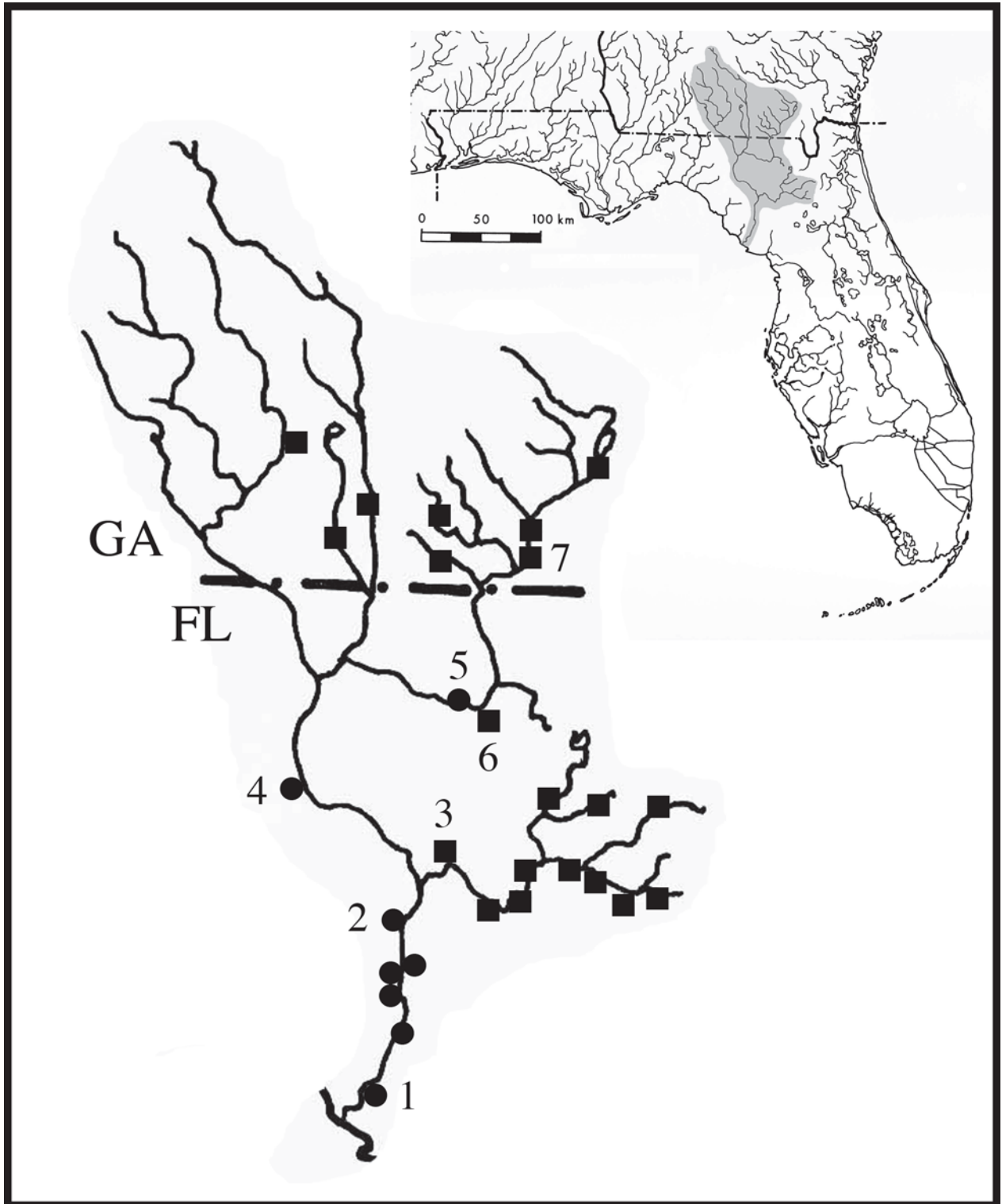
Figure 6. The overall distribution of *Elassoma gilberti* (dark circles) and *E. okefenokee* (dark squares) in Florida and southern Georgia based on material examined. The type locality (TL) for *E. gilberti* is indicated by a black triangle.

middle Suwannee basin, the three lots available clearly separate into *E. gilberti* at the two more downstream locations, Allen Mill Pond (Fig. 7, site 4) and White Springs (Fig. 7, site 5), and into *E. okefenokee* at the more upstream location, Robinson Branch (Fig. 7, site 6). The Robinson Branch and White Springs localities are about six miles apart.

      In summary, the known distribution of the two spe-

cies in the middle Suwannee drainage is as follows. *Elassoma okefenokee* extends down the Suwannee drainage proper to Robinson Branch. *Elassoma gilberti* is then found from White Springs south in the main Suwannee basin, with records scattered sporadically in small tributaries and springs as far south as the mouth of Gopher River (Fig. 7, site 1). The Santa Fe system is occupied by *E. okefenokee* both above and below the

Figure 7. The distribution of *Elassoma gilberti* (dark circles) and *E. okefenokee* (dark squares) in the Suwannee River drainage in Florida and southern Georgia based on material examined. Specific sites referenced in the text are as follows: (1) mouth of Gopher River, (2) Guaranto Springs, (3) Ichetucknee Springs, (4) Allen Mill Pond, (5) White Springs, (6) Robinson Branch, and (7) Suwannee River at Fargo.

Santa Fe Sink and as far downstream as Ichetucknee Springs near the terminus of the Santa Fe. *Elassoma gilberti* occupies springs that are direct tributaries to the Suwannee River both above and below the mouth of the Santa Fe. Thus the ranges of the two species are in close proximity at two places in the middle Suwannee basin, around the Suwannee-Santa Fe confluence and in the Suwannee basin proper between White Springs and Robinson Branch. With the possible exception of Ichetucknee Spring, there is no indication that their ranges overlap or that they occur sympatrically.

Additional material from the middle portions of the Suwannee basin and the lowermost portions of the Santa Fe system, preferably with supporting DNA sequence data, will be needed to refine the distributional pattern of these two species in the middle Suwannee basin. Unfortunately, recent drought, land use changes, and modifications and dewatering of springs has rendered it impossible to duplicate collections at many of the historic sites and difficult to locate new sites where the species might still occur.

The only other sister species that exhibit a similar distribution pattern in Florida are *Fundulus cingulatus* and *F. rubrifrons*. *Fundulus cingulatus* is found primarily in the panhandle of Florida and in extreme southern Alabama and southwestern Georgia, west of the St. Marks drainage. In contrast, *F. rubriforns* is found in southeastern Georgia and northeastern Florida and at widely scattered sites south throughout the Florida peninsula (Gilbert et al. 1992). Both species occur in widely separated areas of the Suwannee River basin. The only area where their populations are in proximity is in the Santa Fe River branch. Like *E. gilberti* and *E. okefenokee*, they have not been taken sympatrically.

Other taxa, although not differentiated to the degree of morphologically diagnosable species, show a similar genetic break across this same geographic area (e.g., Bermingham and Avise 1986). Our inclusion of two disparate samples representing sequence variation within *E. evergladei* likewise suggests substantial divergence across the Suwannee in other pygmy sunfishes. Given these common patterns in diverse taxa, it was expected that genetic divergence across the Suwannee should occur in "okefenokee" as well. However, we are unaware of any substantial morphological differentiation that attends genetic differentiation across this common phylogeographic boundary in species other than *Fundulus cingulatus/rubrifrons* and *Elassoma gilberti/okefenokee*. Indeed, the complete disequilibrium between nuclear DNA, mitochondrial DNA, and morphological characters within samples of *E. okefenokee* and *E. gilberti* from the Suwannee and the absence of het-

erozygous individuals for diagnostic nuclear DNA alleles are compelling support for assigning species-level status to *E. gilberti.*

STATUS OF "*ELASSOMA EVERGLADEI ORLANDICUM*" LÖNNBERG

Einer Lönnberg described this nominal form in 1894 under the text heading *Elassoma evergladei* Jordan. It was based on material collected from several localities in Orange, Osceola, and De Soto counties, Florida. The status of this name was first investigated by Dr. Reeve M. Bailey and Dr. James E. Böhlke (deceased). They examined the known syntypic material and produced a manuscript in 1978 dealing with the name and related nomenclatural issues. That manuscript was never published. The status of the name was commented on by Gilbert (1998) but its assignment was left unresolved because no lectotype was designated. Gilbert later (2004) considered *E. evergladei orlandicum* a *nomen oblitum* under provisions of Article 23.9.1 of the International Code of Zoological Nomenclature (1999), stating that the name had not been used in the primary literature since 1899. He failed to note that Barney and Anson used the name in 1920 (p. 242): "Dr. Einer Lönnberg has published notes on an *Elassoma* found at Orlando, Florida, and named provisionally by him *E. orlandicum.*" We feel that to avoid confusion, the status of this name is best resolved by the selection of a lectotype. With permission (R. M. Bailey, *in litt.* to FFS, June 2007), we paraphrase or quote directly from the draft Bailey and Böhlke manuscript where a lectotype was proposed. Material in brackets embedded within quotes is our addition.

Lönnberg (1894:122-123) wrote: "This little fish seems to be extremely variable. When I obtained my first specimens in Ferncreek [Orlando, Orange County, FL] I surely believed that I had found a new species. I was led to that opinion by the number of spines and soft rays in the vertical fins. Jordan [1884:323] describes *Elassoma evergladei* with four spines and 9 or 10 soft rays in the dorsal and three spines and 5 soft rays in the anal. On my specimens I counted five spines (in one only 4) and 11 or 12 soft rays and the formula of the anal was III, 7. There was thus one spine and 1 or 2 soft rays in the dorsal and 2 soft rays in the anal more than in the typical *E. evergladei*. I therefore believed just to establish a new subspecies with the name 'orlandicum' the more as also the color etc. was different."

Bailey and Böhlke wrote: "Lönnberg's … discussion emphasized the variability of *evergladei*, mentioned sexual differences, and added to the description, including a detailed account of life colors of both sexes. These

were carefully drawn and accurately described *Elassoma okefenokee* Böhlke (1956). The only subsequent use of the name *orlandicum* of which we are aware is by Barney and Anson (1920:242). [They] referred to an *Elassoma* found at Orlando, Florida, named provisionally by Lönnberg as *E. orlandicum*." We add here that Jordan and Evermann (1896) quoted Lönnberg's entire account as a footnote to their species account of *E. evergladei*, with the preface "Dr. Einer Lönnberg gives the following account of the specimens observed by him about Orlando, Florida, and provisionally named "*Elassoma orlandicum*" (*ibid*:984).

Bailey and Böhlke: "In order to investigate the status of *orlandicum*, we have had the privilege, through the courtesy of Dr. Å. Holm, to examine the 19 syntypes from Upsala Universitets Zoologiska Museum. These are labeled "*Elassoma evergladei* var *orlandicum* Lönnb., collected in Orlando, Ferncreek, Orange Co., Florida, Jan. 1893, E. Lönnberg". Examination immediately provided explanation for Lönnberg's impression of variability: the syntypic series is complex, consisting of nine specimens of *Elassoma evergladei* Jordan and ten of *E. okefenokee* Böhlke."

"Lönnberg's fin-ray counts agree better with *okefenokee* than with *evergladei* …, but we are unable to explain his recording of five dorsal spines. Only one of the 19 syntypes (an example of *okefenokee*) has five spines. It seems evident that although Lönnberg had a mixed sample the description was drafted mostly from specimens of *okefenokee*."

At this point, the Bailey and Böhlke manuscript includes two tables, one typed, one handwritten, that give sex, standard length, counts, proportional measurements, and pigmentation traits of the nine *E. evergladei* and ten *E. okefenokee* specimens in the lot. There is no need to duplicate those tables here but we will summarize the major distinctions they noted between the two species that were the basis for their sort. Proportional measurements are given as thousandths of standard length and the range is followed by the mean. Counts are given as range followed by mean. In each case, the first set of values is for the nine *E. evergladei* specimens, the second set is for the ten *E. okefenokee*. Predorsal length: 452-534, 483; 415-450, 430. Caudal peduncle depth: 141-161, 152; 125-144, 132. Longest pectoral ray: 172-214, 182; 130-162, 147. Dorsal fin rays: 10, 10.0; 10-12, 11.0. Anal fin rays: 5-6, 5.6; 6-9, 7.1. Neural spines anterior to first dorsal pterygiophore: 5-6, 5.6; 3-4, 3.9. Top of head: fully scaled versus naked.

Bailey and Böhlke continue: "We elect to preserve current nomenclature for these two species by choosing from the Ferncreek syntypes as lectotype of *Elassoma evergladei orlandicum* Lönnberg, 1894, a male, 19.7 mm in standard length. This specimen agrees with *E. evergladei* Jordan (1884), and the name *orlandicum* should therefore be listed in the synonymy of that species. The lectotype [ZMUU 344a] has counts as follows: dorsal IV, 10; anal III, 6; pectoral 14-14; sum of softray counts of these fins 44 (see Böhlke, 1956:9); body scales 31; vertebrae 28; neural spines anterior to first dorsal pterygiophore 6. The top of the head has a dense investiture of exposed, imbricate scales. The following body proportions are in thousandths of the standard length: predorsal length 487; caudal peduncle depth 157; length of dorsal-fin base 350; length of anal-fin base 188; longest pectoral ray 173; length of pelvic fin 274; diameter of eye 102; snout length 76. The pelvic fins are dusky; the dorsal and anal fins are lightly dusted with melanophores and both fins are notably darkened posteriorly; there are two pale spots at the base of the caudal fin."

"Decision for the selection above was based on the following considerations. (1) *E. e. orlandicum*, [although] the older name, has remained almost unnoticed for nearly 100 years whereas *okefenokee* is established and has received general acceptance. (2) Lönnberg's description was somewhat equivocal: "I surely believed I had found a new species…. I therefore believed just to establish a new subspecies …. The variability of the *E. evergladei* becomes the more evident." [We add, furthermore, that the name "orlandicum" appears only once in Lönnberg's paper. It is embedded in the text under the species account of *E. evergladei* (p. 123), where it is enclosed in quotes and is not italicized, unlike all other scientific names appearing in Lönnberg's paper]. "(3) In view of the above, *orlandicum* might be interpreted as first published as a synonym, therefore unavailable in nomenclature [International Code of Zoological Nomenclature, 1999:article 11.6.] In light of our lectotype selection uncertainty on this question is obviated." Dr. Bailey remains convinced that this lectotype designation is the correct course of action (*in litt.* to FFS, June 2007).

The remaining eight syntypes of *E. evergladei* (ZMUU 344b-i; 16.1-22.1 mm SL) become paralectotypes of *Elassoma evergladei orlandicum* Lönnberg 1894. The other ten syntypic specimens (ZMUU 344j-s; 15.9-20.7 mm SL) are re-identified as *Elassoma okefenokee* Böhlke 1956. FFS has re-examined these 19 specimens and confirms and agrees with Bailey and Böhlke's results, conclusions, and lectotype designation.

Since the Bailey and Böhlke manuscript was writ-

ten, additional original Lönnberg material has been discovered. This material, reported by Gilbert (1998) and also examined by FFS, is as follows: NHRM 14105 (1 specimen, 17.0 mm SL) from Ferncreek, January 1893, re-identified as *E. okefenokee*; NHRM 14106 (5, 17.0-20.0 mm SL) from Ferncreek, January 1893, re-identified as *E. okefenokee*. These two lots appear to represent legitimate syntypes. The lot NHRM 9170 contains three specimens (14.0-17.5 mm SL)) from Bagdad, 27 April, 1893; two are re-identified as *E. okefenokee*, one is dried and unidentifiable. Gilbert (1998) opined that the specimens from Bagdad are questionable syntypes because Bagdad is not mentioned in the original text as a place where Lönnberg collected pygmy sunfish. However, Gilbert (2004) lists NRM 9170 as syntypic material and states that "…all extant types of *E. evergladei orlandicum* are from Fern Creek". We note that the current town of Bagdad is in Santa Rosa County, Florida, in the Blackwater River drainage basin. Neither *E. okefenokee* nor *E. gilberti* are known from that drainage system.

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APPENDIX 1

MATERIAL EXAMINED

Institutional Abbreviations for material examined are as follows: ANSP (Academy of Natural Sciences of Philadelphia), UMMZ (University of Michigan Museum of Zoology), AUM (Auburn University Museum), GMNH (Georgia Museum of Natural History), TU (Tulane University), UF (Florida Museum of Natural History), and WTLC (Walton Taxonomy Laboratory Collection, Georgia Wildlife Resources Conservation Center). We thank the curators and staff of these institutions for specimen loans. The museum number is followed in parentheses by the number of specimens in the lot. Collection numbers followed by an asterisk contain some specimens field fixed and maintained in 95% ETOH and tissue samples are available for molecular study.

Elassoma gilberti
Apalachicola Dr. – **Florida.** Franklin Co.: UF 4594 (15), UF 53382 (3), UF 53386 (6). Jackson Co.: TU 39598 (28), UF 4987 (1), UF 5858 (5), UF 52709 (4), UF 52720 (5), UF 52827 (5), UF 52949 (3), UF 53383 (2), UF 58966 (17), UF 59678 (3), UF 60162 (1), UF 60178 (36), UF 130169 (1). Liberty Co.: UF 117051 (5), UF 120168 (8), UF 144312 (2), UF 144337 (1). **Georgia.** Decatur Co.: WTLC BA05-041 (1), UF 1772 (5). Dougherty Co.: UF 105553 (1). Miller Co.: UF 105442 (1). Mitchell Co.: GMNH 302 (3). Seminole Co.: UF 4863 (81).

Aucilla Dr. – **Florida.** Jefferson Co.: UF 1263 (1), UF 5867 (23), UF 50945 (4), UF 53376 (4), UF 63321 (11), UF 73687 (4), UF 73799 (1), UF 74004 (12), UF 74287 (4), UF 74468 (2), UF 74495 (1), UF 74940 (8), UF 74956 (4), UF 144619 (4), UF 145860 (5), UF 173613* (24). Jefferson-Madison Co.: UF 74037 (15), UF 74539 (39). Madison Co.: UF 53380 (30), UF 63339 (18), UF 73964 (1). **Georgia.** Thomas Co.: UF 66710 (10), UF 75009 (1), UF 75026 (1), UF 75283 (1), UF 75284 (16), UF 75289 (2).

California Creek Dr. – **Florida.** Dixie Co.: UF 63857 (3).

Choctawhatchee Dr. – **Florida.** Holmes Co.: UF 5851 (3). Holmes-Jackson Co.: UF 54162 (1), UF 72411 (2), UF 72548 (8). Jackson Co.: UF 54255 (67). Okaloosa Co.: UF 51936 (7), UF 55605 (2), UF 156374 (13). Walton Co.: TU 111445 (25), TU 124327 (34), UF 50182 (1), UF 50369 (1), UF 130058 (1), UF 144903 (1), UF 145082 (1), UF 145092 (7), UF 145309 (1), UF 145435 (6). Washington Co.: UF 53387 (2), UF 54262 (15), UF 54265 (2), UF 55035 (3).

Econfina Dr. – **Florida.** Taylor Co.: UF 73769 (11), UF 74020 (15), UF 74045 (1), UF 74453 (1), UF 74505 (3), UF 74888 (22), UF 91808 (2), UF 95939 (16), UF 95947 (1), UF 95948 (1), UF 95958 (9), UF 95959 (6), UF 96761 (1), UF 104458 (16).

Fenholloway Dr. – **Florida.** Taylor Co.: UF 74308 (7), UF 74378 (7), UF 74845 (4), TU 36125 (4).

Homosassa Dr. – **Florida.** Citrus Co.: UF 120466 (1).

New Dr. – **Florida.** Liberty Co.: UF 71753 (1), UF 71879 (8), UF 71899 (12), UF 71921 (3).

Ochlockonee Dr. – **Florida.** Gadsden Co.: UF 50093 (10), UF 50309 (34), UF 53388 (4), UF 54266 (10), UF 69793 (2), UF 70149 (11). Leon Co.: UF 53199 (2), UF 53377 (3), UF 53378 (11), UF 53379 (9), UF 61109 (1), UF 71843 (2), UF 75258 (2). Liberty Co.: UF 5859 (14), UF 50168 (1), UF 50232 (31), UF 50248 (2), UF 52264 (16), UF 53375 (1), UF 53383 (1), UF 54261 (14), UF 69794 (2), UF 70076 (8). Wakulla Co.: UF 69776 (5), UF 69926 (7), UF 69997 (21), UF 71779 (7), UF 73291 (1).

Spring Warrior Dr. – **Florida.** Taylor Co.: UF 38823 (25), UF 74817 (1).

St. Marks-Wakulla Dr. – **Florida.** Jefferson Co.: UF 5856 (11), UF 53044 (9), UF 77051 (3). Leon Co.: GMNH

77074 (1), UF 79529 (1), UF 101462 (10), UF 130745 (2), UF 131077 (3), UF 173610* (19).

Steinhatchee Dr. – **Florida.** Dixie Co.: UF 116466 (24). Dixie-Taylor Co.: UF 58463 (11), UF 74198 (21), UF 173612 (2). Lafayette Co.: UF 38124 (2), UF 74324 (20), UF 74703 (1), UF 74809 (1), UF 75058 (4), UF 75085 (4), UF 173611 (8), UF 173614 (4), UF 173609 (77). Taylor Co.: UF 58465 (23), UF 74216 (1).

Suwannee Dr. – **Florida.** Dixie Co.: UF 2490 (9), UF 92238 (11), UF 173607 (26), UF 173608 (36). Gilchrist Co.: UF 58210 (4), UF 58246 (26), UF 173615* (3). Hamilton Co.: UF 4646 (5). Lafayette Co: UF 30238 (9). Levy Co.: UF 90972 (128), UF 110792 (1), UF 120345 (3), UF 120352 (6), UF 120359 (3).

Waccasassa Dr. – **Florida.** Levy Co.: ANSP 151940 (8), GMNH 372 (1), UF 5860 (3), UF 63175 (1), UF 63382 (1).

Elassoma okefenokee
Altamaha Dr. – **Georgia.** Wheeler Co.: GMNH 1073 (1).

Hillsborough Dr. – **Florida.** Hillsborough Co.: UF 173640 (5), UF 173643 (3). Pasco Co.: TU 135521 (1).

Kissimmee Dr. – **Florida.** Glades Co.: UF 118701 (1). Highlands Co.: UF 96451 (1), UF 104843 (1). Okeechobee Co.: UF 2487 (4), UF 96452 (1). Orange Co.: UF 173619 (30), UF 173623 (7), UF 173626 (2), UF 173630 (1).

Satilla Dr. – **Georgia.** Bacon Co.: GMNH 791 (10), GMNH 1474 (4). Brantley Co.: UF 23731 (3), UF 23740 (1). Charlton Co.: AUM 11402 (2). Coffee Co.: GMNH 1573 (1). Wayne Co.: GMNH 1022 (16).

St. Johns Dr. – **Florida.** Alachua Co.: UF 40 (13), UF 2496 (5), UF 2498 (8), UF 2500 (4), UF 5852 (11), UF 5854 (14), UF 5855 (14), UF 5857 (3), UF 5862 (1), UF 5870 (8), UF 9670 (2), UF 17259 (22), UF 25528 (23), UF 25529 (4), UF 32854 (6), UF 43736 (10), UF 45077 (14), UF 81232 (4), UF 90700 (32), UF 97363 (27), UF 146398 (2), UF 146919 (10), UF 173632* (20), UF 173625 (23), UF 173637* (7), UF 173644 (9), UF 173624 (20). Clay Co.: UF 22825 (12), UF 96101 (5). Flagler Co.: AUM 33914 (3). Lake Co.: UF 7652 (11), UF 35235 (2), UF 43315 (2), UF 47209 (5), UF 79514 (1), UF 96179 (3), UF 173633* (17). Lake-Seminole Co.: UF 21519 (2), UF 81257 (2), UF 173620 (7), UF 173617 (19), UF173618 (17), UF 173639 (4), UF 173631 (31), UF 173628 (25), UF 173629 (6), UF 173638* (20). Marion Co.: UF 4428 (1), UF 8753 (1), UF 22905 (6), UF 23175 (5), UF 26267 (1), UF 26355 (1), UF 101463 (1), UF 121949 (4), UF 121950 (4). Marion-Putnam Co.: UF 22927 (3), UF 125369 (1), UF 173636 (17). Orange Co.: UF 173622 (29). Putnam Co.: UF 19 (1), UF 1907 (5), UF 23155 (8), UF 35966 (18), UF 41953 (3), UF 42208 (5), UF 43296 (7), UF 45509 (7), UF 47443 (2). Seminole Co.: UF 173627 (4). Volusia Co.: UF 1308 (4), UF 5871 (79).

St. Marys-Nassau Dr. – **Florida.** Baker Co.: UF 26320 (2), UF 34151 (8), UF 56485 (1). Nassau Co.: UF 56476 (1), UF 56514 (5), UF 145734 (3). **Georgia**. Charlton Co.: AUM 11238 (18), GMNH 1150 (9), GMNH 1149 (9), TU 213320 (94), UF 173635* (23). Ware Co.: GMNH 1476a (17).

Suwannee Dr. – **Florida.** Alachua Co.: UF 9622 (33), UF 25535 (28), UF 34021 (2), UF 38427 (1). Alachua-Bradford Co.: UF 173642 (8). Alachua-Columbia Co.: UF 173641 (3). Bradford Co.: UF 34037 (2), UF 34061 (21). Columbia Co.: UF 25526 (1), UF 101461 (1), UF 101509 (10), UF 173634* (37). Columbia-Suwannee Co.: UF 43728 (1), UF 123566 (1), UF 126467 (1), UF 173621 (2). Columbia-Union Co.: UMMZ 210073 (1). Gilchrist Co.: UF 5865 (1), UF 7645 (28). Suwannee Co.: UF 4984 (1). Union Co.: UF 25536 (1), UF 34140 (10). **Georgia.** Berrien Co.: AUM 10334 (24). Clinch Co.: AUM 4986 (9), GMNH 1312a (22), UF 173616* (50). Echols Co.: GMNH 2049 (24). Lanier Co.: UF 4001 (1). Lowndes Co.: GMNH 1063 (26), UF 50576 (8). Ware Co.: GMNH 1439 (30).

Withlacoochee Dr. – **Florida.** Citrus Co.: UMMZ 176248 (1), UMMZ 176261 (1), UF 7650 (27), TU 12565 (1). Marion Co.: UF 85364 (9). Sumter Co.: GMNH 843 (3).

APPENDIX 2

DNA MATERIAL EXAMINED

Each sample entry for *Elassoma gilberti* and *E. okefenokee* begins with a map code (Eg1, etc.) that corresponds to those in Figure 3.

Elassoma gilberti
Eg1: UF 173656, Ochlockonee drainage, FL. Eg2: UF 173610, UF 173657, St. Marks drainage, FL. Eg3: UF 173613, Aucilla drainage, FL. Eg4: UF 173595, UF 173606, Econfina drainage, FL. Eg5: UF173611, Steinhatchee drainage, FL. Eg6: UF 173615, Suwannee drainage, FL.

Elassoma okefenokee
Eo1: UF 173616, Suwannee drainage, GA. Eo2: UF 173634, Suwannee drainage, FL. Eo3: UF 173641, Suwannee (Santa Fe) drainage, FL. Eo4: UF 173642, Suwannee (Santa Fe) drainage, FL. Eo5: UF 173632, St. Johns drainage, FL. Eo6: UF 173637, St. Johns drainage, FL. Eo7: UF 173636, St. Johns drainage, FL. Eo8: UF 173633, St. Johns drainage, FL. Eo9: UF 173638, St. Johns drainage, FL. Eo10: UF 173643, Hillsborough drainage, FL.

Elassoma evergladei
UF 173757, Apalachicola drainage, FL. (west). UF 173756, Ochlockonee drainage, FL. (west). UF 173759, St. Johns drainage, FL. (east).

Elassoma zonatum
UF 173758, Waccasassa drainage, FL.