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Reappraisal of the *Crangonyx floridanus* species complex, with the description of a new species of *Crangonyx* Bate, 1859 (Amphipoda: Crangonyctidae) from northern Florida, USA

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ABSTRACT

Crangonyx apalachee **n. sp.** Cannizzaro & Sawicki in Cannizzaro, Sisco & Sawicki, is described from Lake Jackson in Leon County, Florida, USA, a prairie lake that periodically drains through karst sinkholes during times of low rainfall. The new species occurs in tall grass along the lake's shoreline. Molecular analyses of the nuclear 18S rDNA and 28S rDNA along with the mitochondrial 16S rDNA, combined with morphological analyses were used to delineate the population from closely related crangonyctids. The occurrence of *C. apalachee* **n. sp.** in Lake Jackson marks the fifth species to be described from the *floridanus* complex in Florida. Collections from other locations across north Florida suggest the presence of additional *floridanus*-complex species still to be described.

KEY WORDS: *Crangonyx floridanus*, Crustacea, *floridanus* complex, species delimitation, 18S rDNA, 28S rDNA, 16S rDNA

INTRODUCTION

Crangonyx Bate, 1859 is a Holarctic genus of Amphipoda comprising 53 species occurring in both epigean and hypogean habitats (Zhang & Holsinger, 2003; Cannizzaro & Sawicki, 2020). In the Nearctic, 47 species have been described, a majority of which (65%) occur in epigean environments. *Crangonyx floridanus* Bousfield, 1963 is one of the most widespread species in the genus. Originally described from specimens collected from a cypress swamp in Highlands Hammock State Park, Highlands County, Florida and from Gerard's Cave in Jackson County, Florida, *C. floridanus* was subsequently recorded throughout North America, ranging from southern Florida north to Massachusetts and west to Missouri (Zhang & Holsinger, 2003; Cannizzaro *et al.*, 2019a). The Gerard's Cave specimens, selected as paratypes by Bousfield (1963), were described as differing from the Highlands Hammock specimens by having only 6—12 eye facets and "in minor points of setation." Recent collections of specimens qualitatively identified as *C. floridanus* from water-filled caves in Jackson County, Florida resulted in the description of *C. manubrium* Cannizzaro & Sawicki in Cannizzaro *et al.*, 2019a). This species description of *C. manubrium* was based on

genetic and morphometric analyses demonstrating the specimens from Jackson County cave were significantly different from epigean populations of *C. floridanus* (Cannizzaro *et al.*, 2019a). In addition, these data suggested that the metapopulation of *C. floridanus* in Florida may be a species complex (Cannizzaro *et al.*, 2019a).

Collections in the Big Bend region of Florida provided support for the *floridanus*-complex hypothesis with the description of two new species *Crangonyx ephemerus* Cannizzaro & Sawicki, 2020 and *C. pseudoephemerus* Cannizzaro & Sawicki, 2020. These two species exist syntopically in ephemeral pools at the St. Marks Headwaters Greenway and were initially qualitatively identified as *C. floridanus*. Genetic and morphometric analyses, however, demonstrated significant divergence between *C. manubrium* and *C. floridanus*. (Cannizzaro & Sawicki, 2020).

Additional support for the *floridanus* complex is provided herein with the description *Crangonyx apalachee* Cannizzaro & Sawicki **n. sp.** in Cannizzaro, Sisco & Sawicki from Lake Jackson, a prairie lake in northern Florida (Fig. 1). Its description brings the total number of species in *Crangonyx* to 54. The new species is supported by molecular genetic analyses of three genes
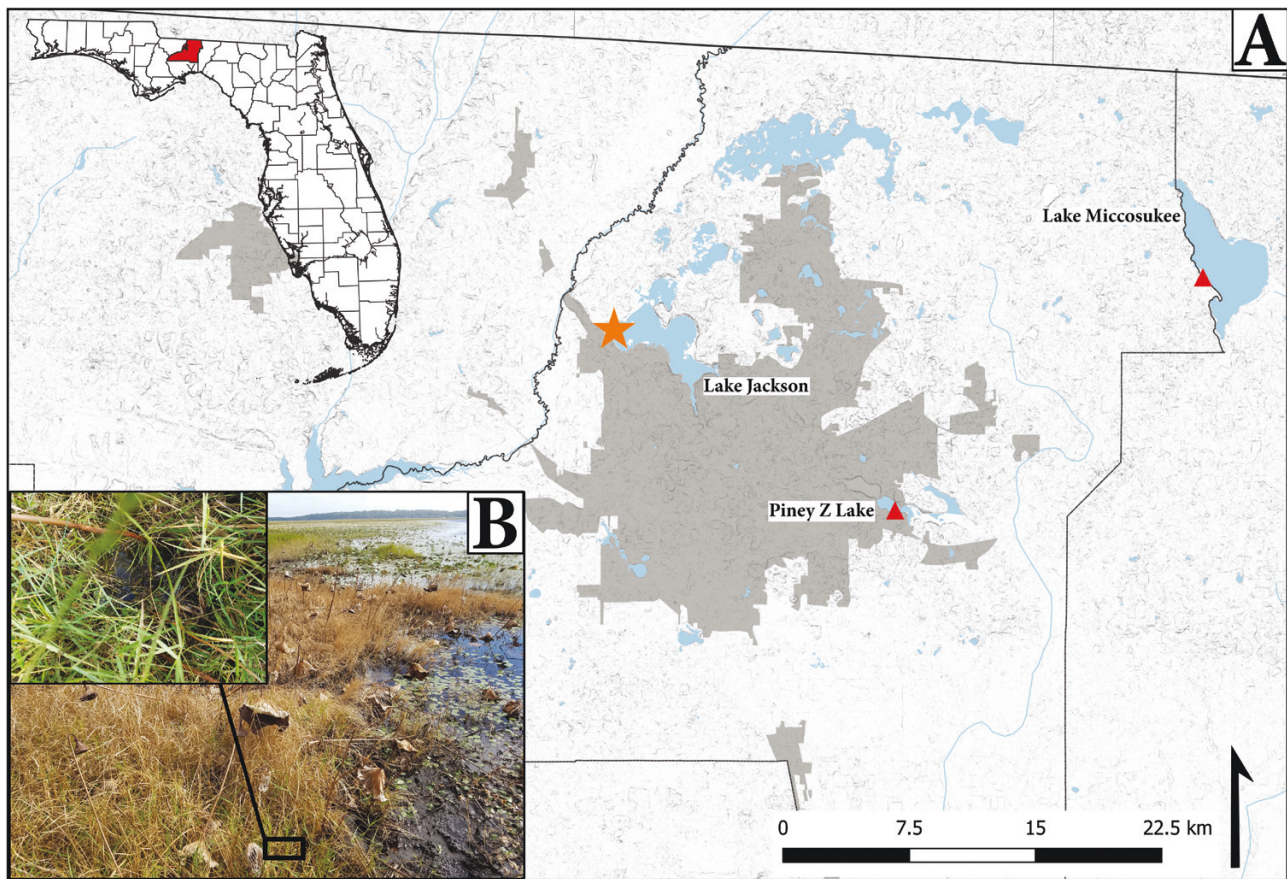
Figure 1. Distribution of *Crangonyx apalachee* **n. sp.** and undescribed *Crangonyx* spp. in Leon/Jefferson Counties, Florida. Star, Sunset Landing, Lake Jackson; triangles, localities where undescribed *Crangonyx* spp. were collected (**A**). Type locality in October 2017, inset showing specific habitat where individuals of *C. apalachee* **n. sp.** were collected (**B**).

commonly used in amphipod molecular phylogenetics, the nuclear 18S rDNA (Englisch & Koenemann, 2001; Macdonald *et al.*, 2005; Hou *et al.*, 2007; Kornobis *et al.*, 2011; White, 2011; Cannizzaro *et al.*, 2019a; Cannizzaro & Sawicki, 2020) and 28S rDNA (Hou *et al.*, 2007; Fišer *et al.*, 2009; Flot *et al.*, 2010; Kornobis *et al.*, 2011; Hou *et al.*, 2014; Cannizzaro *et al.*, 2019a; Cannizzaro & Sawicki, 2020); and the mitochondrial 16S rDNA (Macdonald *et al.*, 2005; Hou *et al.*, 2007; Seidel *et al.*, 2009; Kornobis *et al.*, 2011; Cannizzaro *et al.*, 2019a; Cannizzaro & Sawicki, 2020).

MATERIALS AND METHODS

Collection of specimens

Specimens were live-captured using a dip net, preserved in 100% molecular-grade ethanol and stored at –80 ℃.

Morphological analyses

Morphological analyses of 22 individuals of *C. apalachee* **n. sp.** (15 males, 7 females) (Supplementary material Table S1) were undertaken. Eighteen characteristics were examined and compared to four closely related *Crangonyx* species (Table 1). Specimens were dissected using a Leica M125 stereomicroscope (Leica Biosystems, Wetzlar, Germany) and the appendages mounted in glycerin on microscope slides. Appendages

were then examined and illustrated using a Leica DM 1000 compound microscope fitted with a drawing tube. Plates were prepared using Adobe Illustrator CC 2015ʽ. Body length measurements were taken by measuring the distance from the rostrum to the base of the telson, following the contour of the body using ImageJ software (Abràmoff *et al.*, 2004). Nomenclature for setal patterns on the third article of the mandibular palps follow Karaman (1969). "Defining angle" refers to the posterior margin of the palm and the distalmost point of the posterior margin of the propodus, the area where the tip of the dactyl closes on the propodus; "pereopod 7 gill" to the gill attached between the coxa and basis of pereopod 7 as described by Steele & Steele (1991); "stout setae" on the pereopod dactyli to setae that are intermediate in robustness relative to robust setae (traditionally referred to as "spines") and thinner, more flexible setae (Cannizzaro *et al.*, 2019a).

Material examined is deposited in the Florida Museum of Natural History, University of Florida (UFID), Gainesville, FL, USA.

PCR preparation

Genomic DNA was extracted by removing 3–7 thoracic appendages (generally gnathopods 1, 2 and pereopods 3–7) from one side of the specimen, leaving the other side intact for morphological examination. Extractions were performed using Zymo

Table 1. Morphological differences observed between members of the *Crangonyx floridanus* complex

Character	C. apalachee n. sp.	C. floridanus	C. ephemerus	C. manubrium	C. pseudoephemerus
Maxilliped inner plate armed spine-teeth	1	2–4	1	2	1
♀Gnathopod 1 palmar robust setae	~15	~5	~12–14	~6	~12–14
♀ Gnathopod 2 palmar robust setae	~16	~10	~12–17	~8	~12
♀ Pereopods 3 and 4 dactyl inner margin setation	2	1	1	1	1
♀ Pereopod 5, 6, 7 basis posterior margin serrations	~7, 8, 9	~6, 7, 6	~10, 10, 9	~5, 6, 9	~6, 6, 9
Second epimeron ventral margin	Lacking paired robust setae	With paired robust setae	Lacking paired robust setae	Lacking paired robust setae	Lacking paired robust setae
♂ Uropod 1 outer ramus	With proximal ventral robust seta	With proximal ventral robust seta	Lacking proximal ventral robust setae	With proximal ventral robust seta	With proximal ventral robust seta
♂ Uropod 1 peduncle to outer ramus	~2.07	~1.54	~1.40	~1.45	~1.37
♀ Uropod 1 peduncle robust setae	~12	~9	~10	~12	~10
♀ Uropod 3 inner ramus	With robust seta	With robust seta	With 2 robust setae	With robust seta	With 2 robust setae
♂ Uropod 3 inner ramus	Unarmed	Unarmed	With robust seta	Unarmed	With robust seta
♀ Uropod 3 outer ramus marginal robust setae	~14–21	~17	~17	~12	~11
♂ Uropod 3 outer ramus marginal robust setae	~10	~8	~12	~9	~10
♀ Uropod 3 peduncle length to width	~0.64	~0.55	~0.58	~0.50	~0.59
♂ Uropod 3 peduncle length to width	~0.58	~0.86	~0.70	~0.59	~0.58
♀ Telson length to width ratio	~1.24	~0.98	~0.90	~1.05	~1.0
♂ Telson length to width ratio	~1.35	~0.95	~0.66–1.05	~1.30	~0.66–0.87
♀ Telson cleft depth to length ratio	~0.40	~0.4	~0.33	~0.40	~0.47
♂ Telson cleft depth to length ratio	~0.40	~0.43	~0.39	~0.53	~0.47

Research Quick-DNA™ Tissue/Insect MiniPrep kits (Zymo Research, Irvine, CA, USA) per the manufacturer's protocol. Extracted genomic DNA (gDNA) was stored at –20 °C. Using polymerase chain reaction (PCR) (Saiki *et al.*, 1988), three primer pairs (18SF–18S1000R; 18S700F–18S1250R; 18S1000F–18SR) (Englisch & Koenemann, 2001) were used to amplify 2,078 base pairs of 18S rDNA. Primer pairs 28SF and 28S1000R (Hou *et al.*, 2007) were used to amplify 735 base pairs of 28S rDNA and primer pairs 16STf (Macdonald *et al.*, 2005) and 16Sbr (Palumbi *et al.*, 1991) were used to amplify 278 base pairs of 16S rDNA. Total PCR volumes of 25 µl contained 30–70 ng of extracted gDNA. PCR master mixes contained 12.5 µl APEX™ Hot start 2X Master Mix DNA Polymerase (Genesee Scientific, El Cajon, CA, USA), 1 µl of each 10 µM primer and 8 µl of molecular grade water. PCR was performed on a MultiGene™ OptiMax (Labnet International, Edison, NJ, USA) thermal cycler. A negative control, lacking only genomic DNA, was included for all sets of PCR reactions performed to rule out contamination.

Thermal cycler protocols

Thermal cycler protocols for each gene/gene segment amplified were as follows: 18S rDNA:18SF to 18S1000R: 95 °C initial heating for 15 min, followed by 36 cycles of 94 °C for 45 sec, 68.5 °C for 1 min, and 72 °C for 45 sec, ending with a 10-min extension at 72 °C; 18S700F to 18S1250R: 95 °C initial heating for 15 min, followed by 34 cycles of 95 °C for 45 sec, 72 °C for 1 min 30 sec, ending with a 10-min extension at 72 °C; 18S1000F to 18SR: 95 °C initial heating for 15 min, followed by 35 cycles of 95 °C for 45 sec, 52.5 °C for 30 sec, 72 °C for 1 min 15 sec, ending with a 10-min extension at 72 °C; 28S rDNA: 95 °C initial heating for 15 min, followed by 38 cycles of 94 °C for 30 sec, 59 °C for 45 sec and 72 °C for 1 min 30 sec, ending with a 10-min extension at 72 °C; 16S rDNA: 95 °C initial heating for 15 min, followed by 36 cycles of 94 °C for 1 min, 54 °C for 1 min and 72 °C for 45 sec, ending with a 10-min extension at 72 °C.

Sequencing

PCR products were prepared for sequencing using Zymoclean™ Gel DNA Recovery Kit (Genesee Scientific). DNA sequencing of PCR products was carried out at Eurofins Genomics (Louisville, KY, USA) using industry standard Sanger sequencing methodology.

Phylogenetic analysis

Pairwise sequence alignment was conducted using MAFFT v7.388 (Katoh & Standley, 2013) in Geneious© software v.10.2.3 (Drummond *et al.*, 2017) and further checked by eye. Uncorrected *p*-distances were calculated from sequence alignments using Geneious© software v.10.2.3 (Drummond *et al.*, 2017).

Phylogenetic relationships were reconstructed using a concatenated dataset of three genes (18S rDNA, 28S rDNA, and the mitochondrial 16S rDNA). The best-fit evolutionary substitution models were determined using partitionfinder 2.1.1 using the greedy algorithm (Guindon *et al.*, 2010; Lanfear *et al.*, 2012, 2017). The 18S rDNA and 16S rDNA both used a general time reversible model with a gamma distribution and invariant

sites (GTR+I+G) and the 28S rDNA used a general time reversible model with a gamma distribution (GTR+G). Two Markov chain Monte Carlo (MCMC) simulations were run with each chain consisting of three heated and one cold chain over 100,000,000 generations and sampled every 1,000 generations using Bayesian inference in MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003). In all Bayesian analyses, node support was estimated using posterior probability, with burn-in lengths of 25%.

Sequences of *Crangonyx hobbsi* Shoemaker, 1941; *Crangonyx pseudogracilis* Bousfield, 1958; *Crangonyx grandimanus* Bousfield, 1963; *Crangonyx islandicus* Svavarsson & Kristjánsson, 2006; *Crangonyx manubrium*; *Crangonyx parhobbsi* Cannizzaro & Sawicki in Cannizzaro *et al.*, 2020; *Crangonyx ephemerus*; *Crangonyx pseudoephemerus*; *Crymostigius thingvallensis* Kristjánsson & Svavarsson, 2004; *Stygobromus mackini* Hubricht, 1943; *S. gracilipes* Holsinger, 1978; *S. stegerorum* Holsinger, 1978; *S. floridanus* Holsinger & Sawicki, 2016; *S. doughertyensis* Cannizzaro & Sawicki in Cannizzaro *et al.*, 2019b) were obtained from GenBank and included to root the phylogenies. All sequences generated as a part of this study were submitted to GenBank (Supplementary material Table S1).

Divergence time estimates

Divergence times were estimated using a lognormal relaxed clock in BEAST 2.5.1 (Bouckaert *et al.*, 2014), with the same concatenated sequences as used in the MrBayes analysis. In the concatenated dataset, all models except for the tree prior, were unlinked for each gene. The molecular clock was calibrated using the divergence times estimates by Copilaş-Ciocianu *et al.* (2019, 2020) based on fossil Amphipoda from Baltic amber. The crown age of Crangonyctoidea was modeled under an exponential distribution and was set to a mean of 60 mya, with an offset of 35 mya (HPD = 38–215). A Birth-Death Model tree prior was used, and the best fit evolutionary-substitution models for each gene were independently determined using bMODELTEST (Bouckaert & Drummond, 2017). Two independent Markov chain Monte Carlo (MCMC) simulations were run for 100,000,000 generations and sampled every 5,000 generations. Results from both MCMC chains were combined using LogCombiner 2.4.7 (available with BEAST). Convergence and effective sample size (ESS) were tested using Tracer 1.6 (Rambaut *et al.*, 2014). The first 25% of resulting trees were discarded as burn-in based on parameter estimates determined in Tracer, and the following tree was summarized in TreeAnnotator 1.8.1 (available with BEAST).

Species delimitation

Species delimitation was performed using three methodologies: Generalized Mixed Yule Coalescent (GMYC), Bayesian Poisson Tree Processes (bPTP), and Assemble Species by Automatic Partitioning (ASAP). For GMYC species delimitation the resulting tree from the BEAST analysis was used for species delimitation using the single-threshold GMYC method implemented in the R packages *ape, paran, splits* and *rncl* (Paradis *et al.*, 2004; Dinno, 2012; Ezard *et al.*, 2013; Michonneau, *et al.*, 2016). The algorithm created by Zhang *et al.* (2012) was used with default parameters for bPTP; the algorithm created by Puillandre *et al.* (2021) with default parameters for ASAP.

SYSTEMATICS

Order Amphipoda Latreille, 1816
Suborder Senticaudata Lowry & Myers, 2013
Infraorder Gammarida Latreille, 1802
Parvorder Crangonyctidira Bousfield, 1973
Superfamily Crangonyctoidea Bousfield, 1973
Family Crangonyctidae Bousfield, 1973; emended by
Holsinger, 1977
Genus *Crangonyx* Bate, 1859
***Crangonyx apalachee* Cannizzaro & Sawicki n. sp. in
Cannizzaro, Sisco & Sawicki**
(Figs. 2–9)

Type material

Holotype, female 5.1 mm: Lake Jackson, Leon County, Florida; coll. A.G. Cannizzaro, 6 October 2017; UFID 60028. Allotype, male 4.0 mm: Lake Jackson, Leon County, Florida; coll. A.G. Cannizzaro, 6 October 2017; UFID 60029. Eight paratype males (3.70 mm–5.21 mm) Lake Jackson, Leon County, Florida; coll. A.G. Cannizzaro, 6 October 2017; UFID 60030–60037. Four paratype females (4.81 mm–6.04 mm): Lake Jackson, Leon County, Florida; coll. A.G. Cannizzaro, 6 October 2017; UFID 60038–60041. Additional specimens examined: two females (3.61 mm, Sawicki collection number TRS 160.1; 4.56 mm, TRS 160.8), Lake Jackson, Leon County, Florida; coll. A.G. Cannizzaro, 6 October 2017 (TRS 160.1 completely digested for DNA analysis); six males (3.28 mm–3.94 mm, Sawicki collection numbers TRS 160.2, 160.3, 160,4, 160.11, 160.13, 160.14), Lake Jackson, Leon County, Florida; coll. A.G. Cannizzaro, 6 October 2017 (TRS 160.2–160.4 completely digested for DNA analysis); (Table S1).

Type locality

Sunset Landing, Lake Jackson, Leon County, Florida (30.5336, –84.3554).

Etymology

The specific epithet *apalachee* is in honor of the Apalachee Native Americans who lived in northwest Florida and whose ceremonial mounds are located near Lake Jackson, Florida.

Diagnosis

Small to medium-size epigean species distinguished from other members of the genus *Crangonyx* except *C. floridanus*, *C. manubrium*, *C. ephemerus*, and *C. pseudoephemerus* by pereopods 5–7 possessing deep serrations on posterodistal margins of bases (particularly pereopod 7); strongly produced posterodistal corners of epimera 1–3; comb spines on outer ramus of male uropod 2; lacking ventral spines on inner margin of uropod 2. See Remarks for differential diagnosis between *C. apalachee* and the *floridanus*-complex species *C. floridanus*, *C. manubrium*, *C. ephemerus*, and *C. pseudoephemerus*.

Description

Female (Figs. 2–7): 5.1 mm in length. Eyes full, pigmented, ovate to weakly reniform. Interantennal lobe narrow, with rounded upper, lower margins. Clear/yellowish in live specimens (Fig. 2C).

Antennae. Antenna 1 (Fig. 3A): ~74% body length, 2.1× longer than antenna 2; primary flagellum with 23 articles, aesthetascs on distal articles, aesthetascs shorter than respective articles; accessory flagellum with two articles, subequal in length to first article. Antenna 2 (Fig. 3B): gland cone distinct; peduncle ~2.0× longer than flagellum, with weak, plumose setule on posterodistal margins of articles 4, 5, peduncle article 4 subequal in length to article 5; calceoli absent on both peduncle and flagellum; flagellum with 8 articles.

Mouthparts (Figs. 3–4). Mandibles: left mandible (Fig. 3C) incisor 4-dentate, lacinia mobilis 4-dentate, with 5 robust serrate and numerous plumose accessory setae; molar process with simple seta (damaged in holotype); palp with 3 articles, second article subequal in length to third article, with inner margin

Figure 2. *Crangonyx apalachee* **n. sp.** holotype female (UFID 60028), 5.1 mm, Lake Jackson, Leon County, Florida (**A**); allotype male (UFID 60029), 3.85 mm, Lake Jackson, Leon County, Florida (**B**); female (TRS 210.1), 8.6 mm, showing live coloration, Lake Jackson, Leon County, Florida (**C**). Scale bars = 1 mm
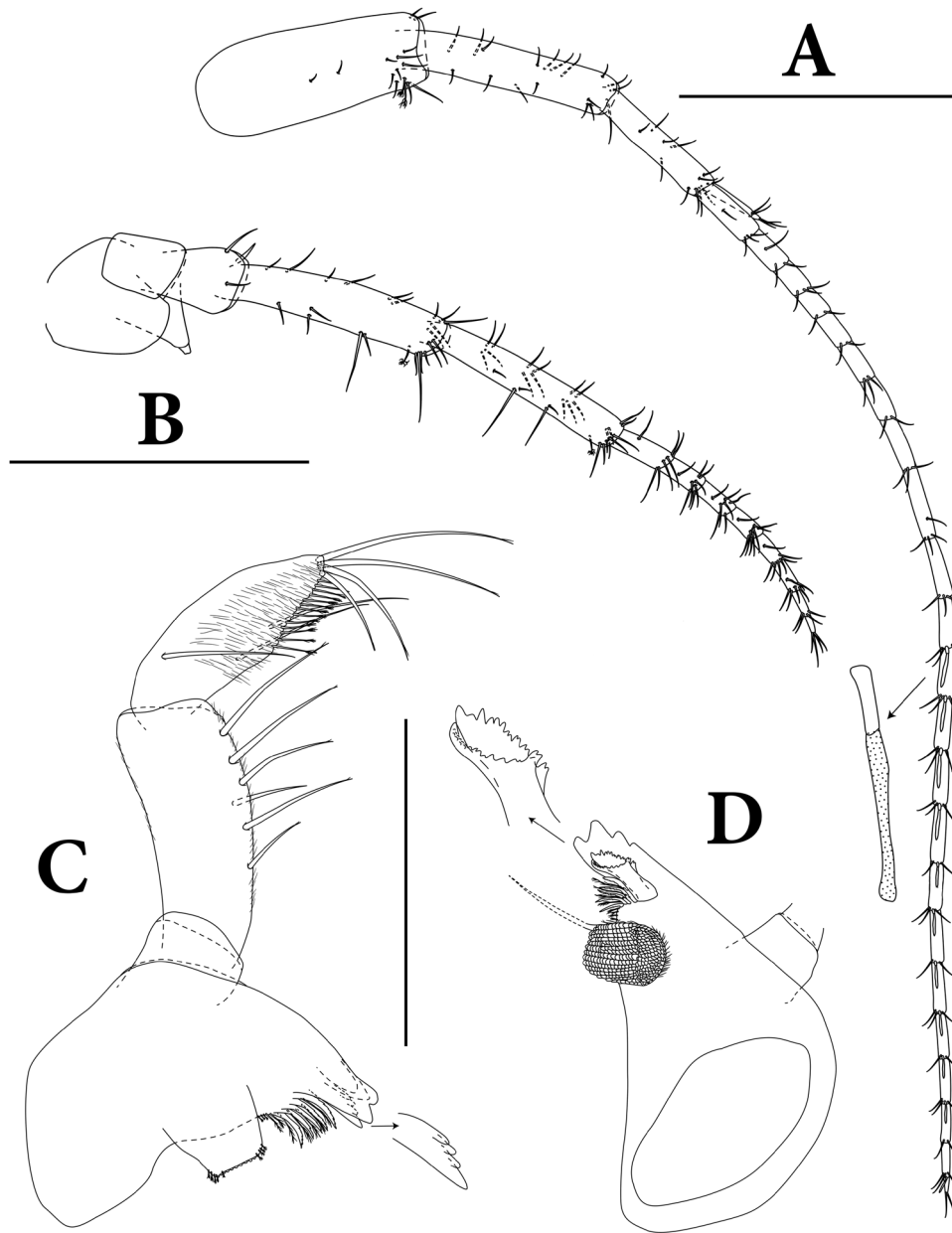
Figure 3. *Crangonyx apalachee* **n. sp.** holotype female, Lake Jackson, Leon County, Florida (UFID 60028), 5.1 mm: antenna 1 (single aesthetasc enlarged) (**A**); antenna 2 (**B**); left mandible, (**C**); right mandible (palp omitted, lacinia mobilis enlarged) (**D**). Scale bars = 1 mm.

bearing 6 setae and numerous fine setae (not illustrated); article 3 rounded distally with 2 C-setae, 4 E-setae, 1 B-seta, and 13 plumose D-setae, lacking A-setae; face of article covered in numerous, fine pubescent setae. Right mandible (Fig. 3D) incisor 4-dentate, lacinia mobilis bifurcate, both lobes with numerous protuberances; accessory setae row with 5 robust, serrate setae and numerous plumose setae; molar process with single simple seta (damaged in holotype). Palp with 3 articles, relative articles lengths and setation patterns as in left mandible. Upper Lip (Fig. 4A): rounded, apical margin of labrum with numerous fine setae. Lower Lip (Fig. 4B): inner lobes highly reduced; outer margin of outer lobe sparsely covered in fine setae; inner margin of outer lobe heavily setose. Maxilla 1 (Fig. 4C): inner plate with 6 plumose marginal setae and numerous fine, pubescent setae covering entire plate; outer plate with 7 apical comb spines, pubescence covering inner margin, decreasing laterally and proximally; palp with 2 articles, distal article covered in pubescence; subapical margin of distal article with 3 long setae, apical margin with 6 robust setae. Maxilla 2 (Fig. 4D): both inner and outer plates covered in pubescent setae; outer plate not as wide as inner plate, not narrowing distally, with numerous distal setae; inner plate narrowing slightly distally, with numerous apical setae and 6 large plumose facial setae. Maxilliped (Fig. 4E): inner plate shorter than outer plate, with 3 naked and plumose cuspidate seta(e) and 2 plumose and naked seta(e) along apical margin, surface of plate covered in fine pubescence; outer plate armed with 4 robust serrate setae; palp second articles with numerous marginal setae, third article with numerous marginal/submarginal setae; dactyl with outer seta and 3 inner setae.
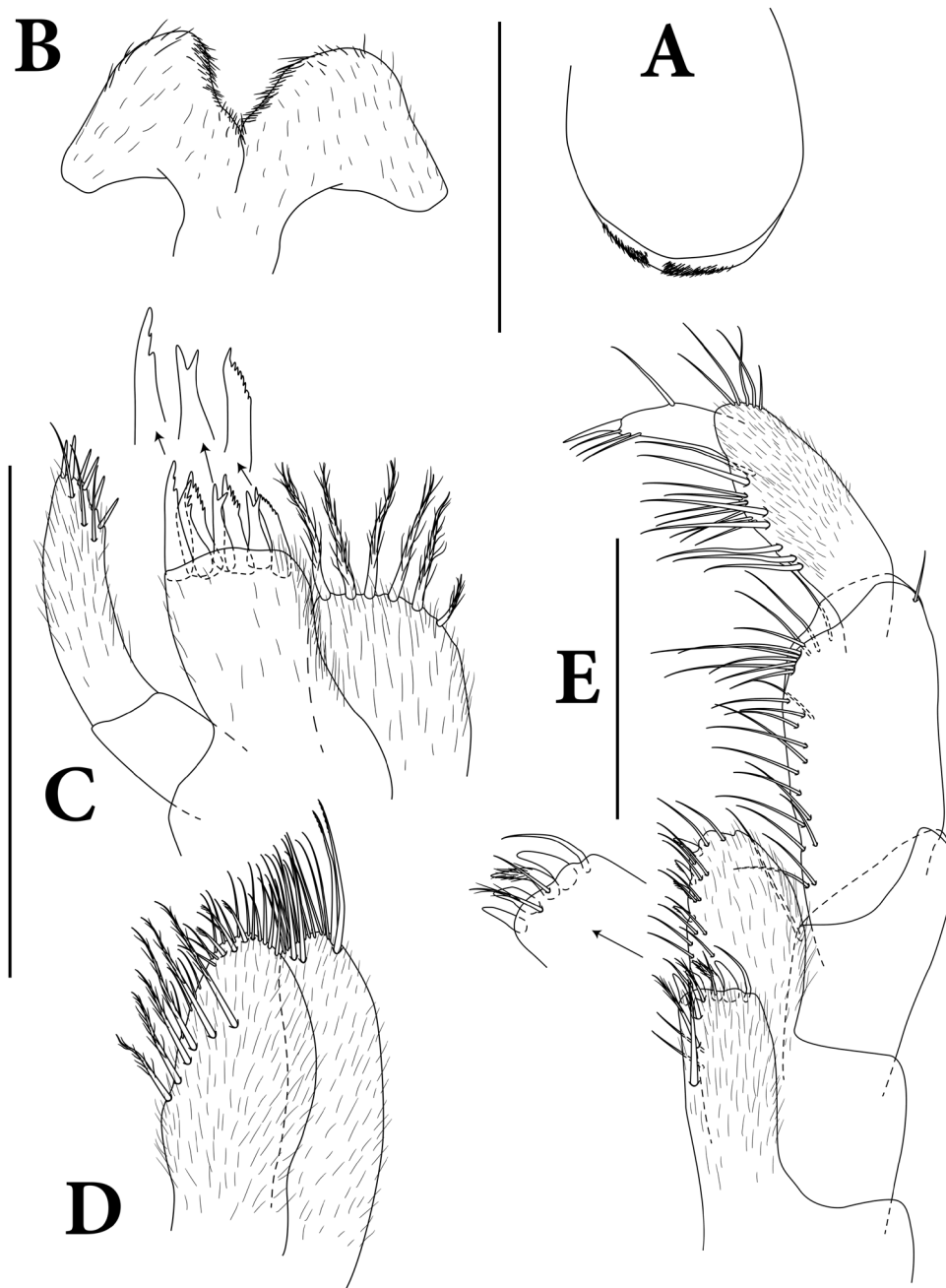
Figure 4. *Crangonyx apalachee* **n. sp.** holotype female, Lake Jackson, Leon County, Florida (UFID 60028), 5.1 mm: upper lip (**A**); lower lip (**B**); maxilla 1 (outer plate spine-teeth enlarged) (**C**); maxilla 2 (**D**); maxilliped (inner plate apical margin enlarged) (**E**). Scale bars = 0.5 mm.

Gnathopods. Gnathopod 1 (Fig. 5A): coxal plate with 6 apical setae; basis with numerous long setae inserted along anterior, posterior, medial margins, shorter setae distally from anterior margin, small patch of pubescence on posterodistal corner; ischium with 6 long setae and pubescence along posterior margin; merus with pubescence covering posterior surface and numerous distal setae; carpus ~80% length of propodus with 2 setae along anterior margin, 3 setae inserted at anterodistal corner, posterior margin with numerous plumose setae and 5 submarginal setae directed distally; propodus 1.5× longer than broad, with 3 marginal anterior setae, 4 superior medial setae, 8 setae inserted at anterodistal corner, 3 inferior medial setae and 8 plumose posterior setae; palm transverse with 8 outer and 7

inner robust setae and 7 long outer setae; inner margin of defining angle with 4 bifid robust setae and 4 outer robust setae; dactylus with outer seta and 4 inner setae. Gnathopod 2 (Fig. 5B): coxal plate with 7 apical setae; basis with numerous long setae inserted along anterior, posterior, medial margins, 4 shorter setae along anterior margin, and small patch of pubescence on posterodistal corner; ischium with 2 setae and pubescence along posterior margin; merus with pubescence covering posterior surface and 4 posterodistal setae; carpus ~75% length of propodus, with 5 anterior setae, group of medial setae and 6 groups of setae along posterior margin; propodus 2.1× longer than broad with 3 marginal anterior setae, 7 superior medial setae, 7 setae at anterodistal margin, 4 inferior medial setae and 4 groups of
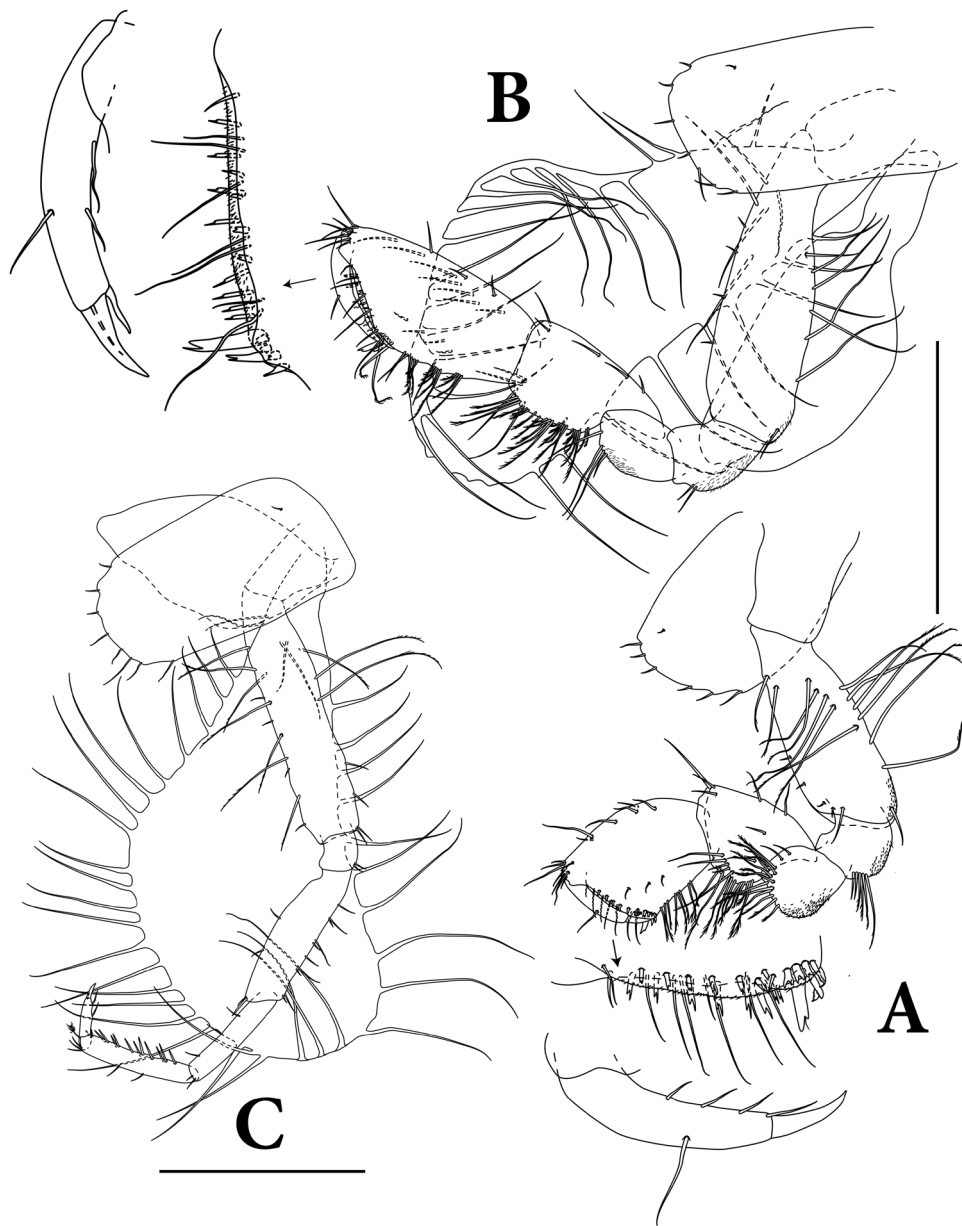
Figure 5. *Crangonyx apalachee* **n. sp.** holotype female, Lake Jackson, Leon County, Florida (UFID 60028), 5.1 mm: gnathopod 1 (palmar margin and dactyl enlarged) (**A**); gnathopod 2 (palmar margin and dactyl enlarged) (**B**); pereopod 3 (**C**). Scale bars = 1 mm.

posterior plumose setae; palm oblique with 8 setae and 4 inner and 8 outer robust setae; defining angle armed with 3 inner, 3 outer robust setae; dactylus with outer seta, 4 inner setae.

Pereopods. Pereopod 3 (Fig. 5C): coxal plate with 8 apical setae; merus 1.4× longer than carpus, carpus and propodus subequal in length; dactylus ～50% length of propodus, with plumose seta on posterior margin, 2 setae along anterior margin followed by thin seta on medial margin. Pereopod 4 (Fig. 6A): subequal to pereopod 3 in length; coxal plate longer than broad, with distinct excavation along posteroproximal margin, armed with 15 or 16 apical setae; merus ～1.7× longer than carpus, propodus ～1.15× longer than carpus; dactylus ～43% length of propodus, with plumose seta on anterior margin, 2 setae on posterodistal margin followed by thin seta on medial margin. Pereopod 5 (Fig. 6B): coxal plate large, bilobate with distinct anterior and posterior lobes, posterior lobe with seta on distal

corner; basis posterior margin weakly convex with 8 shallow serrations, anterior margin with 8 or 9 split-tipped robust setae, 3 distal setae and 1 or 2 anteroproximal setae; merus ～81% length of carpus; carpus ～1.1× longer than propodus, dactylus ～41% length of propodus, with plumose seta on posterior margin, stout seta on distal corner of anterior margin, followed by thin seta on distolateral margin. Pereopod 6 (Fig. 6C): coxal plate bilobate, with produced posterior lobe, posterior lobe bearing apical seta; basis posterior margin weakly convex with 11 serrations and rounded distal corner, anterior margin with 5 split-tipped robust setae, 5 distal robust setae, lacking proximal setae; merus ～85% length of carpus; carpus subequal to propodus in length, dactylus ～36% length of propodus, setation similar to pereopod 5. Pereopod 7 (Fig. 6D): coxal plate small, subtriangular, with 2 posterior setae; basis posterior margin convex with 13 serrations increasing in depth distally and straight distal corner,
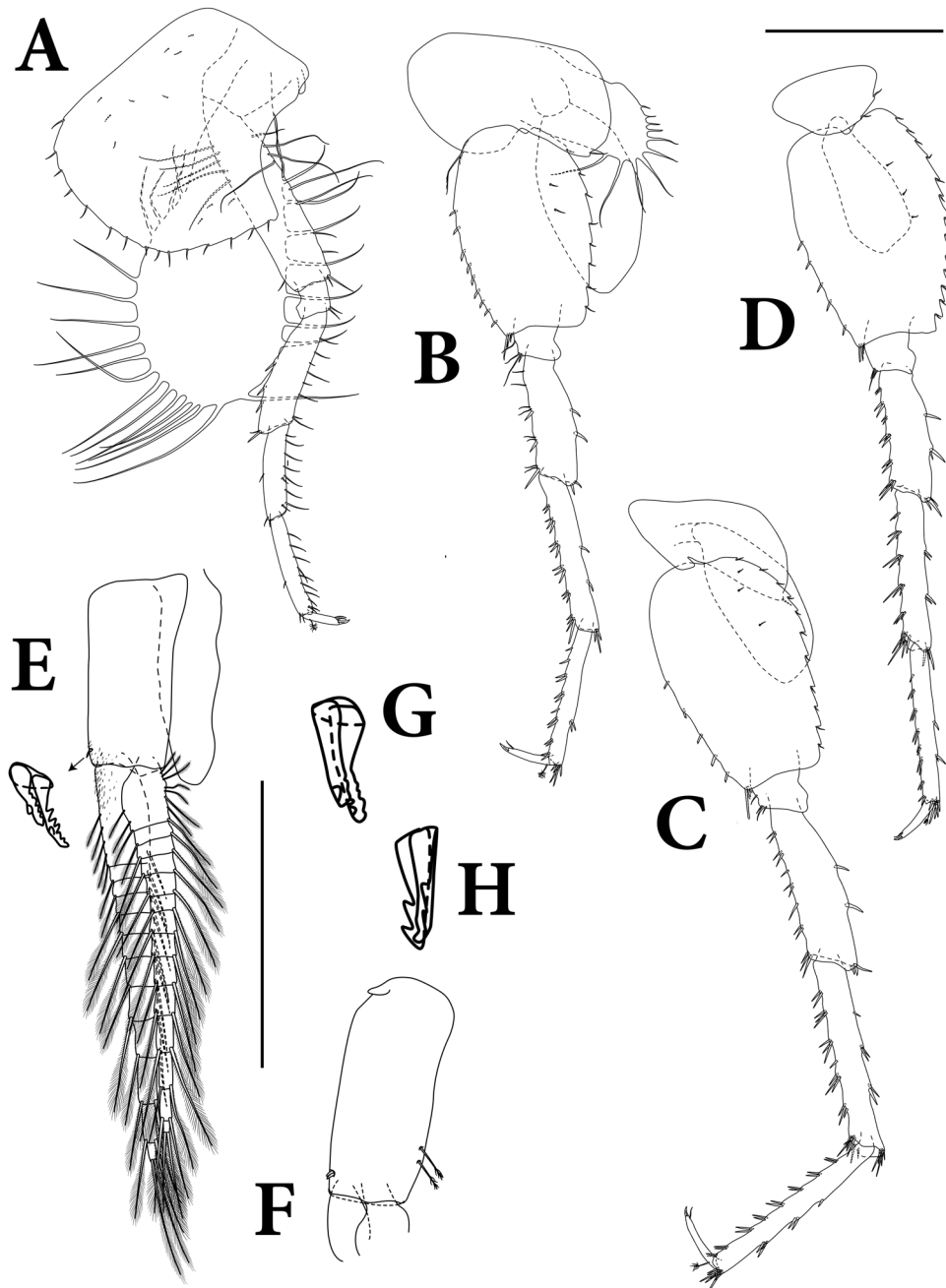
Figure 6. *Crangonyx apalachee* **n. sp.** holotype female, Lake Jackson, Leon County, Florida (UFID 60028), 5.1 mm: pereopod 4 (**A**); pereopod 5 (**B**); pereopod 6 (**C**); pereopod 7 (**D**); pleopod 1 (coupling hooks enlarged) (**E**); pleopod 2 coupling hooks (**G**); pleopod 3 coupling hooks (**H**). Paratype female, Lake Jackson, Leon County, Florida (UFID 60039), 5.47 mm: pleopod 1 peduncle showing variation in setation (**F**). Scale bars = 1 mm.

anterior margin with 5 split-tipped robust setae, 3 distal robust setae, proximal setae absent; merus ∼81% length of carpus; carpus subequal in length to propodus, dactylus ∼36% length of propodus, setation similar to pereopods 5, 6.

Gills and brood plates (Figs. 5B–C, 6A–E): coxal gills on somites 2–6, somite 7 with pereopod 7 gill, subequal in size to coxal gills. Large, setaceous brood plates on somites 2–5, decreasing in size posteriorly. Sternal gills on somites 6 and 8.

Pleon. Pleopods: peduncle of pleopod 1 (Fig. 6E) ∼45% length of rami, lacking setae in holotype, variable in other individuals (Fig. 6F), with 2 coupling hooks; outer and inner rami

with 12 and 14 articles respectively; pleopod 2 (not figured) peduncle lacking setae, with 2 coupling hooks (Fig. 6G); outer and inner rami with 12 and 14 articles respectively; pleopod 3 (not figured) peduncle lacking setae, with 2 coupling hooks (Fig. 6H); outer and inner rami with 10 and 12 articles respectively. Epimera (Fig. 7A): first epimeron ventral margin unarmed, strongly oblique, distoposterior corner distinctly produced, with tooth-like extension, posterior margin with seta placed proximally from distoposterior corner, face of epimeron lacking setae; second epimeron ventral margin with 3 robust setae, distoposterior corner distinctly produced, with tooth-like extension,
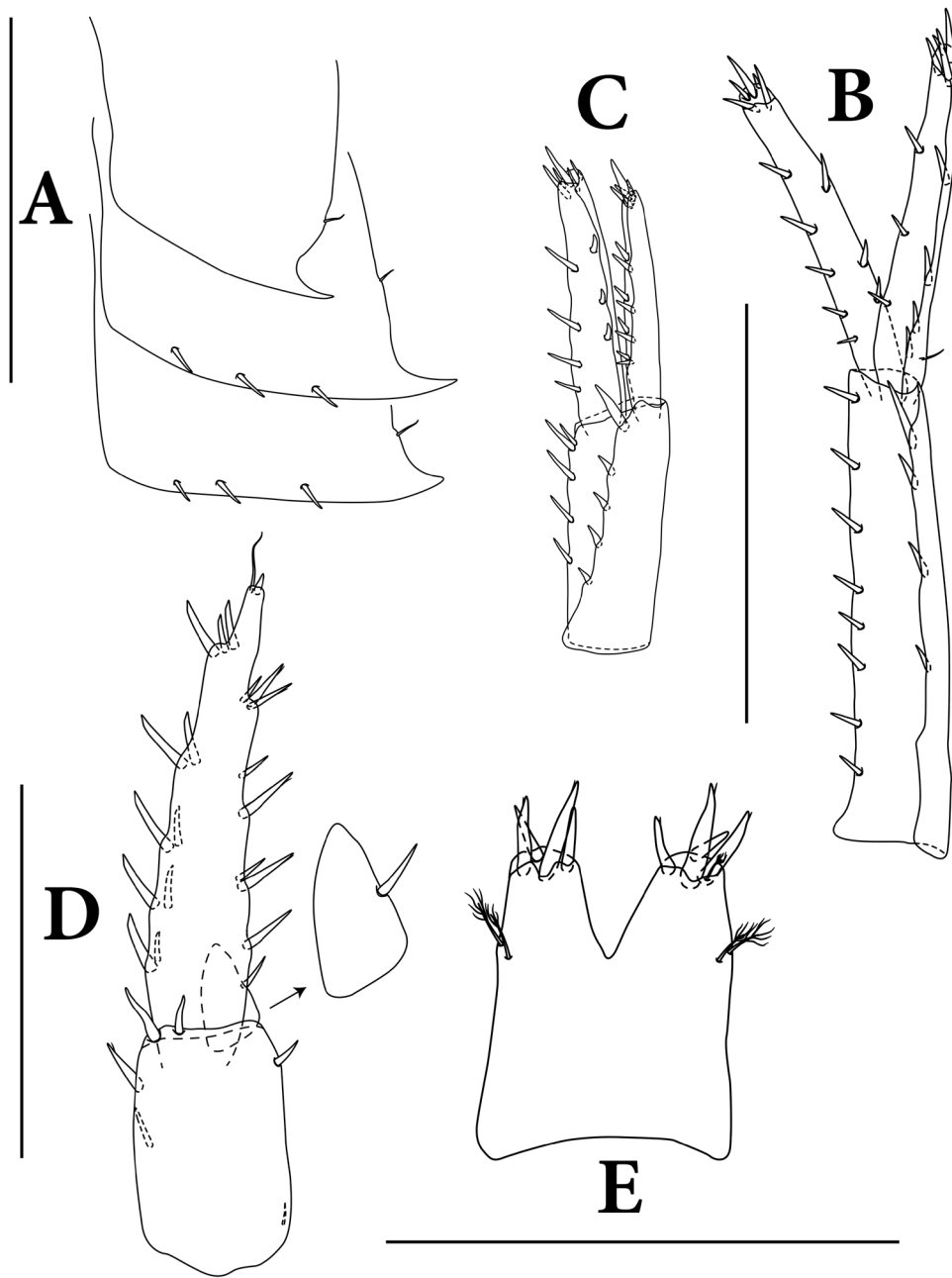
Figure 7. *Crangonyx apalachee* **n. sp.** holotype female, Lake Jackson, Leon County, Florida (UFID 60028), 5.1 mm: epimera 1–3 (**A**); uropod 1 (**B**); uropod 2 (**C**); uropod 3 (inner ramus enlarged) (**D**); telson (**E**). Scale bars = 1 mm.

posterior margin with seta, face of epimeron lacking setae; third epimeron ventral margin with 3 robust setae, distoposterior corner produced, with weaker, rounded tooth-like extension, posterior margin with seta placed near distoposterior corner, face of epimeron lacking setae.

Urosome. Uropod 1 (Fig. 7B): peduncle 1.4× length of rami with 8 and 4 robust setae on inner and outer margins respectively; rami narrowing slightly distally, outer ramus subequal in length to inner ramus, with 3 robust setae on inner margin, 4 robust setae on outer margin, small seta placed proximally on ventral margin and 5 apical robust setae; inner ramus with 3 robust setae on inner margin, 5 robust setae on outer margin, and 5 apical robust setae. Uropod 2 (Fig. 7C): peduncle subequal in length to inner ramus; with 5 robust setae on both outer and inner margins, distalmost inner

robust setae inserted in a pair; rami not narrowing distally, outer ramus ~90% length of inner ramus, with 3 robust setae on inner margin, 4 robust setae on outer margin and 5 apical robust setae; inner ramus with 4 robust setae on inner and outer margins and 5 apical robust setae. Uropod 3 (Fig. 7D): peduncle ~54% length of outer ramus, with 2 robust setae on outer margin, robust seta on inner margin and 2 robust setae inserted distally; inner ramus reduced, scale-like with marginal robust seta; outer ramus 4.7× longer than broad, 4.7× longer than inner ramus, with 8 robust setae on inner and 11 on outer margins, apex with slender seta paired with short robust seta.

Telson (Fig. 7E): ~1.24× longer than broad, cleft ~40% length, with maximum cleft depth to cleft width ratio of 1.22, apices armed with 4 large robust setae and plumose seta on right
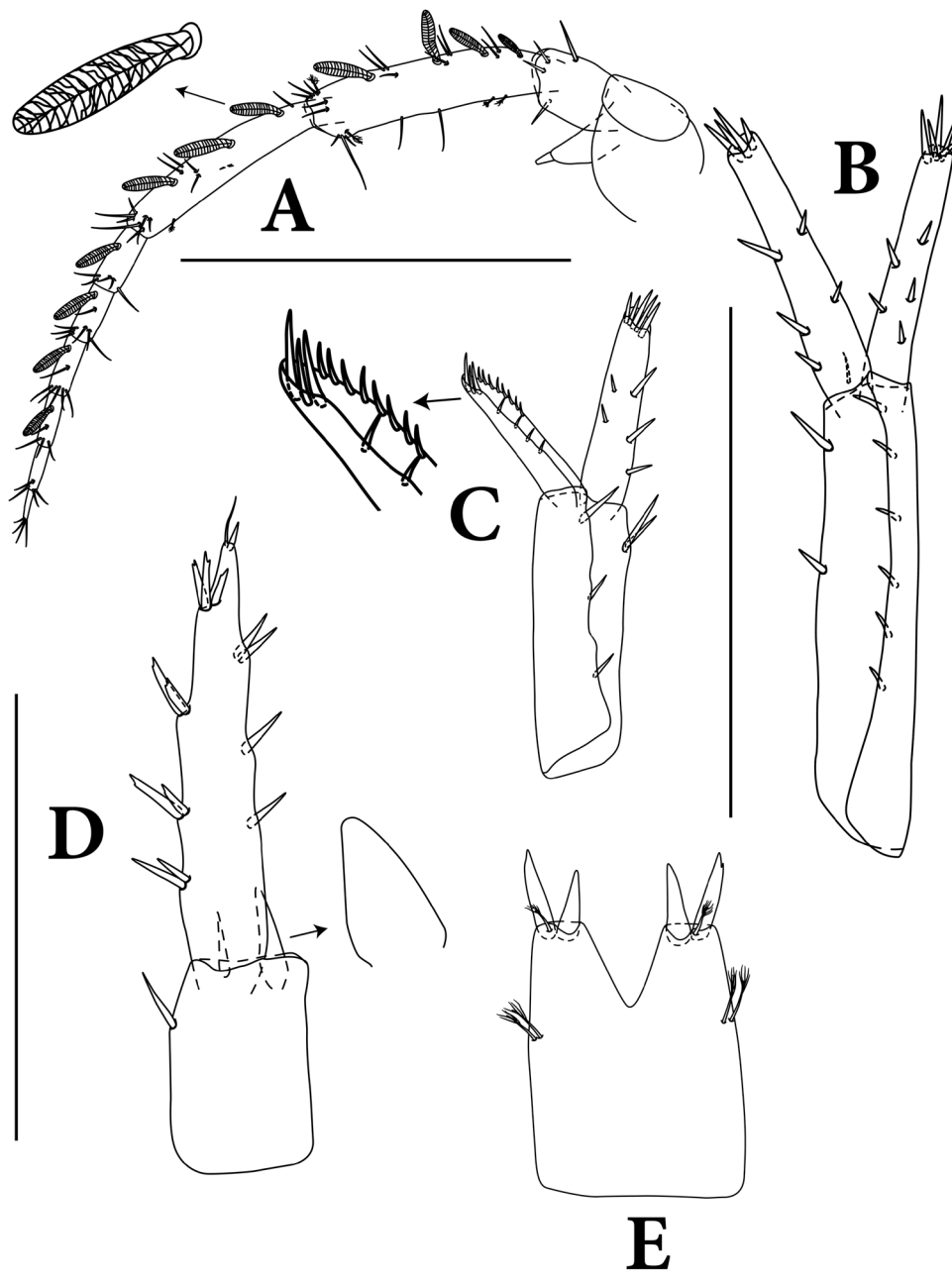
Figure 8. *Crangonyx apalachee* **n. sp.** allotype male, Lake Jackson, Leon County, Florida (UFID 60029), 3.85 mm: antenna 2 (single calceolus enlarged) (**A**); uropod 1 (**B**); uropod 2 (outer ramus comb spines enlarged) (**C**) uropod 3 (inner ramus enlarged) (**D**); telson (**E**). Scale bars = 1 mm.

lobe, 2 additional plumose setae arise dorsolaterally from outer margins of both lobes.

Male (Figs. 8, 9): Allotype (UFID 60029) 3.85 mm in length, differing from female in smaller body length, shorter antennae, presence of calceoli on peduncle and flagellum of antenna 2; more robust gnathopods with enlarged propodi and more robust setae on the palmar margins, propod of gnathopod 1 more oblique; pereopods 5–7 with fewer posterior serrations on bases; setation of uropod 1; setation of uropod 2, including presence of comb spines on inner margin of outer ramus of uropod 2, inner ramus deflected laterally; and uropod 3 setation. Structures not described below as in female.

Antennae. Antenna 1 (not illustrated, but similar to female (Fig. 3A)): 81% body length, 2.7× longer than antenna 2; peduncle and flagellum lacking robust setae; primary flagellum with 21 articles, aesthetascs on distal articles, aesthetascs shorter than respective articles; accessory flagellum with 2 articles, subequal to first article. Antenna 2 (Fig. 8A): gland cone distinct, peduncle ~1.5× longer than flagellum, articles 4, 5 subequal in length; elongated calceoli on articles 4, 5 of the peduncle and most flagellar articles; flagellum with 6 articles.

Gnathopods. Gnathopod 1 (Fig. 9A): coxal plate with 4 apical setae; basis with long seta inserted along anterior and 5 long setae along posterior margins, posterodistal corner with seta; ischium
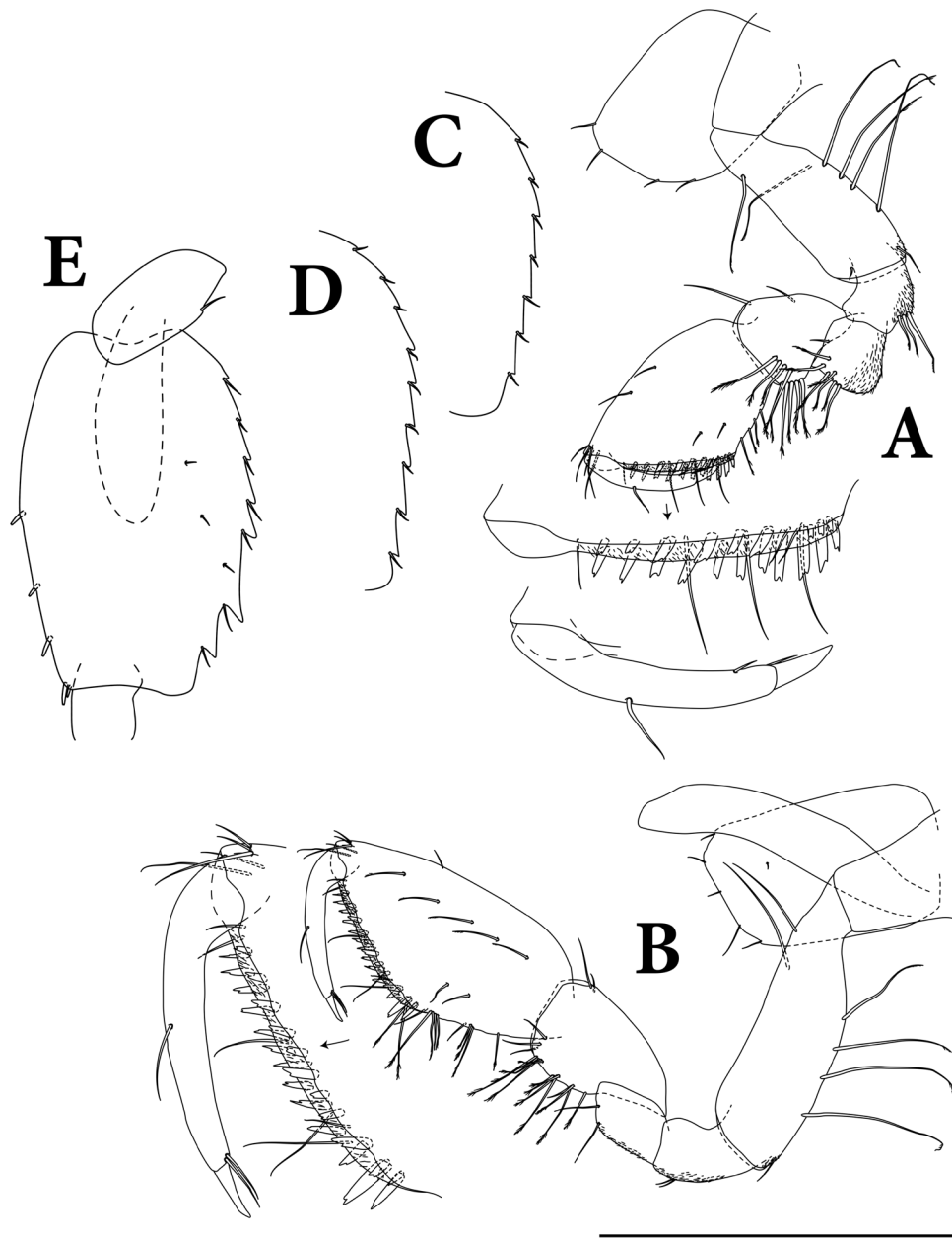
Figure 9. *Crangonyx apalachee* **n. sp.** allotype male, Lake Jackson, Leon County, Florida (UFID 60029), 3.85 mm: gnathopod 1 (palmar margin and dactyl enlarged) (**A**); gnathopod 2 (palmar margin and dactyl enlarged) (**B**); pereopod 5 basis posterior margin (**C**); pereopod 6 basis posterior margin (**D**); pereopod 7 basis posterior margin (**E**). Scale bars = 1 mm.

with 3 setae and pubescence along posterior margin; merus with pubescence covering posterior surface, with 7 plumose setae and smooth seta; carpus ~50% length of propodus with 2 anterior setae, group of plumose medial setae and group of plumose posterior setae; propodus robust, 1.8× longer than broad with 2 superior medial setae, 2 inferior medial setae and 4 plumose posterior setae; palm oblique, with 6 inner and 8 outer bifid robust setae and 3 long outer setae; defining angle armed with 3 inner and outer robust setae; dactyl with outer seta and 2 inner setae. Gnathopod 2 (Fig. 9B): coxal plate with 5 apical setae; basis with 2 long setae inserted along anterior and 4 long setae along posterior margins, posterodistal corner with seta; ischium with 2 setae and pubescence along posterior margin; merus with pubescence covering posterior surface and 2 distal setae; carpus ~54%

length of propodus with 2 anterodistal setae, 3 posterodistal setae, 4 groups of setae along the posterior margin; propodus robust, 2.2× longer than broad with 5 singly-inserted superior medial setae, 2 inferior medial setae, 3 groups of posterior setae; palm oblique with 11 outer and 9 inner bifid robust setae and 4 long outer setae, defining angle armed with 2 inner robust setae and 2 large outer robust setae; dactyl with outer seta and 2 inner setae.

Pereopods. Pereopod 5 (Fig. 9C): basis posterior margin with 7 serrations. Pereopod 6 (Fig. 9D): basis posterior margin with 9 serrations increasing in depth distally. Pereopod 7 (Fig. 9E): coxal plate lobes indistinct, with apical seta; basis posterior margin with 7–9 deep serrations increasing in depth distally, distance between second and third serration from

posterodistal corner notably longer, distal corner weakly rounded, anterior margin with 3 and distal margin with 2 split-tipped robust setae.

Urosome. Uropod 1 (Fig. 8B): peduncle ⁓1.8× length of rami with 2 inner and 6 outer robust setae; outer ramus ⁓86% length of inner ramus, with 2 robust setae on inner margin, 3 robust setae on outer margin and 5 apical robust setae; inner ramus with 2 inner robust setae, 3 outer robust setae, and 4 apical robust setae. Uropod 2 (Fig. 8C): peduncle 1.3× length of inner ramus with 2 setae inserted distally along inner margin and 3 robust setae inserted along outer margin; outer ramus ⁓86% length of inner ramus with 4 outer robust setae and 3 apical setae, inner margin with 8 comb spines that reach ⁓35% length of ramus; inner ramus with 3 outer and 2 inner robust setae, apex with 5 robust setae. Uropod 3 (Fig. 8D): peduncle 46% length of outer ramus, with robust seta on outer margin and robust seta inserted distally; inner ramus reduced, scale-like, lacking seta; outer ramus ⁓6× longer than broad, 4.5× longer than inner ramus, with 4 and 3 groups of robust, split-tipped setae on outer and inner margins respectively, apex with slender seta paired with short, robust seta.

Telson (Fig. 8E): ⁓1.35× longer than broad, cleft ⁓30% of telson length, apices with 2 large robust setae and plumose seta, 2 additional plumose setae dorsolaterally from outer margins of both lobes.

Remarks

The new species is distinguished from *C. floridanus* by palmar margins of gnathopods 1 and 2 having approximately 15 or 16 robust setae respectively; ventral margin of epimeron 2 lacking paired robust setae; male uropod 2 with denser comb spines on outer ramus, reaching less than half the length of the inner margin; male and female telson with length to width ratios of approximately 1.35 and 1.24 respectively.

Distinguished from *C. manubrium* by possessing full eyes; palmar margins of gnathopods 1 and 2 with approximately 15 or 16 robust setae respectively; male uropod 2 peduncle with 4 outer setae and with comb spines on outer ramus reaching less than half the length of the inner margin; female uropod 3 outer ramus with 14–21 marginal robust setae; male telson cleft depth to length ratio of approximately 40%.

It differs from *C. ephemerus* by the outer ramus of the male uropod 1 with proximal ventral robust seta; female uropod 3 inner ramus with robust seta; male uropod 3 inner ramus lacking robust seta. It differs from *Crangonyx pseudoephemerus* by the peduncle of male uropod 2 peduncle having four outer setae; female uropod 3 inner ramus with robust seta; female uropod 3 outer ramus with 14–21 marginal robust setae; male uropod 3 inner ramus lacking robust seta; male telson length to width ratio of approximately 1.35.

Variation

The new species was shown to vary in several nondiagnostic morphological characteristics, even within individuals; one diagnostic characteristic, uropod 3 outer ramus marginal robust setae, demonstrated noteworthy variability (Table 2).

Table 2. Notable morphological variation observed among individuals of *Crangonyx apalachee* **n. sp.**

Character	*C. apalachee* n. sp.
Female antenna 1 length to body length	0.63–0.71
Female antenna 1 flagellar articles	21–24
Female antenna 1 length to antenna 2 length	2.08–2.88
Female antenna 2 flagellar articles	7–9
Male antenna 1 length to body length	0.48–0.88
Male antenna 1 flagellar articles	18–22
Male antenna 1 length to antenna 2 length	1.85–3.09
Male antenna 2 flagellar articles	5, 6
Male pereopod 5 basis posterior margin serrations	6, 7
Male pereopod 6 basis posterior margin serrations	7–9
Male pereopod 7 basis posterior margin serrations	7–10
Male pleopod 1 peduncle length to rami length	0.42–0.78
Female pleopod 1 peduncle length to rami length	0.36–0.62
Female uropod 3 outer ramus marginal robust setae	14–21
Male uropod 3 outer ramus marginal robust setae	8–13

Geographical distribution and ecology

Crangonyx apalachee **n. sp.** is currently endemic to Lake Jackson, Leon County, Florida, where it has been collected in tall grass along the lake's shoreline (Fig. 1B). The new species appears to be restricted to the edges of the lake, occurring among aquatic vegetation. *Crangonyx apalachee* **n. sp.** occurred in the upper littoral zone of the lake, replaced in the lower littoral and limnetic zones by *Hyalella* sp., which was observed to be abundant.

Lake Jackson is a prairie lake that experiences temporary draining episodes through two major sinkholes: Porter Sink and Lime Sink. Sellards (1914) described Lake Jackson as becoming dry in spring 1907 and summer 1909. Hughes (1967) analyzed rainfall patterns and water levels in Lake Jackson, correlating below average rainfall and a lake draining episode that occurred in 1957. Hughes (1967) also documented reports of Lake Jackson being dry in 1932, 1935, and 1936. Wagner (1984) noted a correlation between lake and groundwater levels with rainfall amounts in response to a major lake draining event in 1982. Lake Jackson has had multiple lake draining events in the 21st century, with the most recent ones in 2021 (Fig. 10). In addition, references to Lake Jackson going dry in the 19th century can be found in local newspaper reports. It can thus be inferred that *C. apalachee*
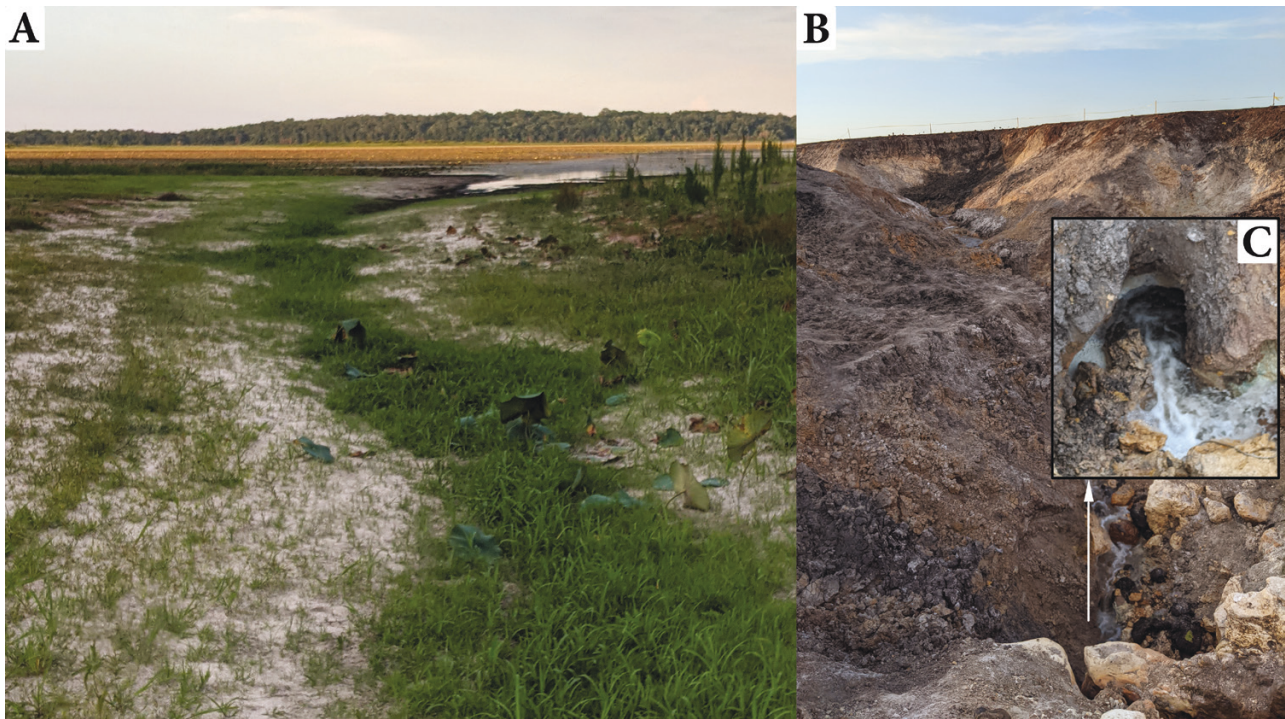
Figure 10. Type locality of *C. apalachee* **n. sp.** during lake draining event on 16 June 2021. Image taken at the same location and from the same vantage point as Fig 1B (**A**); bottom of Porter Sink in Lake Jackson during lake draining event on 16 June 2021 (**B**); close-up of Porter Sink showing lake water entering the ground (**C**).

n. sp. is likely adapted to these periodic draining episodes, which, although unpredictable, occur repeatedly.

Unpublished dye trace studies suggest that at least some of the water that drains from Lake Jackson travels approximately 32 km south to Wakulla Springs via subterranean cave systems (S. McGlynn, personal communication, 26 January 2022). Despite collection efforts by one of us (TRS) in Wakulla Springs State Park, near the Wakulla Spring vent, and downstream in the Wakulla River, individuals of the new species have not been discovered. Other collection efforts in the Wakulla River have documented the presence of species belonging to multiple amphipod families, but no crangonyctids (Drumm & Knight-Gray, 2019). The presence of individuals of *C. apalachee* **n. sp.** in surface waters of Lake Jackson but not from groundwater or Wakulla Springs suggests that individuals may not get washed into groundwater during lake draining events. It is unclear how the new species survives lake-draining events, but it may have an adaptation to reside interstitially during these episodes such as *Crangonyx pseudogracilis* (Harris *et al.,* 2002). Efforts to collect during the 2021 draining episode using a Bou-Rouch pump were nevertheless unsuccessful, suggesting this may not be their method of survival. It is also possible that they may survive in small pools of water that may persist during these draining episodes; however, this leaves the question of how *C. apalachee* **n. sp.** and *Hyalella* sp. that also occur in the lake reestablish and maintain their niche partitioning during times of higher lake water levels.

It appears that populations of *Crangonyx* living in the nearby Lakes Piney Z and Miccosukee may represent a separate, undescribed species (Figs. 11, 12). Additional studies are needed to determine their status in relation to *C. apalachee* **n. sp.** and other geographically proximate populations of species of *floridanus* complex found in similar habitats.

Little is known about the ecology of *C. apalachee* **n. sp.** or the other members of the *floridanus*-complex species in northern Florida. The new species seems to exhibit an unusual male-biased sex ratio. Out of the 22 specimens collected, 7 were female and 15 were male. This contrasts with *C. ephemerus* and *C. pseudoephemerus* with an approximate 50:50 sex ratio (Cannizzaro & Sawicki, 2020). *Crangonyx manubrium* exhibited an extreme female-biased sex ratio with only 11 males out of 76 total specimens examined (Cannizzaro *et al.,* 2019a). Collections at other times of the year are needed to confirm the male-biased sex ratio of the new species.

RESULTS

Crangonyx apalachee **n. sp**. differs from other members in the *floridanus* complex in several taxonomically significant characters. Of note are differences between *C. apalachee* **n. sp.** and *C. floridanus* sensu stricto for which no genetic data is available. These include the number of robust setae along the palmar margins of gnathopods 1, 2, setation of the dactyl inner margin of pereopods 3 and 4, number of serrations along the basis of posterior margin of pereopod 7, armament of second epimeron ventral margin, telson structure, and ratios such as the uropod 1 peduncle length to outer ramus length ratio (Table 1).

Molecular analyses

All phylogenetic trees reconstructed using Bayesian Inference based on the concatenation of three genes (nuclear 18S rDNA and 28S rDNA, and mitochondrial 16S rDNA) clearly identify
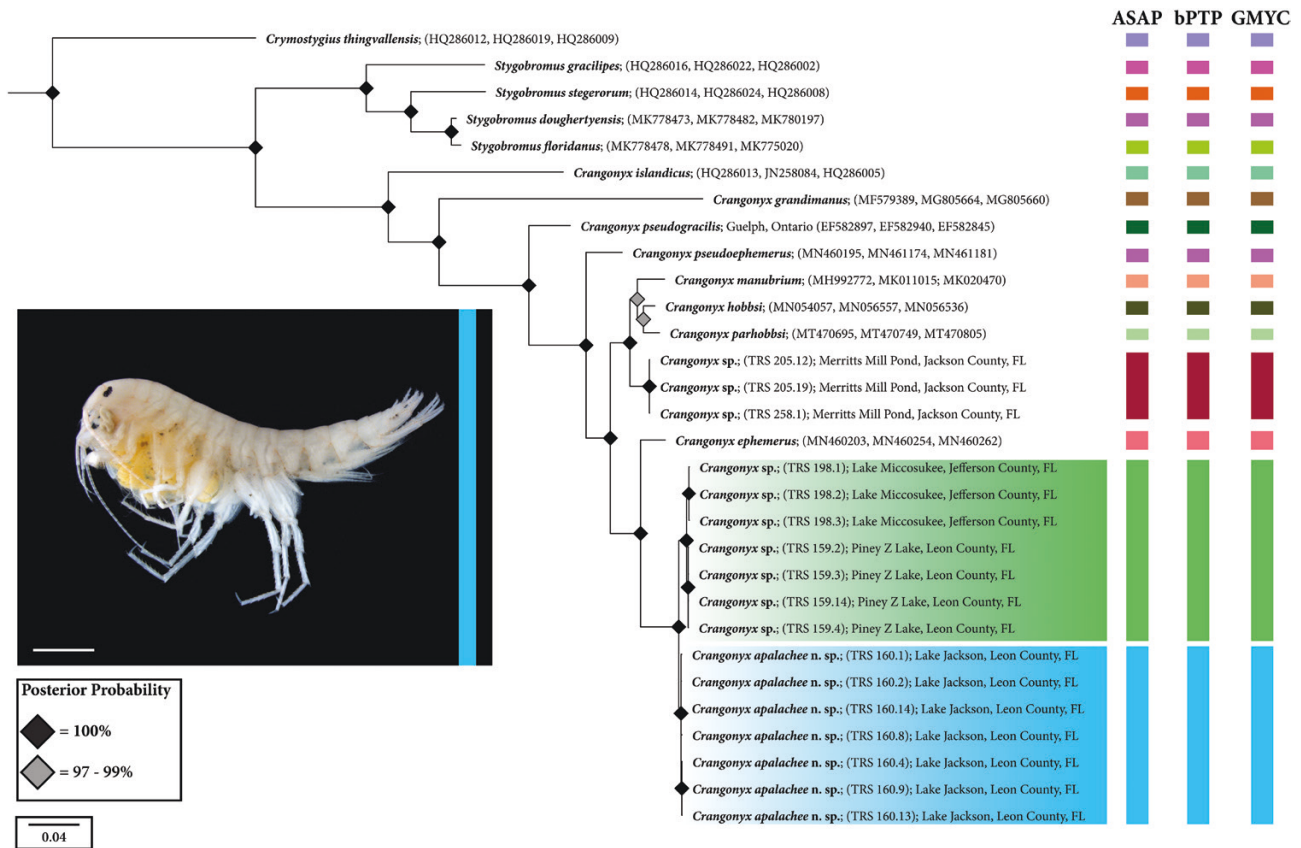
Figure 11. Multi-locus Bayesian phylogeny of selected members of Crangonyctoidea based on a concatenation of the nuclear 18S, 28S, and mitochondrial 16S rDNA. Posterior probability is indicated by colored diamonds placed at nodes (black 1.0, grey 0.97–0.99). Results of species delimitations presented right of tree. ASAP, Assemble Species by Automatic Partitioning; bPTP, Bayesian Poisson Tree Processes; GMYC, generalized mixed yule coalescent. Inset *Crangonyx apalachee* **n. sp.**, holotype female, Lake Jackson, Leon County, Florida (UFID 60028). Scale bar = 1 mm.

individuals of *C. apalachee* **n. sp.** as a separate clade from all closely related *Crangonyx* species (Figs. 11, 12). The genetic differences between *C. apalachee* **n. sp.** and other congenera were also identified as species-level using three different species delimitation methodologies (GMYC, bPTP, and ASAP). These models also delimit *C. apalachee* **n. sp.** as distinct from populations that morphologically align as still-undescribed *floridanus*-complex species in the nearby Lake Miccosukee and Piney-Z Lake (Figs. 11, 12).

Based on the results of the BEAST analysis, *C. apalachee* **n. sp.** diverged from its closet congener (*C. ephemerus*) during the Neogene, approximately 10–15 mya (Fig 12). *Crangonyx apalachee* diverged from populations in the nearby lakes Piney-Z and Miccosukee in the early Pleistocene approximately 2.5 mya (Fig 12).

DISCUSSION

Zhang & Holsinger (2003) redescribed *Crangonyx floridanus* based on individuals collected from Orangeburg County, South Carolina, USA and noted that *C. floridanus* is morphologically similar to *C. pseudogracilis*, broadly aligning with the *gracilis*-complex species. In their diagnosis, they indicated several characteristics that differentiated *C. floridanus* from other *gracilis*-complex species, including the depth of serrations on posterodistal corners on bases of pereopods 5–7, well-developed posterodistal corners of epimeral plates, and comb spines on the outer ramus of male uropod 2, but lacking ventral spines. Cannizzaro *et al.* (2019a) redescribed *C. floridanus* based on type material collected from Highlands Hammock State Park, Florida by Bousfield (1963) and demonstrated significant morphometric differences between these specimens and those collected from caves in Jackson County, Florida. Based on these data, Cannizzaro *et al.* (2019a) described the Jackson County cave populations as *C. manubrium*. Bousfield (1963) originally described these cave populations as *C. floridanus* paratypes based on their similarities. *Crangonyx ephemerus*, *C. pseudoephemerus*, and *C. apalachee* **n. sp.** share the *floridanus* diagnostic characters described by Zhang & Holsinger (2003), although they significantly differ morphometrically and genetically (Table 1; Figs. 11, 12; Cannizzaro & Sawicki, 2020). These data demonstrate that the characters defined by Zhang & Holsinger (2003) cannot be used to diagnose *C. floridanus*, but instead refer to a morphological cryptic-species complex described here as the *floridanus* complex.

White (2011) demonstrated the utility of the 18S rDNA as a species-level marker in leucothoid amphipods, noting an uncorrected *p*-distance of 0.005 as a minimum threshold for species delimitation. Table 3 shows the uncorrected *p*-distance of the 18S rDNA between crangonyctid amphipods from Florida. For
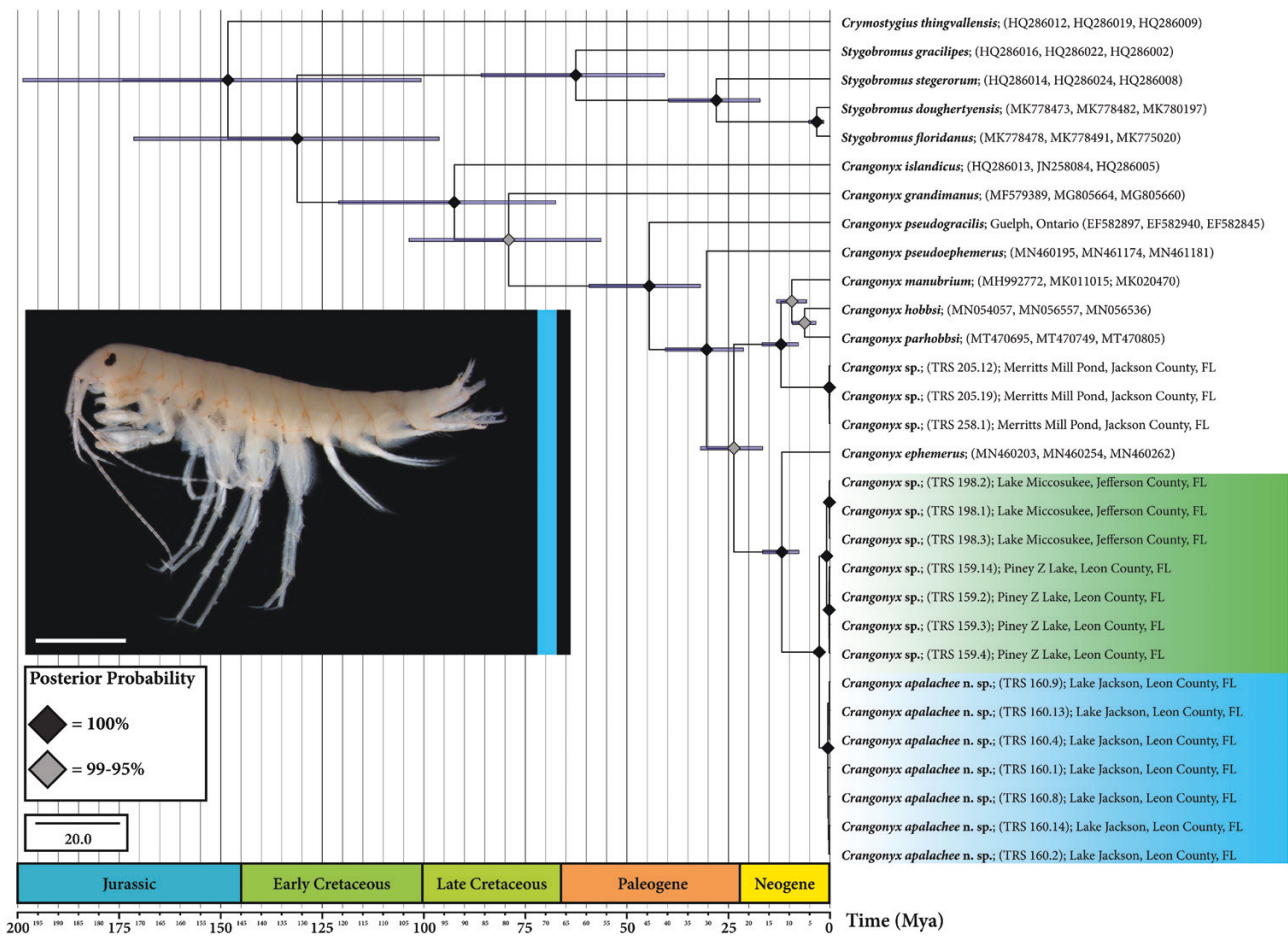
Figure 12. Time-calibrated multilocus phylogeny of selected members of Crangonyctoidea. Posterior probability is indicated by colored diamonds at nodes (black 1.0, grey 0.95–0.99), blue bars indicate the 95% highest posterior density (HPD) interval of clade age. Inset: *Crangonyx apalachee* **n. sp.**, allotype male, Lake Jackson, Leon County, Florida (UFID 60029). Scale bar = 1 mm.

instance, the uncorrected *p*-distance of the 18S rDNA between the morphologically variant *Stygobromus floridanus* and *S. dough-ertyensis* is approximately 0.0028. The uncorrected *p*-distance between the stygobitic species *Crangonyx hobbsi* and an epigean *Crangonyx* sp. from Merritt's Mill Pond is approximately 0.007, which might represent a minimum species-level genetic distance at this locus for the genus, particularly given the comparison is between *Crangonyx hobbsi*, considered the most stygomorphic species in the genus (Zhang & Holsinger 2003), and an epigean species. It is noteworthy that the uncorrected *p*-distance of the 18S rDNA between *C. apalachee* **n. sp.** and the four *florida-nus*-complex species for which 18S rDNA data is available is between approximately 0.028 and 0.039. These data provide strong support for the validity of *C. apalachee* **n. sp.**.

The phylogenetic analyses performed herein indicate that of all the described *floridanus*-complex species for which genetic data is available, *C. apalachee* **n. sp.** is most closely related to *C. ephemerus.* (Figs. 11, 12). *Crangonyx apalachee* **n. sp.** diverged from *C. ephemerus* around 10 to 15 mya during the mid to late Miocene (Fig. 12). These data indicate individuals from Lakes Miccosukee and Piney-Z represent a putative sister species to *C. apalachee* **n. sp.**, suggesting divergence approximately 2.5 mya during the early Pleistocene (Fig. 12). Additionally, these data suggest that the crown age of the species in the *floridanus*

complex is sometime during the Paleogene to early Neogene, which is remarkable given their morphological similarity. Future collection and sequencing efforts across the range of *C. flori-danus* sensu lato may provide further insight into the timing of the divergence of this complex from closely related *Crangonyx* species.

The discovery of species-level genetic divergence between *C. apalachee* **n. sp.** and a putative *Crangonyx* sp. collected from nearby Lakes Miccosukee and Piney-Z is noteworthy as *C. apalachee* **n. sp.** was collected along the shoreline of Lake Jackson in heavy vegetation (Fig. 1B). This habitat is frequented by wading birds, strongly suggesting a mechanism of dispersal between lakes. If *C. apalachee* **n. sp.** occurs interstitially, par-ticularly to survive lake draining events, it is possible that they emerge at certain times of the year to reproduce. If true, tempo-ral variation in emergence for reproduction may account for the documented genetic discontinuity between these nearby and ecologically similar lakes. Additional research should determine how such high genetic divergence is maintained across nearby and similar lake habitats

The description of *C. apalachee* **n. sp.** brings the total number of described species of the *floridanus* complex in Florida to five. While *floridanus*-complex species broadly align morphologically, they are not monophyletic (Figs. 11, 12). The *floridanus*-complex

Table 3. Uncorrected *p*-distances for the nuclear 18S rDNA observed between crangonyctid amphipods in Florida. Values in bold highlight distances observed between *Crangonyx apalachee* **n. sp.** and other members of the *C. floridanus* complex

	Crangonyx sp. Lake Miccosukee	*Crangonyx* sp. Piney-Z Lake	*Crangonyx apalachee* n. sp.	*Crangonyx hobbsi*	*Crangonyx parhobbsi*	*Crangonyx* sp. Merritt's Mill Pond	*Crangonyx manubrium*	*Crangonyx pseudoephemerus*	*Crangonyx ephemerus*	*Crangonyx grandimanus*	*Stygobromus doughertyensis*	*Stygobromus floridanus*
Crangonyx sp. Lake Miccosukee												
Crangonyx sp. Piney-Z Lake	0.00264											
Crangonyx apalachee **n. sp.**	0.00528	0.00528										
Crangonyx hobbsi	0.03838	0.03838	0.03425									
Crangonyx parhobbsi	0.03838	0.03838	0.03425	0.00507								
Crangonyx sp. Merritt's Mill Pond	0.03628	0.03628	**0.03267**	0.00668	0.00709							
Crangonyx manubrium	0.03889	0.03889	**0.03476**	0.01014	0.00868	0.01039						
Crangonyx pseudoephemerus	0.04295	0.04295	**0.03937**	0.01836	0.01864	0.01599	0.01993					
Crangonyx ephemerus	0.03258	0.03258	**0.02844**	0.01841	0.0187	0.01887	0.01953	0.02377				
Crangonyx grandimanus	0.11082	0.11082	0.10928	0.09502	0.09417	0.09478	0.0975	0.09581	0.09969			
Stygobromus doughertyensis	0.11801	0.11698	0.11562	0.10612	0.10555	0.10782	0.10818	0.10503	0.1066	0.11344		
Stygobromus floridanus	0.11852	0.1175	0.11614	0.1069	0.10612	0.10808	0.10879	0.10583	0.10789	0.11427	0.00285	

species exist in a wide range of habitats including cypress swamps (*C. floridanus* sensu stricto), phreatic water (*C. manubrium*), ephemeral pools along river floodplains (*C. ephemerus* and *C. pseudoephemerus*), and prairie lakes (*C. apalachee* **n. sp.**). Additionally, multiple populations of *Crangonyx* spp., occurring in both lake and cave habits in Florida alone, and possessing *floridanus*-complex characters, need description (Figs. 11, 12). These undescribed species may have varying ecological functions within these habitats. The origin of this remarkable diversity awaits future investigation.

Previous collection efforts over the past few years at the type locality of *C. floridanus*, Highlands Hammock State Park, Highlands County, Florida, resulted in the absence of specimens of *C. floridanus* or any other amphipod species. Collections made by one of us (TRS) on 22–23 January 2022 at Highlands Hammock State Park nevertheless collected four *Hyalella* sp. individuals, but none of *C. floridanus*. In addition, previous collections in lakes near the type locality documented the presence of *Hyalella* sp. in high densities; however, Bousfield (1963) makes no mention of *Hyalella* sp. in the cypress swamp at Highlands Hammock State Park. The documentation of *Hyalella* sp. within this cypress swamp, and the continued absence of *C. floridanus* is concerning, particularly given the high level of endemism within the *floridanus* complex being discovered in northern Florida. It is possible that *C. floridanus* sensu stricto may have been endemic to Highlands Hammock State Park. If that is true, extirpation of this population would mean extinction of the species. Future collection efforts in the type locality and surrounding region should shed light on the status of this species.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. Material examined.

ACKNOWLEDGMENTS

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