Phylogenetic analyses of a new freshwater amphipod reveal polyphyly within the Holarctic family Crangonyctidae, with revision of the genus *Synurella*

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A new genus and species of crangonyctid amphipod, ***Sicifera cahawba* gen. & sp. nov.**, is described from Dallas County, AL, USA, based on both morphological and molecular comparison with similar crangonyctids. These data, with the application of four species delimitation models, identify the taxon as distinct when compared with related species. Nearctic members of the crangonyctid genus *Synurella* form a separate, well-supported monophyletic lineage when compared with Palaearctic members, which differ considerably in both molecular and morphological markers. Nearctic members, with the exception of the enigmatic Synurella (*Eosynurella) johanseni*, are placed in the newly erected *Sicifera*. The separation of these two genera implies that Palaearctic and Nearctic crangonyctid lineages might not be as closely related as once thought, and their evolutionary and biogeographical history requires further review. In addition, a key to Nearctic members of the genera *Eosynurella*/*Sicifera* is presented to aid in future identification.

ADDITIONAL KEYWORDS: Alabama – Cahawba Prairie – Nearctic – phylogeny – species delimitation – *Sicifera*.

INTRODUCTION

Amphipod crustaceans represent one of the most abundant and diverse invertebrate taxa known to occupy freshwater systems, with > 2000 species in 30+ families currently described from all continents except Antarctica (Väinölä *et al.*, 2007; Horton *et al.*, 2021). Despite their ubiquity and high diversity, the majority of amphipod taxa are known from the Holarctic, with many of the most species-rich and wide-ranging taxa (Gammaridae, Niphargidae and Crangonyctidae) occurring solely in this realm. In contrast, taxa occurring in the Southern Hemisphere are often endemic to narrow areas and are, more often than not, relictual in nature (Väinölä *et al.*, 2007; Horton *et al.*, 2021). Amphipods are thought to have entered continental freshwaters and diversified by several methods, the most common of which being either invasion from marine systems, as observed in the gammarids, or continental vicariance, which has been observed in the crangonyctids (Hou *et al.*, 2011; Copilaş-Ciocianu *et al.*, 2019).

In the Holarctic, several taxa are shared between the Nearctic and Palaearctic realms, but the amphipod fauna of each realm is distinct. In the Nearctic, crangonyctids are the most diverse taxon, with 150+ species, in contrast to the gammarids that are often locally abundant but not as species rich (< 20 described species). In the Palaearctic, gammarids and niphargids show high diversity, whereas crangonyctids are more limited, mostly restricted to hypogean habitats (Väinölä *et al.*, 2007). Of these taxa, the crangonyctids are an ideal model for examining biogeographical forces in the Holarctic Realm. They are represented in both the Nearctic and the Palaearctic and occupied freshwater habitats in these areas before the break-up of Laurasia (Copilaş-Ciocianu *et al.*, 2019). These facts, combined with the poor dispersal ability noted among amphipods, allow their evolution to be linked tightly to the development of the regions they occupy.

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As a result, a better understanding of the evolutionary history of the crangonyctids has broad implications for their systematics, while also being of great utility to understand freshwater habitats, organisms and circumpolar lineages as a whole.

Synurella Wrześniowski, 1877 is a genus of freshwater amphipod crustacean in the family Crangonyctidae that can be found throughout the Northern Hemisphere (Holsinger, 1977). Originally proposed as the Palaearctic species *Synurella ambulans* (Müller, 1846), an additional ~20 species were described subsequently from localities throughout the Northern Hemisphere. Although the genus has Nearctic representatives, it is primarily a Palaearctic taxon, with ~80% of species occurring in the Palaearctic (Holsinger, 1977; Horton *et al.*, 2021). In the Nearctic, three of the four described species are restricted to the eastern/central USA, with the unusual *Synurella* (*Eosynurella*) *johanseni* (Shoemaker, 1920) being endemic to Alaska. The genus *Synurella s.l.* has undergone considerable revision since its original description, with increased investigation into the evolutionary and phylogenetic history of taxa in the genus revealing noteworthy polyphyly. From the taxa originally representing *Synurella s.l.*, four genera have now been erected: the Siberian/Alaskan *Eosynurella* Martynov, 1931; the Caucasian *Diasynurella* Behning, 1940; *Pontonyx* Marin & Palatov, 2021 from Turkey and Ukraine; and *Volgonyx* Marin & Palatov, 2021 from Saratov Oblast, Russia. Collectively, these 'synurellids' share a similar morphology, including reduced third uropods and deep coxal plates. Given that they differ in characteristics of the uropods, pleopods and epimera and in genetic characteristics, they are likely to represent distinct lineages of Crangonyctidae, not a monophyletic group (Holsinger, 1977; Marin & Palatov, 2021). The revision of *Synurella* is not unusual for crangonyctids; traditional morphological analyses have failed to capture generic-level relationships in the family, resulting in either the erection of new genera or the identification of polyphyly indicative of generic-level separation in large genera, such as *Crangonyx* Bate, 1859 and *Stygobromus* Cope, 1872 (Copilaş-Ciocianu *et al.*, 2019; Palatov & Marin, 2020). Uncertainties are still present in several taxa, including 'revised' taxa such as the 'synurellids'. Taxa occurring in the two realms occupied by 'synurellids' are notably distinct, with Nearctic and Palaearctic species exhibiting consistent morphological and molecular phylogenetic differences, suggesting their potential status as separate crangonyctid lineages (Holsinger, 1977; Copilaş-Ciocianu *et al.*, 2019; Palatov & Marin, 2020; Marin & Palatov, 2021).

While conducting routine field investigations, we collected an unknown Nearctic species of 'synurellid' amphipod and described it using morphological features. The description was supported by the results from multiple species delimitation models that used genes commonly assessed for amphipod molecular phylogenetics: the nuclear 18S ribosomal DNA (rDNA) (Englisch & Koenemann, 2001; Macdonald *et al.*, 2005; Kornobis *et al.*, 2011; White, 2011; Cannizzaro *et al.*, 2019) and the mitochondrial cytochrome *c* oxidase subunit I (*COI*; Hou *et al.*, 2007; Seidel *et al.*, 2009; Flot *et al.*, 2010; Kornobis *et al.*, 2011). We then performed phylogenetic analyses of members of the genus *Synurella* and other confamilial species and used the results of these analyses to describe a new genus and infer the evolutionary relationships of Nearctic and Palaearctic crangonyctids.

MATERIAL AND METHODS

COLLECTION OF SPECIMENS

Specimens were collected live from macrophytes and substrata in an unnamed first-order stream in Dallas County, AL, USA (see site details in the species description) using hand nets, preserved in 95% ethanol and stored at –20 °C. Examined specimens were deposited at the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

MORPHOLOGICAL ANALYSES

Specimens were dissected using a Nikon SMZ-8000 stereomicroscope and the appendages mounted on temporary glycerin slides to facilitate examination using an AmScope M620 compound microscope. Plates were prepared using Adobe Illustrator CC. 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 second and third segments of the mandibular palps follows that of Cole (1980) and Karaman (1969), respectively. The term 'defining angle' refers to the posterior margin of the palm and the distal-most point of the posterior margin of the propodus, the area where the tip of the dactylus closes on the propodus. The term 'pereopod 7 gill' refers to the gill attached between the coxa and basis of pereopod 7 as described by Steele & Steele (1991). 'Clothes-pin setae' refer to notched robust setae present on the basal segments of the pleopod inner rami as illustrated by Holsinger *et al.* (2008).

POLYMERASE CHAIN REACTIONS

Genomic DNA (gDNA) was extracted by removing three to seven thoracic appendages (generally, gnathopods 1

and 2 and pereopods 3–7) from one side of the animal, leaving the other side intact for morphological examination. Extractions were performed using Tissue & Insect DNA MiniPrep kits (Zymo Research), according to the manufacturer's protocol. Extracted DNA was stored at –20 °C and quantified using Qubit fluorometry.

Polymerase chain reactions (PCRs) using the primer pair 18SF and 18S700R (Englisch & Koenemann, 2001) amplified a 495 bp segment of the 18S rDNA gene; the primer pair LCO1490 and HCO2198 (Folmer *et al.*, 1994) was used to amplify a 487 bp segment of *COI*. Total PCR volumes of 20 µL contained 30–70 ng of extracted gDNA. The PCR master mix contained 10 µL of GoTaq Master mix (Promega), 1 µL of each 10 µM primer and 6 µL of molecular grade water. The PCR was performed on a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories). A negative control lacking only gDNA was included for all sets of PCRs performed to rule out contamination. Thermal cycler protocols for 18S rDNA were 95 °C for 15 min, followed by 32 cycles of 94 °C for 45 s, 68.5 °C for 1 min and 72 °C for 45 s, ending with a 5 min final extension at 72 °C. For *COI*, the following protocol was followed: 95 °C for 5 min, followed by 38 cycles of 94 °C for 45 s, 42°C for 45 s and 72 °C for 45 s, ending with a 5 min final extension at 72 °C.

SANGER SEQUENCING

The PCR products were prepared for Sanger sequencing using illustra ExoProstar (GE Healthcare) exonuclease I and alkaline phosphatase, or by purification from a 1% agarose gel following an EZNA gel extraction protocol (Omega Bio-Tek), with a final elution of 35 µL. The same primers that were used for amplification were used for cycle sequencing reactions, following the default protocol with the Big Dye Terminator v.3.1 cycle sequencing kit (Applied Biosystems). Purification followed the EDTA/sodium acetate/ethanol protocol from the Big Dye kit; sequencing was performed using an ABI Genetic Analyzer (Applied Biosystems). All sequences generated as a part of this study were submitted to GenBank (Table 1).

SEQUENCE PREPARATION AND ALIGNMENT

Pairwise sequence alignment was conducted using MUSCLE (Edgar, 2004) in GENEIOUS PRIME (www.geneious.com) and checked further by eye. For *COI*, amino acid translation was used to screen for pseudogenes (indicated by the presence of multiple stop codons). Multiple sequence alignment of the 18S rDNA was performed separately using SATÉ v.2.2.6 (Liu *et al.*, 2012) owing to its accuracy with complex datasets, such as those observed in nuclear genes (Mirarab *et al.*, 2015). Within SATÉ, MAAFT (Katoh *et al.*, 2005) was selected as the aligner and OPAL (Wheeler & Kececioglu, 2007) as the merger, because this combination has been demonstrated to provide high phylogenetic accuracy (Liu & Warnow, 2014). Tree inference within SATÉ was conducted using the maximum-likelihood method estimated in FASTTREE (Price *et al.*, 2010), under the GTR+2O substitution model. The tree building–alignment cycle was repeated ten times, and the iteration with the best likelihood score was selected for final analysis. GBLOCKS v.0.9 (Talavera & Castresana, 2007) was used to refine the 18S rDNA alignment further by removing regions of poor alignment and ambiguous homology; minimum restrictive positions were applied, and gap positions were allowed in the final alignments. All markers were concatenated into a final alignment using SEQUENCE MATRIX v.1.8 (Vaidya *et al.*, 2011).

SPECIES DELIMITATION

Species delimitation was performed using four methodologies: Bayesian Poisson tree processes (bPTP); automatic barcode gape discovery (ABGD); assemble species by automatic partitioning (ASAP); and generalized mixed Yule coalescence (GMYC). These methodologies were selected owing to their common use in delimiting crustacean taxa. For bPTP, the algorithm created by Zhang *et al.* (2012) was used with default parameters. For ABGD and ASAP, the algorithms created by Puillandre *et al.* (2012, 2021) were used with default parameters. For GMYC species delimitation, an ultrametric tree was created in BEAST v.2.5.1 (Bouckaert *et al.*, 2014). Best-fitting evolutionary substitution models for each gene used

Table 1. GenBank accession numbers for sequences generated as a part of this study

Catalogue number	Species	18S accession number	*COI* accession number
AGC-34.1	*Sicifera cahawba*	OK491121	OK489438
AGC-34.3	*Sicifera cahawba*	OK491120	OK489441
AGC-44.3	*Sicifera cahawba*	OK491119	OK489440
AGC-44.6	*Sicifera cahawba*	OK491118	OK489439
AGC-53.1	*Sicifera dentata*	OK491117	OK489437

(18S rDNA and *COI*) were determined independently using bMODELTEST (Bouckaert & Drummond, 2017), with the tree prior set to Yule process and the molecular clock set to log normal. A Markov chain Monte Carlo (MCMC) simulation was run for 100 million generations and sampled every 1000 generations. Convergence and effective sample size (ESS) were checked using TRACER v.1.6 (Rambaut *et al.*, 2014). The first 25% of resulting trees were discarded as burn-in, and the remaining trees were summarized in TREEANNOTATOR v.1.8.1 (available in BEAST). The resulting tree was used for species delimitation implemented in the R packages *ape*, *paran*, *splits* and *rncl* (Paradis *et al.*, 2004; Dinno, 2012; Ezard *et al.*, 2013; Michonneau *et al.*, 2016).

PHYLOGENETIC ANALYSES

Phylogenetic relationships were reconstructed using both maximum likelihood and Bayesian inference methods. Maximum likelihood analyses were conducted using IQTREE v.1.6 (Nguyen *et al.*, 2015). The IQTREE search was run using the MODELFINDER algorithm to select best-fitting substitution models for individual partitions, which were analysed under an edge-linked model. For *COI*, HKY with empirical base frequencies was selected for the first and second codon positions and TIM with empirical base frequencies for the third codon position. For the 18S rDNA, Tamura–Nei with equal base frequencies (TNe) was selected. Statistical support was estimated using 1000 ultrafast bootstrap replicates (Minh *et al.*, 2013) and the Shimodaria–Hasegawa approximate likelihood ratio test (Shimodaria & Hasegawa, 1999; Guindon *et al.*, 2010).

Bayesian inference was performed in MRBAYES v.3.2.6 (Ronquist & Huelsenbeck, 2003). The best-fitting evolutionary substitution models were selected based on those identified using the MODELFINDER algorithm in IQTREE. All parameters, except branch lengths, were unlinked and allowed to vary independently. Two MCMC simulations, each consisting of three heated chains and one cold chain, were set to run until the point of convergence, as determined by an average standard deviation of split frequencies < 0.01. Convergence was also checked in TRACER v.1.6 (Rambaut *et al.*, 2014). The MCMC ran for 20 million generations before stopping, with sampling occurring every 1000 generations. The first 25% of resulting trees were discarded as burn-in, based on convergence determined in TRACER.

RESULTS

SPECIES DELIMITATION

Delimitation performed using four methodologies (GMYC, bPTP, ABGD and ASAP) identified *Sicifera*

cahawba as a separate, well-supported species (confidence > 95%) in all cases (Fig. 1).

MOLECULAR PHYLOGENETIC ANALYSES

All reconstructed phylogenetic trees clearly identify individuals of *Sicifera* as a well-supported clade that is separate from other members of the genus (Fig. 1). *Sicifera cahawba* appears sister to the Ohio River basin species *Sicifera dentata* (Hubricht, 1943) comb. nov. despite these two taxa being separated by an overland distance of ~460 km (Figs 1, 2). In addition, all eastern North American 'synurellids' do not cluster phylogenetically with Palaearctic 'synurellids'; instead, they form a well-supported clade sister to the genera *Crangonyx* and *Amurocrangonyx* Sidorov & Holsinger, 2007 (Fig. 1). In contrast to this '*Crangonyx* clade', the remaining Palaearctic 'synurellids' occur in two other clades of the family, with *Synurella*, *Eosynurella*, *Lyurerlla* and *Palearcticarellus* forming the Palaearctic '*Synurella* clade' and *Diasynurella*, *Volgonyx* and *Pontonyx* allying with *Stygobromus* to form the Nearctic/Palaearctic '*Stygobromus* clade' (Fig. 1).

SYSTEMATICS

ORDER AMPHIPODA LATRIELLE, 1816
SUBORDER SENTICAUDATA LOWRY & MYERS, 2013
INFRAORDER GAMMARIDA LATRIELLE, 1802
PARVORDER CRANGONCTIDIRA BOUSFIELD, 1973
SUPERFAMILY CRANGONYCTOIDEA BOUSFIELD, 1973
FAMILY CRANGONYCTIDAE BOUSFIELD, 1973;
EMENDED BY HOLSINGER, 1977

SICIFERA GEN. NOV.

Zoobank registration: urn:lsid:zoobank. org:act:9E1F37D8-3DF2-458A-B7E7-3A3B43B43738

Type species: Sicifera cahawba sp. nov.

Included species (four): Sicifera bifurca (Hay, 1882) comb. nov., *Sicifera cahawba* sp. nov., *Sicifera chamberlaini* (Ellis, 1941) comb. nov. and *Sicifera dentata* (Hubricht, 1943) comb. nov.

Diagnosis

Medium-sized epigean species, with full eyes and integumentary pigment; interantennal lobe narrow, with rounded upper and lower margins; antenna 1 longer than antenna 2, aesthetascs present on flagellar segments, accessory flagellum two-segmented;
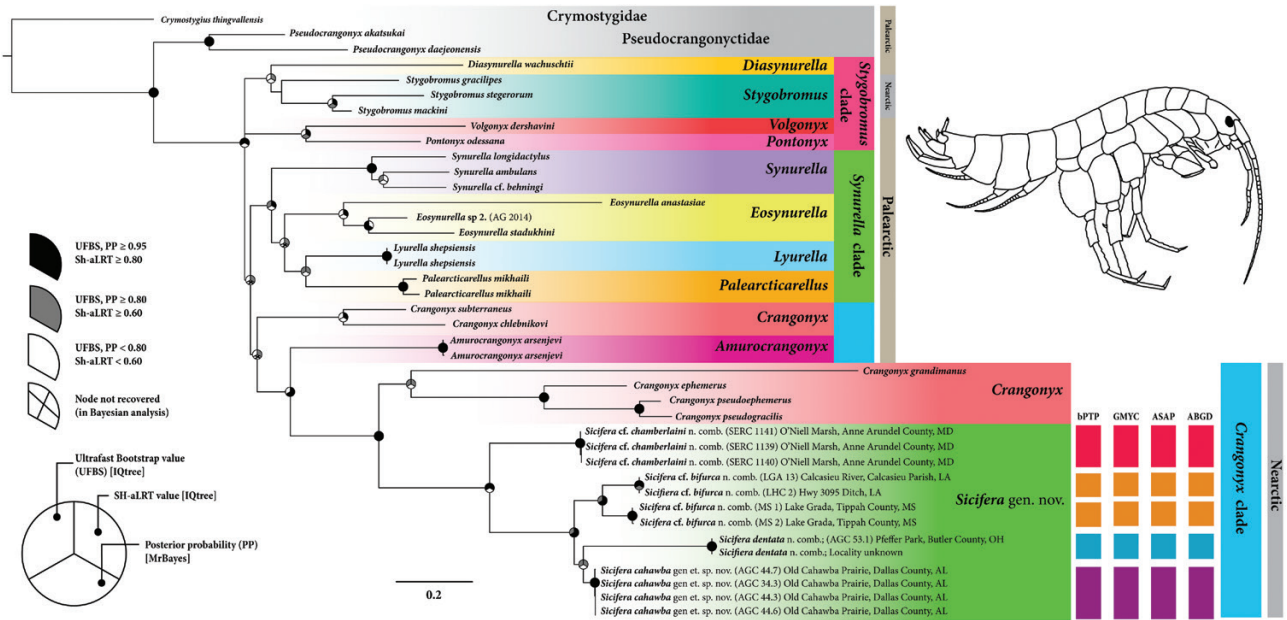
Figure 1. Maximum- likelihood phylogeny acquired from concatenated dataset (18S ribosomal DNA and *COI*) using IQTREE. Node support for each of the three support methodologies used is indicated by shaded circles placed on nodes; weakly supported nodes are not marked. Results of four species delimitation analyses (bPTP, GMYC, ASAP and ABGD) are presented to the right of the tree for members of the genus *Sicifera*. Vertical lines to the right of the tree illustrate both major clades within the family and biogeographical realms occupied by members of the Crangonyctidae. The line illustration depicts a male *Sicifera cahawba*. Abbreviations: ABGD, automatic barcode gap discovery; ASAP, assemble species by automatic partitioning; bPTP, Bayesian Poisson tree processes; GMYC, generalized mixed Yule coalescent; PP, posterior probability; SHaLRT, Shimodaria–Hasegawa approximate likelihood ratio test; UFBS, ultrafast bootstrap. New taxa and new combinations indicated.

antenna 2 of males bearing calceoli on peduncle and flagellum; mandibular molar, incisor and lacinia mobilis well developed, palp three-segmented; maxilla 1 outer plate with seven apical comb-spines; maxilla 2 inner plate with more than five plumose facial setae; propodus of second gnathopod larger than or subequal to first, rastellate setae present or absent on posterior margins of carpus and propodus, propodi anterodistal corners bearing spine-like projections that reduce in size until maturity, palmar margins straight or weakly oblique, armed with multiple robust, bifid setae; coxal plates 1–4 deeper than corresponding somites, longer than broad; pereopod 6 longer than pereopod 7 and much longer than pereopod 5; pereopod dactyli bearing two to five inner marginal setae; subovate coxal gills present on somites 2–6, lanceolate sternal gills present on somites 6 and 7, single pereopod 7 gill present on somite 7; epimera with distinct posterodistal corners, posterior margins with few setae; uronites free or coalesced; uropods 1 and 2 weakly sexually dimorphic; uropod 3 uniramous, ramus one-segmented, shorter than peduncle, with apical robust setae, peduncle often with robust setae; telson of males > 50% cleft-to-base, apices with numerous robust setae, lateral margins bearing plumose setae.

Remarks

Closely allied morphologically with the 'synurellid' genera (*Synurella*, *Eosynurella*, *Diasynurella*, *Volgonyx* and *Pontonyx*), but can be distinguished from these by using the combination of the following characteristics: the presence of spine-like projections on the anterodistal corner of the gnathopod propodi; presence of two to five inner marginal setae on pereopod dactyli; and male telson > 50% cleft-to-base. Differs from the molecularly allied *Amurocrangonyx* and the sympatric *Crangonyx* in the presence of a reduced, uniramous uropod 3; presence of spine-like projections on the anterodistal corners of the gnathopod propodi; and the presence of multiple inner marginal setae on the pereopod dactyli. Differs from other Nearctic genera (*Stygobromus*, *Stygonyx* Bousfield & Holsinger, 1989 and *Bactrurus* Hay, 1902) in possessing pigmented eyes, a uniramous uropod 3 and the presence of spine-like projections on the anterodistal corner of the gnathopod propodi.

Etymology

The genus name *Sicifera* is formed from the Latin *sica* (dagger) and *ferre* (to bear/carry), in reference to the
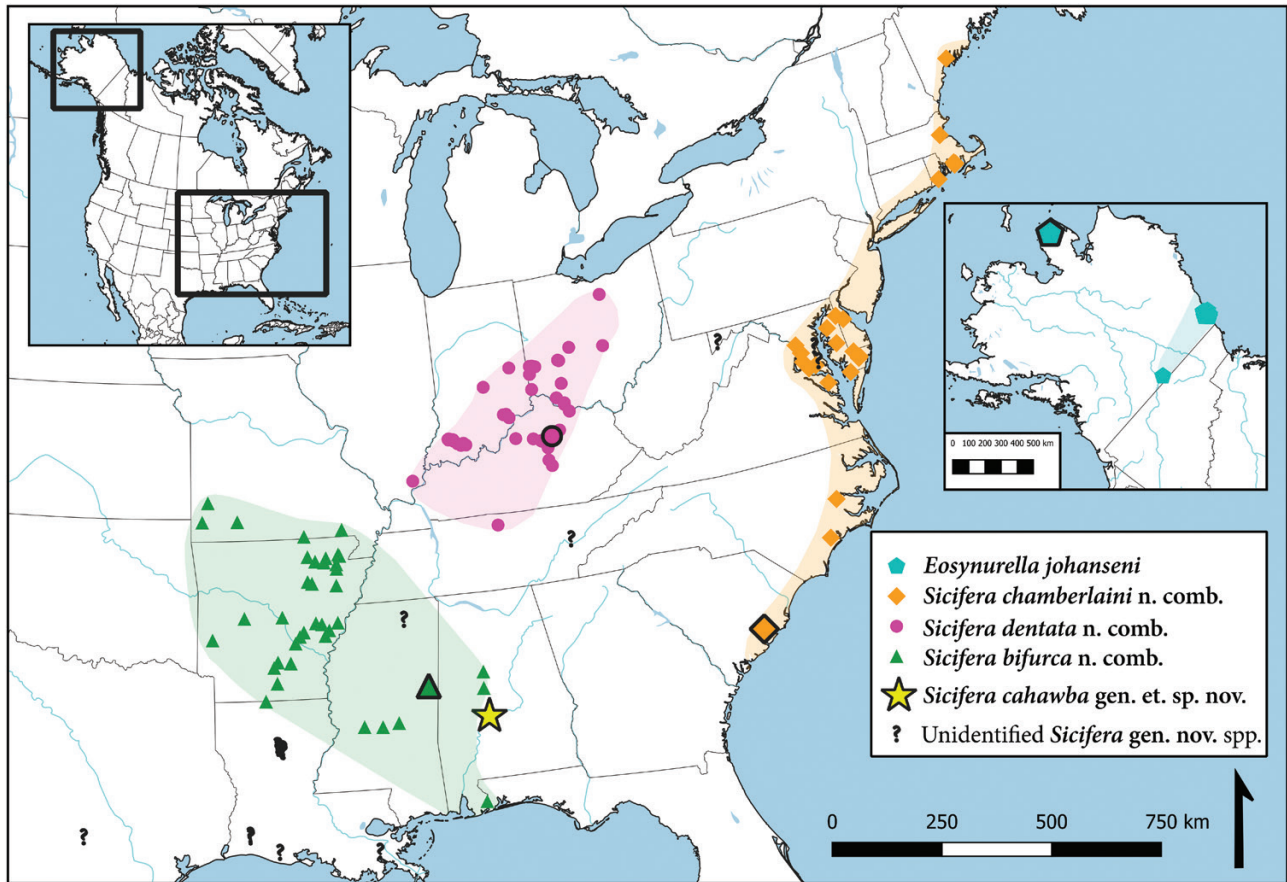
Figure 2. Recorded distribution of 'synurellids' in North America. Symbols with outlines denote type localities for the respective taxa.

characteristic spine-like projections originating from the anterodistal corner of the gnathopod propodi.

SICIFERA CAHAWBA SP. NOV.
(FIGS 3–10)

Zoobank registration: urn:lsid:zoobank.org:act:AF8DF95F-3679-4C14-B754-506892A1CED5

Type material
Holotype, male, 6.38 mm: Old Cahawba Prairie Forever Wild Tract, Dallas County, AL, USA (32.319696, −87.104513); collector: James D. Daniels, 20 February 2020; *USNM 1660542*. Allotype, female, 8.27 mm: Old Cahawba Prairie, Dallas County, AL, USA (32.32048, −87.10624); collector: James D. Daniels, 20 February 2020; USNM 1660543. Paratype female, 5.15 mm: Old Cahawba Prairie Forever Wild Tract, Dallas County, AL, USA (32.319696, −87.104513); collector: James D. Daniels, 28 February 2019; USNM 1660544. Paratypes, three females: Old Cahawba

Prairie Forever Wild Tract, Dallas County, AL, USA (32.319696, −87.104513); collector: James D. Daniels, 20 February 2020; USNM 1660545-47.

Type locality: Old Cahawba Prairie, Dallas County, AL, USA (32.32048, −87.10624).

Etymology
The specific epithet *cahawba* is given in direct reference to the distribution of the species, which is currently endemic to the Old Cahawba Prairie in Dallas County, AL, USA. The name is probably a corruption of two Choctaw words: *oka*, meaning water, and *uba*, above (Owen & Owen, 1921).

Diagnosis
Medium-sized epigean species distinguished from all other members of the genus *Sicifera* by the combination of the following characteristics: maxilla 1 inner plate with up to eight setae; gnathopod propodi rectangular, 1.5–1.7× longer than tall; palmar margins
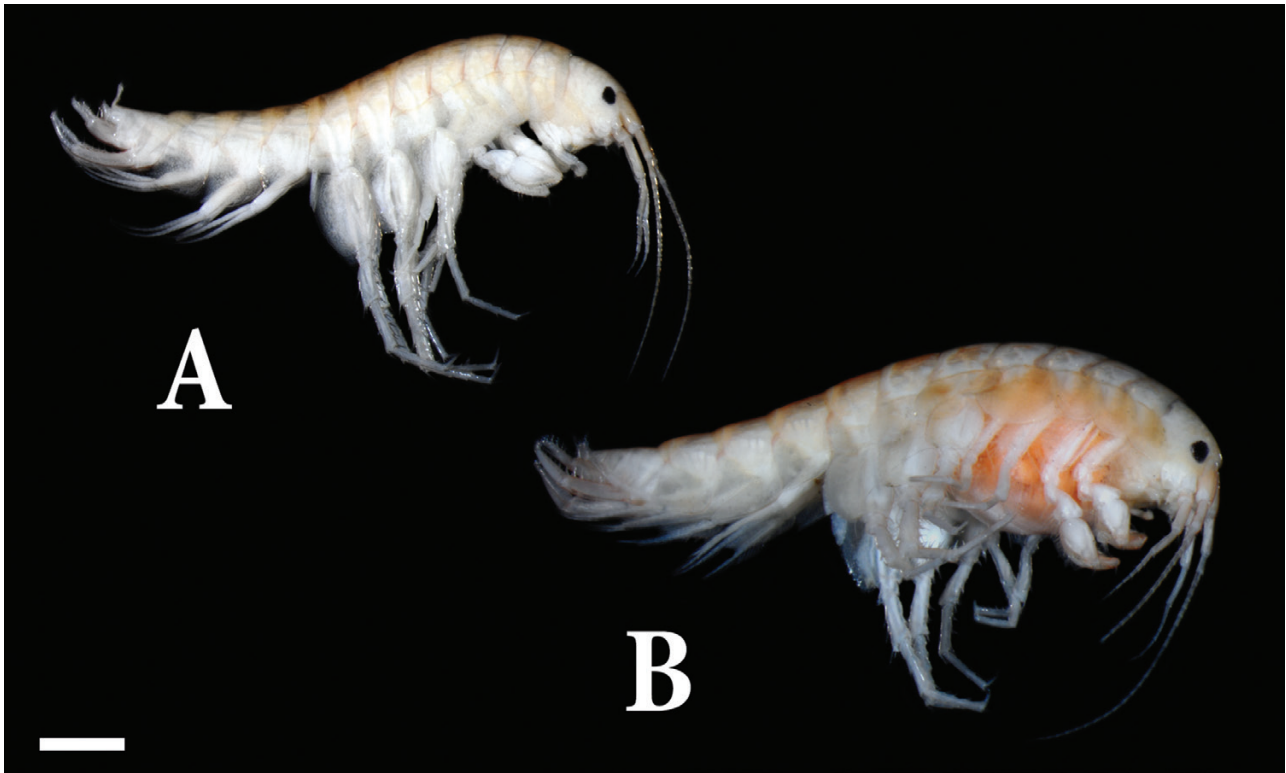
Figure 3. Habitus photographs of *Sicifera cahawba*. A, holotype, male (USNM 1660542), 6.38 mm, Old Cahawba Prairie, Dallas County, AL, USA. B, allotype, female (USNM 1660543), 8.27 mm, Old Cahawba Prairie, Dallas County, AL, USA. Scale bar: 1 mm.

concave, armed with ≤ 19 large robust setae; propodi with superior medial setae singly or doubly inserted; inner margin of gnathopod dactyli of females with six serrations; gnathopod bases of females armed with numerous long setae; uronites fused (not free); telson of male with weakly bifurcate apices, cleft width-to-depth ratio of four, 50% cleft; female telson 40% cleft. Females ≤ 8.5 mm long, males ≤ 6 mm long.

Description: male (*Figs 3–8*)

Length 6.38 mm. Eyes circular to ovate in shape, pigmented (Fig. 3A). Interantennal lobe narrow, with rounded upper and lower margins. Integumentary pigment bluish grey or brown when alive.

Antennae: Antenna 1 (Fig. 4A): 58% body length, 1.9× longer than antenna 2; peduncle segment 1 with three lateral setae, plumose setae absent; primary flagellum with 21 segments, aesthetascs present on distal segments, aesthetascs shorter than respective segments; accessory flagellum two-segmented, shorter than first flagellar segment in length. Antenna 2 (Fig. 4B): gland cone distinct; peduncle 1.5× longer

than flagellum, with one robust seta placed laterally on segment 3 and plumose setae placed on distal margins of segments 4 and 5, peduncular segment 4 subequal in length to segment 5; calceoli present on flagellum and peduncular segments 4 and 5; flagellum eight-segmented.

Mouthparts: Mandibles (Fig. 4C, D): left mandible incisor five-dentate, lacinia mobilis six-dentate, with seven robust and plumose accessory setae; molar process developed with one plumose seta; palp three-segmented, second segment subequal in length to third, with nine alpha setae and three beta setae, outer margin of segment covered in fine setae; third segment rounded distally, inner margin straight, with two C-setae, five E-setae, two A-setae, two B-setae and 11 plumose D-setae, face of segment covered in numerous, fine pubescent setae. Right mandible, incisor three-dentate, lacinia mobilis bifurcate, proximal lobe with numerous fine dentations, distal lobe with four dentations; accessory setae row with six robust and plumose setae; molar process and palp as in left mandible. Upper lip (Fig. 5A): rounded, apical margin of labrum with numerous fine setae. Lower
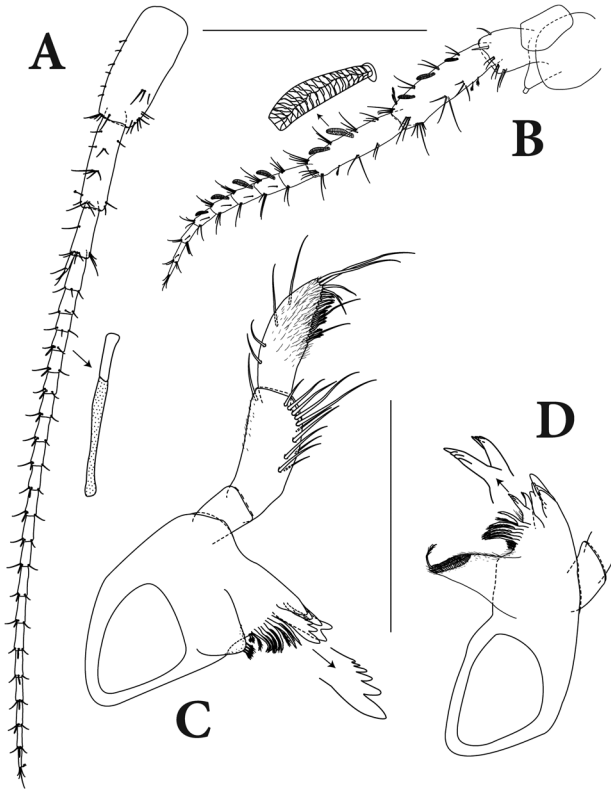
Figure 4. *Sicifera cahawba*; holotype male, Old Cahawba Prairie, Dallas County, Alabama (USNM 1660542), 6.38 mm. A, antenna 1 (single aesthetasc enlarged). B, antenna 2 (single calceolus enlarged). C, left mandible (lacinia mobilis enlarged). D, right mandible (palp omitted, lacinia mobilis enlarged). Scale bars: 1 mm in A, B; 0.5 mm in C, D.

Figure 5. *Sicifera cahawba*; holotype male, Old Cahawba Prairie, Dallas County, AL, USA (USNM 1660542), 6.38 mm. A, upper lip. B, lower lip. C, maxilla 1 (outer plate spine teeth enlarged). D, maxilla 2. E, maxilliped (apical margin of inner plate enlarged). Scale bars: 0.5 mm.

lip (Fig. 5B): inner lobes reduced, outer margin of both inner and outer lobes covered in numerous fine setae; face of lip pubescent. Maxilla 1 (Fig. 5C): inner plate with eight plumose marginal setae and fine pubescence covering entire plate; outer plate with seven apical comb-spines, pubescence covers entire plate, decreasing laterally; palp two-segmented, distal segment covered in pubescence; apical margin of distal segment with three submarginal setae and seven marginal setae. Maxilla 2 (Fig. 5D): both inner and outer plates covered in pubescence; outer plate subequal in length to inner plate, with 23 apical setae; inner plate narrowing slightly distally, with 20 apical setae and nine plumose facial setae. Maxilliped (Fig. 5E): inner plate much shorter than outer plate, with three unarmed spine-teeth along apical margin and five plumose inner marginal setae, surface of plate covered in fine pubescence; outer plate armed with numerous setae, surface of plate covered in fine pubescence; palp four-segmented, second segment with 25 marginal and submarginal setae on inner margin and one distal outer marginal seta, third segment
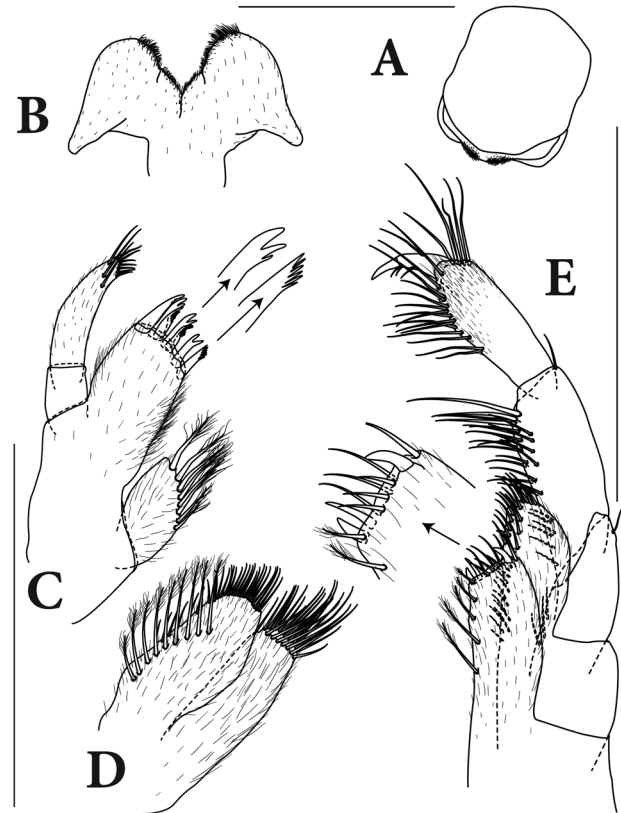
with numerous distal setae, lateral surface pubescent marginally; dactylus with three inner setae.

Gnathopods: Gnathopod 1 (Fig. 6A): coxal plate with seven apical setae and sparse amounts of short facial setae; basis with one anterior and five posterior setae, along with two shorter posterodistal setae, small patches of pubescence are present on posterodistal and posteroproximal corners; ischium with five setae and pubescence along the posterior margin; merus with pubescence covering posterior surface and 15 plumose posterodistal setae; carpus 70% length of propodus, with two anterior setae, six posterior setae and five medial setae; propodus 1.6× longer than broad, with four superior medial setae, six inferior medial setae and five posterior setae, anterodistal margin of propodus ending with small spine-like projection covered by tuft of setae; palm slightly convex, with seven inner robust setae and ten outer robust setae; defining angle armed with four inner and outer robust setae; dactylus inner margin not dentate, outer margin with two setae. Gnathopod 2 (Fig. 6B): coxal plate with seven
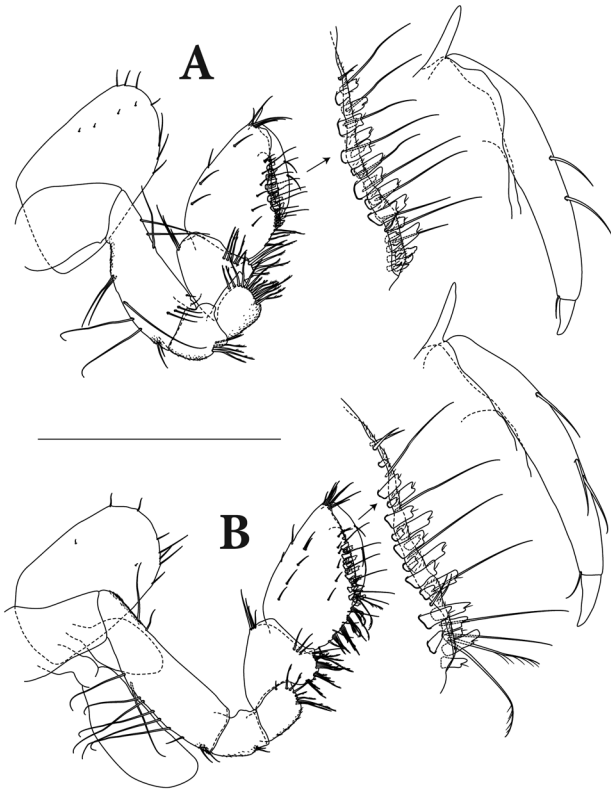
Figure 6. *Sicifera cahawba*; holotype male, Old Cahawba Prairie, Dallas County, AL (USNM 1660542), 6.38 mm. A, gnathopod 1 (palmar margin and dactylus enlarged). B, gnathopod 2 (palmar margin and dactylus enlarged). Scale bar: 1 mm.

Figure 7. *Sicifera cahawba*; holotype male, Old Cahawba Prairie, Dallas County, AL, USA (USNM 1660542), 6.38 mm. A, pereopod 3. B, pereopod 4. C, pereopod 5. D, pereopod 6. E, pereopod 7. Scale bar: 1 mm.

apical setae and sparse amounts of short facial setae; basis with two anterior and five posterior setae, along with two shorter posterodistal setae, a small patch of pubescence is present on posterodistal corner; ischium with two setae and pubescence along the posterior margin; merus with pubescence covering posterior surface and eight plumose posterodistal setae; carpus 55% length of propodus, with four anterior setae, four groups of posterior setae and three medial setae; propodus 1.7× longer than broad, with two anterior setae, five superior medial setae (distal-most paired), six inferior medial setae and four groups of posterior setae, anterodistal margin of propodus ending with a small spine-like projection covered by a tuft of setae; palm slightly convex, with nine inner robust setae and five outer robust setae; defining angle armed with two inner and five outer robust setae; dactylus inner margin not dentate, outer margin with three setae.

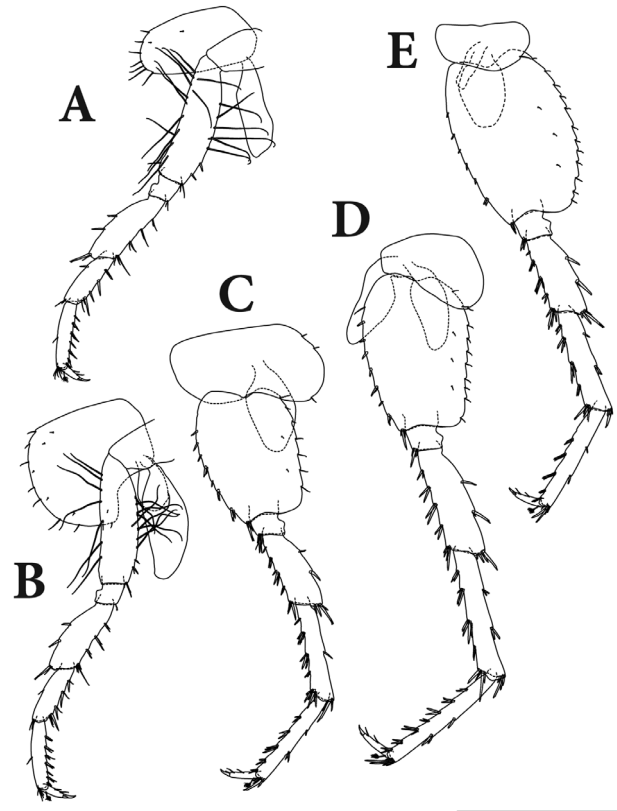*Pereopods:* Pereopod 3 (Fig. 7A): coxal plate with seven apical setae and sparse facial setae; basis with numerous anterior and posterior setae; merus 1.4× longer than carpus, carpus 90% length of propodus; dactylus 42% length of propodus, with one plumose seta on outer margin and two inner marginal setae. Pereopod 4 (Fig. 7B): subequal to pereopod 3 in length; coxal plate 2× longer than broad, with distinct excavation along the posteroproximal margin, armed with nine apical setae and sparse facial setae; merus 1.2× longer than carpus, carpus 90% length of propodus; dactylus ~53% length of propodus, setation like pereopod 3. Pereopod 5 (Fig. 7C): coxal plate large, bilobate, with distinct anterior and posterior lobes, posterior lobe with three setae; basis posterior margin weakly convex with seven shallow serrations and a convex distal corner, anterior margin with seven split-tipped robust setae and three distal setae, face of segment with sparse setae; merus 90% length of carpus; carpus subequal to propodus in length; dactylus 40% length of propodus, setation like other pereopods. Pereopod 6 (Fig. 7D): coxal plate bilobate, with produced posterior lobe, posterior lobe bearing one apical seta; basis posterior margin slightly convex with
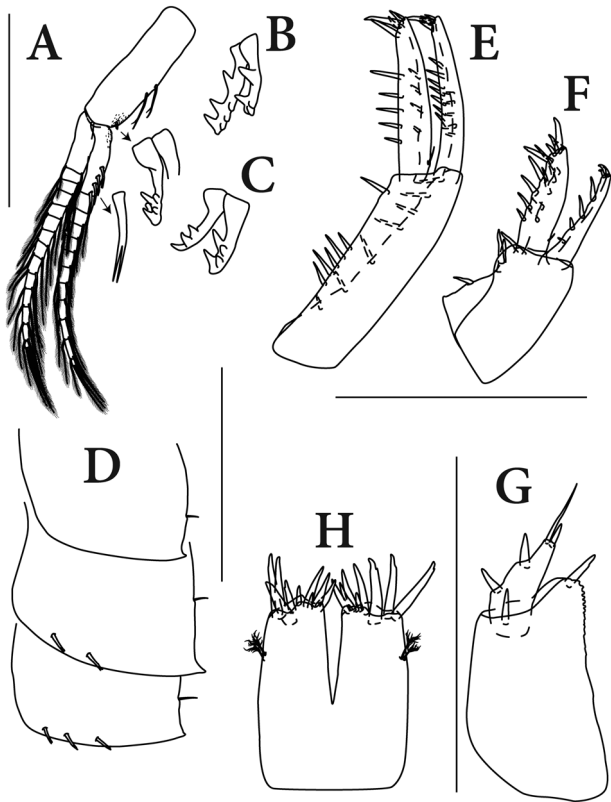
Figure 8. *Sicifera cahawba*; holotype male, Old Cahawba Prairie, Dallas County, AL, USA (USNM 1660542), 6.38 mm. A, pleopod 1 (coupling spines and clothes-pin setae enlarged). B, pleopod 2 coupling spines. C, pleopod 3 coupling spines. D, epimera. E, uropod 1. F, uropod 2. G, uropod 3. H, telson. Scale bars: 1 mm.

ten shallow serrations and a straight distal corner, anterior margin with seven split-tipped robust setae, face of segment with sparse setae; merus 85% length of carpus; carpus 80% length of propodus; dactylus 30% length of propodus, setation like other pereopods. Pereopod 7 (Fig. 7E): coxal plate lobes indistinct, with a single posterior seta; basis posterior margin convex with 12 serrations and a slightly convex distal corner, anterior margin with six split-tipped robust setae and three distal robust setae, face of segment with sparse setae; merus 73% length of propodus; carpus subequal to propodus in length; dactylus 30% length of propodus, setation like other pereopods.

Gills (Figs 6B, 7A–E): Coxal gills present on somites 2–5; somite 7 with pereopod 7 gill subequal in size to coxal gills. Sternal gills present on somite 6.

Pleosome: Second and third segments with one or two setae arising on dorsodistal margins. Pleopods (Fig. 8A–C): peduncle of pleopod 1 55% length of rami, with
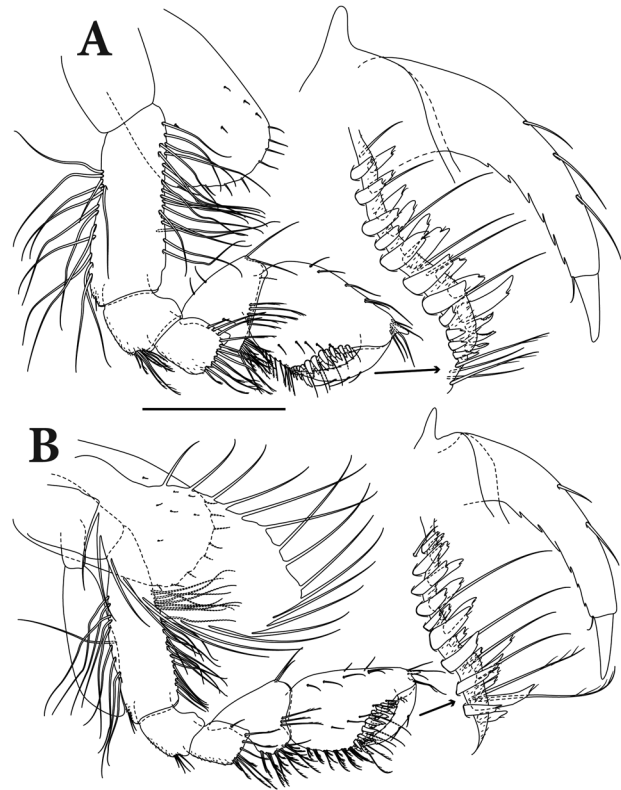

Figure 9. *Sicifera cahawba*; allotype female, Old Cahawba Prairie, Dallas County, AL, USA (USNM 1660543), 8.27 mm. The arrows in this figure associate the enlargement of the palmar margin/dactylus with the respective gnathopod. A, gnathopod 1 (palmar margin and dactylus enlarged). B, gnathopod 2 (palmar margin and dactylus enlarged). Scale bar: 1 mm.

two setae and two coupling hooks; outer and inner rami with 12 and 14 segments, respectively, basal segment of outer ramus with clothes-pin setae. Pleopod 2 peduncle similar to first, with two coupling hooks; outer and inner rami with 12 and 13 segments, respectively. Pleopod 3 peduncle like first and second, with two coupling hooks; outer and inner rami with 12 and 13 segments, respectively. Epimera (Fig. 8D): first epimeron ventral margin unarmed, distoposterior corner with small tooth-like extension, posterior margin with one seta placed proximally from distoposterior corner; second epimeron ventral margin with two robust setae, distoposterior corner with tooth-like extension, posterior margin with one seta; third epimeron ventral margin with three robust setae, distoposterior corner with small tooth-like extension, posterior margin with one seta.

Urosome: Bare dorsally, segments 1–3 fused. Uropod 1 (Fig. 8E): peduncle 1.3× length of rami, with six inner robust setae and five outer robust
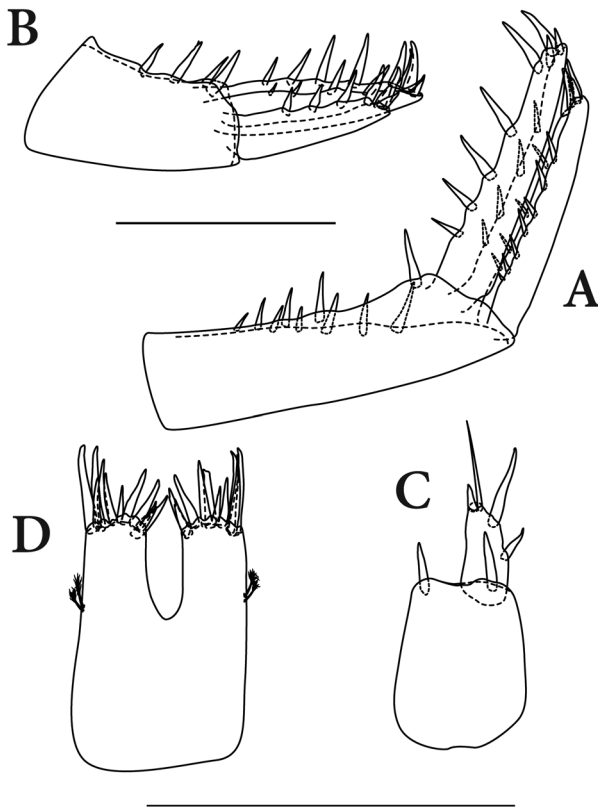
Figure 10. *Sicifera cahawba*; allotype female, Old Cahawba Prairie, Dallas County, AL, USA (USNM 1660543), 8.27 mm. A, uropod 1. B, uropod 2. C, uropod 3. D, telson. Scale bars: 1 mm.

setae; rami narrowing slightly distally, outer ramus subequal in length to inner ramus, with five robust setae on inner margin, seven robust setae on outer margin and five apical robust setae; inner ramus with four robust setae on inner margin, five robust setae on outer margin, five apical setae and one small seta placed proximally on ventral margin. Uropod 2 (Fig. 8F): peduncle 1.3× length of rami, with two inner/outer robust setae; rami not narrowing distally, outer ramus subequal in length to inner ramus, with four robust setae on inner margin, one robust seta on outer margin and three apical robust setae; inner ramus with five inner/outer robust setae, three apical robust setae and one small seta placed proximally on ventral margin. Uropod 3 (Fig. 8G): small, 90% length of telson, uniramous; peduncle 2× the length of ramus, with two apical robust setae, apical corner of peduncle produced with shallow serrations on surface; ramus with two marginal robust setae and two apical setae. Telson (Fig. 8H): quadrate, 1.3× longer than broad, lobes fused, cleft ~50% of length, apices armed with eight or nine large robust setae,

two plumose setae arising dorsolaterally from outer margins of both lobes.

Description: female (Figs 3, 9, 10)
Length 8.27 mm. Differing from male in larger body length; lack of calceoli on peduncle and flagellum of antenna 2; presence of more robust gnathopods with more setae on bases, enlarged propodi, more robust setae on palmar margins, convex palms and dentate inner dactyli margins; uropod 1 setation; uropod 2 setation; uropod 3 structure; and telson shape. Structures not described below are as in male.

Antennae: Antenna 1 (Fig. 3B): ~52% body length, 2.2× longer than antenna 2; primary flagellum with 24 segments; accessory flagellum two-segmented, shorter than first flagellar segment in length. Antenna 2 (Fig. 3B): gland cone distinct, peduncle 1.2× longer than flagellum, calceoli absent on both peduncle and flagellum; flagellum seven-segmented.

Gnathopods: Gnathopod 1 (Fig. 9A): coxal plate with eight apical setae and sparse amounts of short facial setae; basis with numerous anterior and posterior setae, along with two shorter posterodistal setae, and small patches of pubescence are present on posterodistal corner; ischium with eight setae and pubescence along the posterior margin; merus with pubescence covering posterior surface and 13 plumose posterodistal setae; carpus 42% length of propodus, with two anterior setae, ten posterior setae and five medial setae; propodus 1.7× longer than broad, with five superior medial setae, five inferior medial setae and seven posterior setae, anterodistal margin of propodus ending with small spine-like projection covered by tuft of setae; palm distinctly convex, with seven inner robust setae and eight outer robust setae; defining angle armed with four inner and five outer robust setae; dactylus inner margin with six dentations, outer margin with three setae. Gnathopod 2 (Fig. 9B): coxal plate with eight apical setae and sparse amounts of short facial setae; basis with numerous anterior and posterior setae, along with two shorter posterodistal setae, a small patch of pubescence present on posterodistal corner; ischium with four setae and pubescence along the posterior margin; merus with pubescence covering posterior surface and eight plumose posterodistal setae; carpus 70% length of propodus, with two anterior setae, five groups of posterior setae and three medial setae; propodus 1.7× longer than broad, with two anterior setae, five superior medial setae (distal-most paired), five inferior medial setae and six groups of posterior setae, anterodistal margin of propodus ending with small spine-like projection covered by tuft of setae; palm distinctly convex, with eight inner

Table 2. Notable morphological variation observed among individuals of *Sicifera cahawba*

Character	Variability (%)
Ratio of antenna 1 to body length	55–58
♀ Antenna 1 flagellar segments	22–24
♀ Antenna 2 flagellar segments	7–8
Maxilla 1 inner plate facial setae	7–8
Maxilla 2 inner plate facial setae	8–9
Epimeron 2/3 ventral robust setae	2–3
♀ Telson cleft	38–42

robust setae and 11 outer robust setae; defining angle armed with one inner seta and two outer robust setae; dactylus inner margin with six dentations, outer margin with two setae.

Brood plates: (Fig. 9B): large, setaceous brood plates present on somites 2–5, decreasing in size posteriorly.

Urosome: Bare dorsally, segments 1–3 fused. Uropod 1 (Fig. 10A): peduncle 1.4× length of rami, with four inner robust setae and six outer robust setae; rami narrowing slightly distally, outer ramus 95% length of inner ramus, with six robust setae on inner margin, four robust setae on outer margin and four apical robust setae; inner ramus with five robust setae on inner margin, four robust setae on outer margin and four apical setae. Uropod 2 (Fig. 10B): peduncle subequal in length to rami, with three inner and one outer robust seta(e); rami narrowing slightly distally, outer ramus 75% length of inner ramus, with three outer robust setae (inner robust setae lacking) and four apical robust setae; inner ramus with four inner/outer robust setae and four apical robust setae. Uropod 3 (Fig. 10C): small, 95% length of telson, uniramous; peduncle 2× the length of ramus, with two apical robust setae; ramus with two marginal robust setae and two apical setae. Telson (Fig. 10D): quadrate, 1.5× longer than broad, lobes fused, cleft ~40% of length, apices armed with nine or ten large robust setae, two plumose setae arise dorsolaterally from outer margins of both lobes.

Variation
Individuals examined were shown to vary in several morphological characteristics (Table 2).

Distribution and ecology
Sicifera cahawba is currently known only from its type locality in the Old Cahawba Prairie in Dallas County, AL, USA. Specimens were collected from the 1217 ha (3007 acre) Old Cahawba Forever Wild Tract (OCFWT). This area is managed by the Alabama Department of Conservation and Natural Resources (AL-DCNR). The unnamed watercourse where specimens were collected is a first-order stream that drains Blackland Prairie remnants and mixed hardwood forest, emptying into the Cahaba River. During the seasonally heavy rains of winter, sheet flow across the soil surface is common. The watercourse is essentially permanent, with deeper pockets and pools holding water even in the driest months (J.D.D., pers. obs.).

DISCUSSION

Both morphological and molecular analyses reveal *Sicifera cahawba* as a separate species when compared with its congeners. Morphologically, *Sicifera cahawba* appears most similar to *Sicifera dentata* but can be distinguished easily from this species and all others in the genus using several characters, including telson shape and gnathopod shape/armament [see below, Key to species of 'synurellids' (*Eosynurella/Sicifera*) in the Nearctic]. Molecular analyses are congruent with morphological observations, with *Sicifera cahawba* forming a well-supported monophyletic lineage sister to *Sicifera dentata*. These results are curious, given the distributions of these species and others in the genus. Geographically, *Sicifera cahawba* occurs closest to the Mississippi embayment species *Sicifera bifurca*, with the two taxa separated by a distance of ~65 km (Fig. 2). In contrast, *Sicifera cahawba* is separated from its sister-taxon *Sicifera dentata*, which occurs in the Ohio River Basin, by ~460 km (Fig. 2). This result suggests that more complete sampling across eastern North America is required to delineate the ranges of described species in the genus *Sicifera*. Such sampling might also reveal additional undescribed congeners.

Owing to their size, habits and lack of a dispersing larval stage, amphipods are noted for their poor ability to disperse over long ranges, leading to high richness and endemicity among taxa. In the Nearctic, no family exemplifies this better than Crangonyctidae, with > 50 species of *Crangonyx* and 130 species of *Stygobromus* currently recognized (Cannizzaro & Sawicki, 2019; Gibson *et al.*, 2021). Given that amphipods lack taxonomically significant reproductive organs and are prone to display large ranges of morphological variation, morphological identification is difficult even for experts (Cannizzaro *et al.*, 2020). As a result, examination with molecular tools has often uncovered large amounts of cryptic diversity in numerous taxa (Witt & Herbert, 2000; Adams *et al.*, 2018). In the south-eastern USA, this has been demonstrated with *Crangonyx*, the sister

genus of *Sicifera*, where molecular techniques have revealed that formerly wide-ranging taxa, such as *Crangonyx floridanus* Bousfield, 1963, are, in fact, species complexes (Cannizzaro *et al.*, 2019; Cannizzaro & Sawicki, 2019). It is likely that continued analyses of the genus *Sicifera* will also uncover species complexes in its North American range.

Differences between Nearctic and Palaearctic members of the genus *Synurella* have long been discussed. Despite morphological similarity in taxonomically important characters, such as the third uropod, a review of the Crangonyctidae (Holsinger, 1977) noted the unique morphology present in Nearctic 'synurellids', highlighting features such as the spine-like projections on the gnathopods and suggesting possible subgeneric affinity. All eastern/central North American 'synurellids' possess these unique spine-like projections on their gnathopods, which are present in juveniles and reduce in size until adulthood, when they are concealed by the anterodistal setae of the propodi. These spine-like projections have yet to be observed in Palaearctic taxa or any other crangonyctids (Holsinger, 1977). In addition, although not present uniquely in Nearctic 'synurellids', these taxa tend to show at least two setae on the inner margins of pereopods 3–7, and male telsons which are > 50% cleft-to-base, characters which are present in a minority of Palaearctic 'synurellids'.

Our molecular results corroborate the distinctive morphology previously observed, with the Nearctic 'synurellids' forming a separate, well-supported clade, far removed from Palaearctic taxa (Fig. 1). The Nearctic taxa show affinity to members of the genera *Crangonyx* and *Amurocrangonyx*, together forming the '*Crangonyx* clade' in the family. The remaining Palaearctic 'synurellids' align closer to other Palaearctic genera, such as *Lyurella* Dershavin, 1939 and *Palearcticarellus* Palatov & Marin, 2020, forming a separate '*Synurella* clade' (Fig. 1). Similar topologies have also been recovered in other studies examining phylogenetic relationships among crangonyctids (Kornobis *et al.*, 2011; Copilaş-Ciocianu *et al.*, 2019; Marin & Palatov, 2021). In total, our data identify *Synurella* as non-monophyletic, justifying generic-level separation for the Nearctic taxa in order to better capture evolutionary relationships in the family. It is likely that further investigations into the phylogenetic and evolutionary history of the crangonyctids will help to resolve additional polyphyly among genera, particularly for 'Holarctic' genera, such as *Crangonyx* and *Stygobromus*.

With the inclusion of DNA sequence data in taxonomic and systematic analyses, our understanding of evolutionary and biogeographical relationships in the Crangonyctidae has increased substantially, but many features remain poorly understood. The family

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KEY TO SPECIES OF 'SYNURELLIDS' (*EOSYNURELLA*/*SICIFERA*) IN THE NEARCTIC

[Modified from Holsinger (1972, 1977)]

1a. Dactyli of pereopods 3–7 with two to five setae on inner margin(s), male telson > 50% cleft-to-base. Juveniles with spine-like projections on anterodistal corners of gnathopod propodi, which decrease in size until maturity ....…… 2. *Sicifera*

1b. Dactyli of pereopods 3–7 with one or two inner marginal setae, male telson < 50% cleft-to-base. Lacking spine-like projections on anterodistal corners of gnathopod propodi … *Eosynurella johanseni* (Alaska)

2a. Uronites not fused (free) …............................. *Sicifera chamberlaini* (Atlantic Coastal Plain of the USA)

2b. Uronites fused …... 3

3a. Palmar margins of gnathopod propodi straight to weakly convex; male telson apices distinctly bifurcate, cleft width-to-depth ratio of 1.6; inner margin of female gnathopod dactyli lacking teeth … *Sicifera bifurca* (Mississippi Embayment and surrounding regions)

3b. Palmar margins of gnathopod propodi distinctly concave; male telson apices weakly bifurcate, cleft width-to-depth ratio of three to four; inner margins of female gnathopod dactyli dentate ....… 4

4a. Mature males larger than mature females; gnathopods subquadrate, 1.3× longer than tall; female gnathopod bases similar in setation to those of males; telson of female 60% cleft; gnathopods 1 and 2, propodi with triply/doubly inserted superior medial setae; maxilla 1, inner plate with 12 plumose setae …... *Sicifera dentata* (Ohio River Basin)

4b. Mature females larger than mature males; gnathopods rectangular, 1.5–1.7× longer than tall; female gnathopod bases with numerous setae; telson of female 40% cleft; gnathopods 1 and 2, propodi with singly/doubly inserted superior medial setae; maxilla 1, inner plate with eight plumose setae ....…… *Sicifera cahawba* (Old Cahawba Prairie, Dallas County, AL, USA)

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lacks close marine relatives and is thought to have colonized freshwaters before the break-up of Laurasia (Copilaş-Ciocianu *et al.*, 2019). After this, the points of origin and movements of major clades are still poorly resolved (Copilaş-Ciocianu *et al.*, 2019). It is likely that the clade containing Nearctic *Crangonyx* and *Sicifera* originated in what is now North America, as opposed to the clade containing *Synurella* and similar genera, which is likely to have a Palaearctic origin. In addition, the enigmatic Nearctic species *Eosynurella johanseni* is retained in the genus *Eosynurella* owing to its morphological similarity to Palaearctic taxa. Biogeographically, it follows that this taxon would be an extension of the Palaearctic fauna, because Alaska is home to several crustacean taxa with Palaearctic origins. For example, the only native Nearctic member of the mainly Palaearctic genus *Asellus*, *Asellus alaskensis* Bowman & Holmquist, 1975, is endemic to Alaska, with all other genera in the family Asellidae being endemic to the Nearctic; this mirrors the distributions observed with *Eosynurella* and *Sicifera*. Other crangonyctid taxa, such as *Stygobromus*, *Bactrurus* and the Palaearctic *Crangonyx* spp., are harder to place with any confidence owing to taxonomic uncertainty and polyphyly present in these taxa. As relationships among these (and other) taxa are resolved further, improved understanding of the evolutionary and biogeographical history of clades in Crangonyctidae will result.

The age, diversity and distribution of Crangonyctidae make the members of the family excellent model taxa for examining biogeographical hypotheses on both continental and local scales. The family represents one of the richest freshwater crustacean taxa in the Nearctic, with crangonyctids occupying similar areas and showing patterns of diversity similar to other highly diverse Nearctic freshwater taxa, such as the cambarid crayfish and unionoid mussels. Continued analyses of crangonyctid taxonomy, biogeography and evolutionary relationships will demonstrate the utility of these organisms for examining patterns of diversity in freshwater systems. Given that it is likely that hidden endemism in these groups will be uncovered, often from threatened freshwater habitats, many of these species will also be of considerable conservation concern.

ACKNOWLEDGEMENTS

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