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Redescriptions have consequences: biogeographic and conservation insights from the redescription of a widespread freshwater amphipod (Amphipoda, Gammaridae)

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Abstract

Redescriptions are opportunities to “update” species hypotheses based on modern methodology. As such, they can also lead to new insights into biogeography and evolutionary history. We redescribe *Gammarus fasciatus* Say, 1818, a freshwater amphipod from the eastern Nearctic, based on material from near its type locality. Our redescription is supported by both molecular phylogenetic and morphometric analyses. Molecular relationships and shallow genetic divergence indicate a recent expansion of *G. fasciatus* into the interior of the USA, suggesting the potential for anthropogenically aided dispersal. Individuals of *G. fasciatus* split from their closest congeners during the Miocene, likely promoted by embayments present on the Atlantic coastal plain. While molecular analyses suggest little genetic divergence within the species, especially in the northern part of its range, morphometric analyses suggest that populations at the edges of the range are misidentified and likely belong to other species. Based on trends observed within *Gammarus*, it seems plausible that other populations transitioned to freshwater and speciated in the southern USA, also utilizing embayments present during the Miocene. We discuss insights afforded by the redescription of *G. fasciatus* and highlight the utility of such analyses for other taxa and for understanding regional biogeography and evolutionary history.

Key Words

Gammarus fasciatus, *G. fasciatus* complex, *Gammarus tigrinus*, Miocene, Salisbury Embayment

Introduction

Despite over 250 years of study, understanding the scale of Earth’s biodiversity remains elusive. To date, over one million species of eukaryotes have been identified and described, yet estimates suggest that the total number of eukaryotic species likely present on the planet ranges from 8 million (Mora et al. 2011) to over 100 million (Larsen et al. 2017), implying that the vast majority of the world’s species remain undescribed. It is clear that not only is there significant work to be done, but progress has been slow – due in part to the process of species description. Such descriptions are often laborious because they require extensive knowledge of the taxon’s morphology and history, as well as the ability to produce illustrations of that morphology.

An additional factor slowing the taxonomic assessment of species lies in the taxonomic process itself. Names follow the law of priority – the first name proposed for a species (or other taxon) is the one that is retained – and any subsequent names must be synonymized if individuals are revealed to be conspecific (Schenk and McMasters 1948). This law functions to stabilize nomenclature but can create uncertainty if species are described poorly or lack clear type material. This problem is especially amplified when cryptic species are present. As a result, taxonomic assessments cannot proceed without a clear understanding of which species is represented by the type specimen, because new names risk future synonymy. In such cases, the redescription of a species provides great utility, allowing investigators to employ modern methods and understandings to reexamine a type series or freshly collected material from a type locality to avoid confusion regarding identity. While seemingly routine and of low "impact," a redescription can significantly change the view of a species and its relationships with other taxa. For example, analyses of freshwater isopods from the type locality of *Lirceus fontinalis* Rafinesque, 1820 revealed that the species had been confused with the similar *L. richardsonae* Hubricht & Mackin, 1949, and as a result, the distributions of both species were greatly modified (Samsa et al. 2024). Such analyses are particularly needed for the many invertebrate taxa that harbor cryptic species and may have brief or unusable original descriptions.

The genus *Gammarus* Fabricius, 1775 is one of the most speciose amphipod taxa, with nearly 300 described species found throughout the Palearctic and Nearctic (Horton et al. 2024). While members of the genus are known from marine and freshwater habitats in both realms, the great majority of species are Palearctic freshwater species. Owing to this, Palearctic species are well represented in the literature, especially in regions such as western Europe and China, where taxonomic efforts have been focused (Karaman and Pinkster 1977a, 1977b, 1987; Hou and Li 2010; Wattier et al. 2020; Zhang et al. 2022). Conversely, species occupying other regions remain relatively understudied, and much uncertainty still exists in reference to taxonomy, distribution, and ecology. This is especially true in the Nearctic, where at least 17 species of freshwater *Gammarus* have been recorded to date (Horton et al. 2024; Cannizzaro and Berg 2024). A number of these species, particularly those occupying the eastern portion of the realm, have not received much attention outside of their original descriptions and limited treatment in publications targeting amphipod fauna as a whole, many of which were published 40 or more years ago (Bousfield 1958; Holsinger 1972; Cole 1980). A lack of targeted or modern references can lead to unclear distributions and taxonomy, factors that can act as significant roadblocks for understanding the evolution and natural history of a species. This is particularly important because members of the genus *Gammarus* exhibit high levels of endemism and include significant numbers of cryptic species, a trend demonstrated in the western Palearctic (Wattier et al. 2020). It is likely that Nearctic taxa display similar trends, but they have not been examined critically with modern phylogenetic and taxonomic tools.

Gammarus fasciatus Say, 1818, was one of the first members of the genus described from Nearctic freshwaters. Since its original description from Pennsylvania, USA, individuals identified as *G. fasciatus* have been recorded from an area spanning Quebec south to South Carolina, as well as the Great Lakes and parts of the Ohio and Mississippi rivers (Fig. 1). The species is benthic and thought to prefer larger, turbid riverine systems and the upper parts of estuaries, where it is known from salinities of up to 3 parts per thousand (Bousfield 1958; Bousfield 1973). It is also known from lakes, small streams, and occasionally spring runs (Bousfield 1958; Holsinger 1972). However, the extent to which this distribution is natural or represents valid identifications remains unclear. Several factors contribute to these uncertainties. First, it is thought that the occurrences in the Great Lakes and Ohio/Mississippi rivers represent recent introductions, as records of the species in the region do not exist prior to the 1960s (Beckett et al. 1998; Grigorovich et al. 2005). Second, *G. fasciatus* shows significant morphological similarity to other species in the genus, such as *G. tigrinus* Sexton, 1939 in Sexton and Cooper (1939), a euryhaline species with overlapping distribution and habitat requirements. As a result, the two species have often been confused (Bousfield 1958; Grigorovich et al. 2005).

Phylogenetically, *Gammarus fasciatus* belongs to a basally derived clade within the genus that includes euryhaline eastern seaboard species such as *G. tigrinus* and members of the *Gammarus pecos* complex, an assemblage of spring-endemic species from New Mexico and Texas (Cole 1985; Cannizzaro and Berg 2024). Intriguingly, members of both lineages show a proclivity for cryptic diversity and endemism. The *G. pecos* complex has long been recognized for its high degree of endemism, being comprised solely of unique species restricted to single springs or spring systems in the Chihuahuan Desert (Cole 1985; Seidel et al. 2009). However, even within the small geographic ranges occupied by these species, recent phylogenetic analyses have uncovered cryptic species (Adams et al. 2018; Cannizzaro and Berg 2024). Likewise, both *G. tigrinus* and the similar *G. daiberi* Bousfield, 1969 (an Atlantic euryhaline species), have recently been shown to contain unique clades that probably represent undescribed species (Smith et al. 2024). Given the trends observed in other species within the clade and the relatively large range occupied by *G. fasciatus*, it is likely that this species is no exception. In fact, authorities have suggested that populations at the edges of the species range, especially the southern edge, may represent separate species (Holsinger 1972). However, *G. fasciatus* has not been subject to much taxonomic attention, and this has likely been hindered by a poor understanding of the species.

Say's original description of *Gammarus fasciatus* was brief, lacked figures, and is no longer adequate to diagnose the species. This is particularly clear when comparing it with closely related species such as *G. tigrinus* and *G. daiberi*. In order to facilitate future taxonomic work on the species, it needs to be redescribed from its type locality. While no clear type specimens or type locality were established for the species, Say (1818) noted that *G. fasciatus* inhabits "larger streams of fresh water" and was a "common inhabitant of our rivers and is frequently introduced into Philadelphia in the Schuylkill water." Given these statements, it can be reasonably assumed that the material examined by Say was collected from the Schuylkill River as it enters Philadelphia. As such, a redescription should be based on collections as close as possible to that location. No type material of *G. fasciatus* currently exists at the Academy of Natural Sciences of Drexel University; however, two syntype specimens,

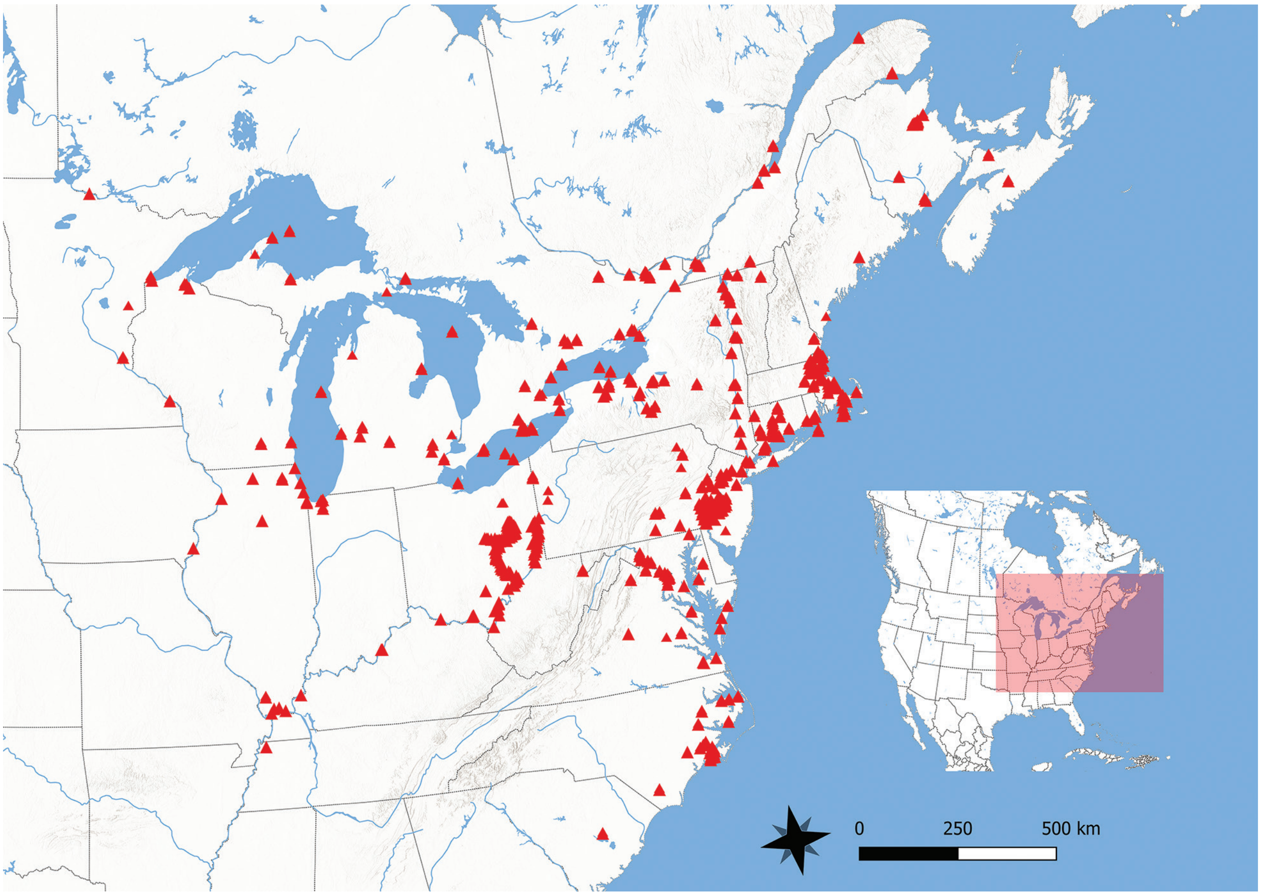

Figure 1. Recorded distribution of *Gammarus fasciatus* sens. lat., including introduced regions in the Laurentian Great Lakes and Mississippi River basins, and records currently identified as *G. fasciatus* from the southern United States, which may represent cryptic or pseudocryptic species. Distributional data presented here were collected from a variety of sources, including personal collections, museum records, literature searches, and iNaturalist observations.

"White 1 178.a-b," collected by Say, exist at the Natural History Museum in London. This material is dried, partly fragmentary, and pin mounted. We redescribe *G. fasciatus* based on freshly collected individuals from the Schuylkill River outside of Philadelphia that serve as topotypes. This redescription is supported by both molecular phylogenetic and morphometric analyses based on museum populations from selected portions of its range. Our results lead to a new understanding of *G. fasciatus* and illustrate the usefulness of species redescriptions for elucidating regional biogeography and reconstructing the evolutionary history of taxa.

Materials and methods

Collection of specimens

Specimens were live-collected using hand nets, preserved in 95% ethanol immediately upon collection, and stored at −20 °C. Topotypic individuals were collected on 6 June 2024 from the Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA (40.04379, −75.2527). Additional individuals were collected on the same day from Spring Creek, Donald R. Taylor Park, Dauphin County, Pennsylvania, USA (40.25893, −86.82501), and on 21 April 2024 from the Ohio River, Gallipolis, Gallia County, Ohio, USA (38.80599, −82.20491).

Morphological analyses

To aid in morphological analysis, specimens were cleared and stained prior to dissection. This process began with digestion in a mixture of 400 µL of molecular-grade water, 360 µL of 2× digestion buffer (Zymo Research, Irvine, CA, USA), and 40 µL of Proteinase K; samples were incubated in this mixture at 37 °C for 2 h and then at room temperature overnight. Once sufficiently digested, specimens were transferred to a solution of Lignin Pink for 2–3 h to stain; after sufficient staining, specimens were transferred to glycerin for dissection using a Nikon SMZ-800 stereomicroscope. The appendages were mounted on temporary glycerin slides to facilitate examination using a Nikon Alphaphot-2 YS2 compound microscope with an attached drawing tube. Body length was determined 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 Cole (1980) and Stock (1974), 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 (Steele and Steele 1991). "Stout setae" on pereopod dactyli refers to setae that are intermediate in robustness relative to robust setae (traditionally referred to as spines) and thinner, more flexible setae (Cannizzaro et al. 2019).

In order to assess morphological variability across the presumed range of *G. fasciatus*, we examined 39 preserved individuals of the species from nine localities housed at the National Museum of Natural History (USNM), Smithsonian Institution (Suppl. material 1). Morphological variation was quantified using five morphometric and 14 meristic characteristics (Suppl. material 2). Measurements were log-transformed before principal component analyses (PCA) were performed using Paleontological Statistics (PAST; Hammer et al. 2001); 95% bootstrap confidence intervals were calculated using 1,000 replicates. To examine variation within populations of *G. fasciatus*, we performed a nonparametric multivariate analysis of variance (PERMANOVA) on Euclidean dissimilarity with 50,000 permutations using PAST.

DNA extraction and sequencing

Genomic DNA (gDNA) was extracted from the digested material prepared during the specimen clearing process. Extractions were performed using QIAGEN DNeasy Blood and Tissue kits (QIAGEN N.V., Hilden, North Rhine-Westphalia, Germany) following the standard protocol after digestion. Extracted DNA was stored at −20 °C and quantified using Qubit fluorometry (Invitrogen, Waltham, MA, USA).

Polymerase chain reactions (PCRs) using primer pairs 18SF/18S1000F and 18S700F/18S1250R (Englisch and Koenemann 2001) amplified ~1000 base pair (bp) segments of nuclear 18S rDNA; 28SF/28S1000R (Hou et al. 2007) amplified ~850 bp of nuclear 28S rDNA; 16StF (Macdonald et al. 2005) and 16SBR (Palumbi et al. 1991) amplified ~350 bp of mitochondrial 16S rDNA; LCO1490/HCO2198 (Folmer et al. 1994) or UCOIF/UCOIR (Costa et al. 2009) amplified ~650 bp segments of mitochondrial cytochrome c oxidase subunit I (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. Reactions were run on a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories). A negative control lacking only gDNA was included in all PCR sets to screen for contamination. The following thermal cycler protocols were followed: 18S, 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 68.5 °C for 30 s, and 72 °C for 2 min, ending with a 10-min final extension at 72 °C; 28S, 95 °C for 5 min, followed by 38 cycles of 94 °C for 30 s, 59 °C for 45 s, and 72 °C for 90 s, ending with a 5-min final extension at 72 °C; 16S, 95 °C for 5 min, followed by 34 cycles of 94 °C for 40 s, 42 °C for 40 s, and 72 °C for 45 s, ending with a 5-min final extension at 72 °C; and COI, 95 °C for 5 min, followed by 38 cycles of 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 45 s, ending with a 5-min final extension at 72 °C.

PCR products were purified using Exo-CIP Rapid PCR Cleanup (New England Biolabs, Ipswich, MA, USA). Sanger sequencing of purified products was performed at Eurofins Genomics (Louisville, KY, USA) using standard methodology. All sequences generated in this study were submitted to GenBank (Table 1).

Phylogenetic analyses

Pairwise sequence alignment was conducted using MUSCLE (Edgar 2004) in Geneious Prime (www.geneious.com) and checked visually. For COI, amino acid translation was used to screen for pseudogenes (indicated by the presence of multiple stop codons). Multiple sequence alignment was conducted using MUSCLE after confirmation. Multiple sequence alignment of nuclear 18S/28S rDNA and mitochondrial 16S rDNA was performed

Table 1. GenBank accession numbers and collection information for individuals sequenced as a part of this project. * Sequence not included due to the presence of pseudogenes.

Catalog #	Species	Locality	Date	Coordinates	Collector	18S Acc. #	28S Acc. #	16S Acc. #	COI Acc. #
AGC-1096.1	*Gammarus fasciatus*	Schuylkill River @ Flat Rock Park, Montgomery County, PA	6/2/2024	40.04379, -75.2527	A.G. Cannizzaro	PX297348	PX297356	PX297352	PX280269
AGC-1096.2	*Gammarus fasciatus*	Schuylkill River @ Flat Rock Park, Montgomery County, PA	6/2/2024	40.04379, -75.2527	A. G. Cannizzaro	PX297349	PX297357	PX297353	PX280268
AGC-1093.1	*Gammarus fasciatus*	Spring Creek, Donald R. Taylor Park, Dauphin County, PA	6/2/2024	40.25893, -76.82501	A.G. Cannizzaro	PX297347	PX297355	PX297351	PX28067
AGC-1065.1	*Gammarus fasciatus*	Ohio River, Gallipolis, Gallia County, OH	4/21/2024	38.80599, -82.20491	A.G. Cannizzaro	PX297346	PX297354	PX297350	*

separately using the same parameters; gaps and indels were retained for these loci. Both markers were concatenated into a final alignment using SequenceMatrix v.1.8 (Vaidya et al. 2011). Median-joining haplotype networks were independently constructed and visualized for both 16S rDNA and COI using default settings in POPART 1.7 (Bandelt et al. 1999; Leigh et al. 2015).

Phylogenetic relationships were reconstructed using both maximum likelihood and Bayesian inference. Maximum likelihood analyses were conducted using IQ-TREE v.2.2 (Minh et al. 2020). The search was run with the ModelFinder algorithm to select the best-fitting substitution models, analyzed under an edge-linked model. The following models were selected: for 18S, a General Time Reversible model with equal/empirical base frequencies and a FreeRate model with four categories (GTR+F+R4); for 28S/16S/COI, a General Time Reversible model with equal/empirical base frequencies, a proportion of invariant sites, and a gamma distribution with four categories (GTR+F+I+G4). Statistical support was estimated using 1,000 ultrafast bootstrap replicates (Minh et al. 2013) and the Shimodaira–Hasegawa approximate likelihood ratio test (Shimodaira and Hasegawa 1999; Guindon et al. 2010). Accession numbers for individuals included in phylogenetic analyses are provided in the supporting information (Suppl. material 3).

Bayesian inference was performed in BEAST v.2.5.2 (Bouckaert et al. 2019) using bModelTest (Bouckaert and Drummond 2017) to select the best-fit substitution model for each partition, with evolutionary models unlinked for each gene. A relaxed log-normal clock was applied to each partition, and the tree prior was set to a birth–death model. Divergence times were estimated using a calibration scheme similar to that used in an analysis of *Gammarus lacustris* (Hou et al. 2022). The divergence time of the genus *Niphargus* from the genera *Microniphargus* and *Pseudoniphargus* was set to a log-normal distribution (mean 45, sigma 0.5, offset 35; 95% range 58.5–38.3 Ma) based on previous age estimates (Jażdżewski and Kuprjanowicz 2010). The root of the family Gammaridae was set to a uniform distribution (110–40 Ma) based on maximum (Hou et al. 2014) and minimum (Copilaş-Ciocianu et al. 2020) estimates determined for the family. The common ancestor of the members of the Lake Ohrid *Gammarus* clade was set to a normal distribution (mean 1.2, sigma 0.079; 95% range 1.36–1.05 Ma) based on previous divergence estimates (Mamos et al. 2016). The divergence between clades of the North American estuarine species *G. tigrinus* was set to a log-normal distribution (mean 3.0, sigma 0.5, offset 1.5; 95% range 7.5–2.6 Ma) based on the formation of the Labrador Current (Kelly et al. 2006) and supported by previous divergence estimates (Smith et al. 2024). Markov chain Monte Carlo (MCMC) simulations were run for 50 million generations, sampling every 1,000 generations. Convergence and effective sample size (ESS) were checked using Tracer v.1.7 (Rambaut et al. 2018). The first 25% of trees were discarded as burn-in based on parameter estimates determined in Tracer, and the final tree was summarized using TreeAnnotator v.1.8.1 in BEAST.

Systematics

Order Amphipoda Latrielle, 1816
Suborder Senticaudata Lowry & Myers, 2013
Infraorder Gammarida Latreille, 1802
Parvorder Gammaridira Latreille, 1802
Superfamily Gammaroidea Latreille, 1802
Family Gammaridae Latreille, 1802
Genus *Gammarus* Fabricius, 1775

Gammarus fasciatus Say, 1818

Figs 2–8

Gammarus fasciatus Say, 1818 p. 874; Smith, 1874 p. 653; Weckel, 1907 p. 48; Huntsman, 1911 p. 151; Pentland, 1930; Hubricht & Mackin, 1940 p. 193; Hubricht, 1943 p. 6; Clemens, 1950; Bousfield, 1958 p. 69; Cole, 1970 p. 338; Cole, 1980 p. 72; Bousfield, 1973 p. 53; Holsinger, 1972 p. 21. *Gammarus* nr. *fasciatus* Beckett, 1998. *Gammarus* sp. Nicholson, 1873, p. 500.

Material examined. USA • topotype male, 13. 12 mm; Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania; 40.04379°N, 75.2527°W; 6 June 2024; A. G. Cannizzaro leg.; USNM 1694137 (AGC-1096.1) • topotype female, 5.91 mm; same data as for topotype male; USNM 1694138 (1096.2) • 3 topotype males; same data as for topotype male; USNM 1694139 • 3 topotype males; same data as for topotype male; USNM 1694140.

Additional material examined. USA • 3 males; Ohio River, Gallipolis, Gallia County, Ohio; 38.80599°N, 82.20491°W; 21 April 2024; A. G. Cannizzaro leg.; USNM 1694140 (AGC-1065 series) • 2 males; Spring Creek, Donald R. Taylor Park, Dauphin County, Pennsylvania; 40.25893°N, 86.82501°W; A. G. Cannizzaro leg; USNM 1694141 (AGC-1093 series) • 3 males, 2 females; Lake Superior, Ashland, Ashland County, Wisconsin; 26 June 1957; E. L. Bousfield leg.; USNM 191207 • 2 males, 2 females; Lake Champlain, Burlington, Chittenden County, Vermont; 44.75°N, 73.33°W; 19 July 1956; E. L. Bousfield leg.; USNM 191214 • 5 males; Grand Rapids, Kent County, Michigan; 11 September 1909; R. C. Coker leg.; USNM 41754. CANADA • 4 females, 1 male; St. Lawrence River, Beauharnois Power Dam Outlet, Montérégie, Quebec; 1 July 1968; R. K. Lee leg.; USNM 191209.

Diagnosis. distinctly banded, setiferous species; antenna 1 0.9–1.5× length of antenna 2; antenna 2 setiferous, lacking calceoli in males; mandibular palp with C-setae on third segment, C-setae 2–2.5× D-setae in length; mandibular palp α-setae inserted parallel to margin; B-setae on mandibular palp longer than A-setae; coxal plates 1–4 with numerous long facial setae; pereopods setiferous, pereopod 7 basis posterior margin convex, posterior setae 35–40% length of basis width; second and third epimera lacking or with limited robust setae, facial surface of epimeron 2 and ventral margin of epimera 2+3 with long setae; uronites distinctly humped; uropod 3 of male inner ramus 75–80% length of outer ramus.

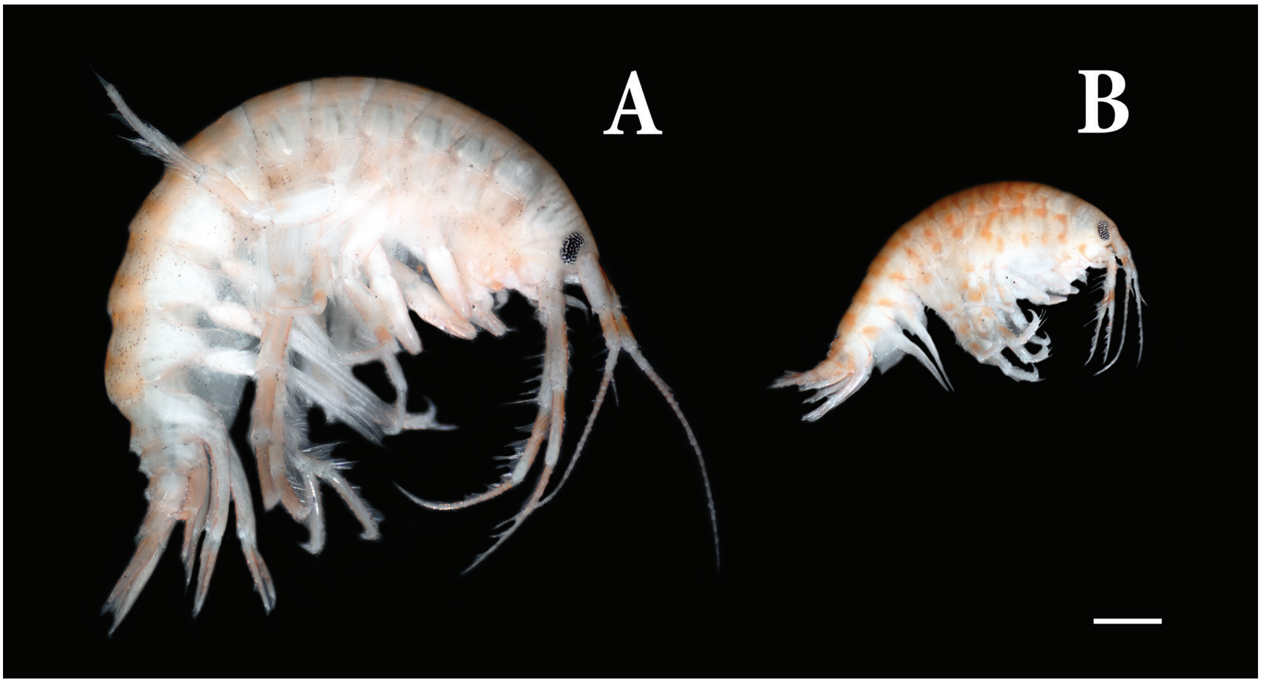

Figure 2. Habitus images of *Gammarus fasciatus*, **A.** Topotype male (USNM 1694137), 13.12 mm, Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA; **B.** Topotype female (USNM 1694138), 5.91 mm, Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA. Scale bar: 1 mm.

Description. Male: 13.12 mm in length. Eyes reniform, integumentary pigment present when alive and in freshly preserved specimens (Fig. 2A).

Antennae. Antenna 1 (Fig. 3A): approximately 40% body length, subequal in length to antenna 2; peduncle segment 1 with 1 marginal ventral seta and 3 robust setae on distoventral corner; peduncle segment 2 with 3 clusters of marginal ventral setae; primary flagellum with 23 segments, aesthetascs present on all but proximal segments, aesthetascs shorter than respective segments; accessory flagellum with 5 segments, approximately 20% length of primary flagellum. Antenna 2 (Fig. 3B): gland cone developed; peduncle approximately 1.4× length of flagellum, peduncular segment 4 with 6 clusters of ventral setae, 6 clusters of lateral setae, and 6 clusters of submarginal ventral setae; peduncular segment 5 subequal in length to segment 4 with 6 clusters of submarginal ventral setae and 5 clusters of dorsal setae, and an additional 3 clusters of submarginal setae inserted laterally near the dorsal margin; flagellum with 9 segments, dorsal and ventral setae decreasing in length distally, all segments lacking calceoli.

Mouthparts. Left mandible (Fig. 3C): incisor 5-dentate, lacinia mobilis 4-dentate; accessory setae row with 5 plumose setae; molar process well developed, cylindrical triturative with plumose seta; palp 3-segmented, second segment approximately 1.2× length of third segment, with 7 α-setae, 6 β-setae, and 6 γ-setae; third segment weakly rounded distally, inner margin straight, with 8 A-setae set in two rows, 3 B-setae, 2 C-setae, 18 D-setae, and 5 E-setae; E-setae 1.25× length of longest α-seta, C-setae 2× D-setae in length, longest A-seta approximately 60% length of B-setae; distal face of segment covered in numerous fine pubescent setules. Right mandible (Fig. 3D): incisor 4-dentate, lacinia mobilis bifurcate, lobes with numerous fine dentations; accessory setae row with 6 plumose setae; molar similar in form to left mandible, palp as in left mandible. Upper lip (Fig. 4A): rounded, apical margin of labrum with numerous setules. Lower lip (Fig. 4B): inner lobes indistinct, outer margin of inner, outer lobes covered in setules; face of lip covered in pubescent setules. Maxilla 1 (Fig. 4C, D): inner plate with 13 pappose setae and numerous fine pubescent setules covering entire plate; outer plate with 11 apical serrate setae, pubescent setules covering plate, decreasing laterally and proximally; right palp with 2 segments, subapical margin of distal segment with 1 long seta, apical margin with 6 robust setae; left palp with 2 segments, subapical margin of distal segment with 3 long setae, apical margin with 7 robust setae, weaker than those of left palp. Maxilla 2 (Fig. 4E): both inner and outer plates covered in pubescent setules; outer plate approximately 1.1× length of inner plate, with numerous apical setae; inner plate narrowing slightly distally with numerous apical setae and 12 pappose facial setae. Maxilliped (Fig. 4F): inner plate shorter than outer plate, apical margin with 3 naked cuspidate setae, and an additional naked cuspidate seta inserted toward inner margin, surface of plate covered in pubescent setules; outer plate armed with numerous naked cuspidate setae covering inner margin along with setae inserted submarginally, apical margin with 5 plumose setae, increasing in robustness towards inner margin; palp second segment with numerous marginal setae, third segment with numerous marginal/submarginal setae, apical

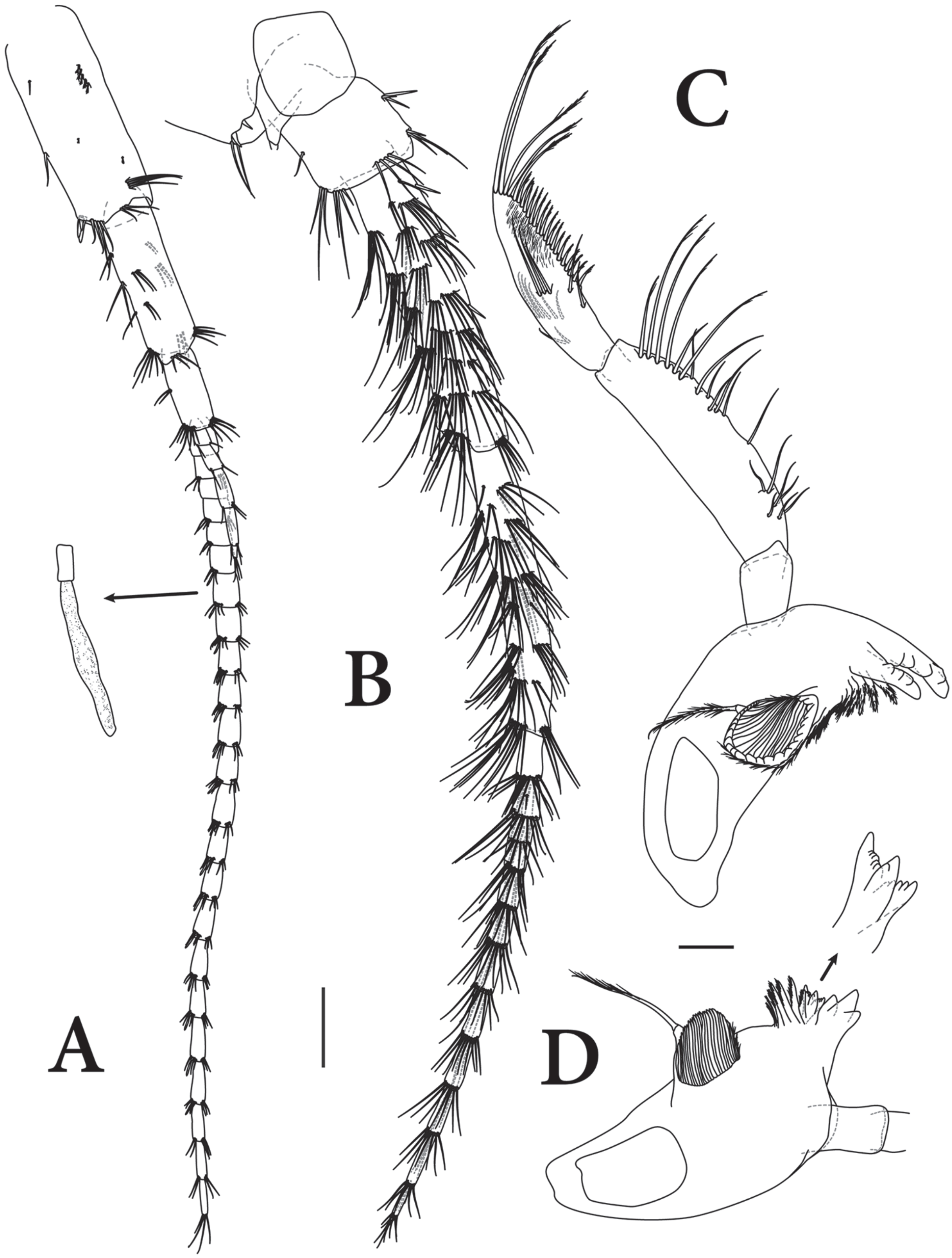

Figure 3. *Gammarus fasciatus* topotype male, 13.12 mm (USNM 1694137), Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA. **A**. Antenna 1 (aesthetasc enlarged); **B.** Antenna 2; **C.** Left mandible; **D.** Right mandible (palp omitted, lacinia mobilis enlarged). Scale bars: 0.25 mm **(A, B)**, 0.1 mm **(C, D)**.

margin with robust serrate setae, distal surface of segment with pubescent setules; dactylus with numerous setae lining inner margin.

Gnathopods. Gnathopod 1 (Fig. 5A): coxal plate with 5 anterior setae, 3 posterior setae, and 4 facial setae, additional smaller, submarginal setae line margin of plate;

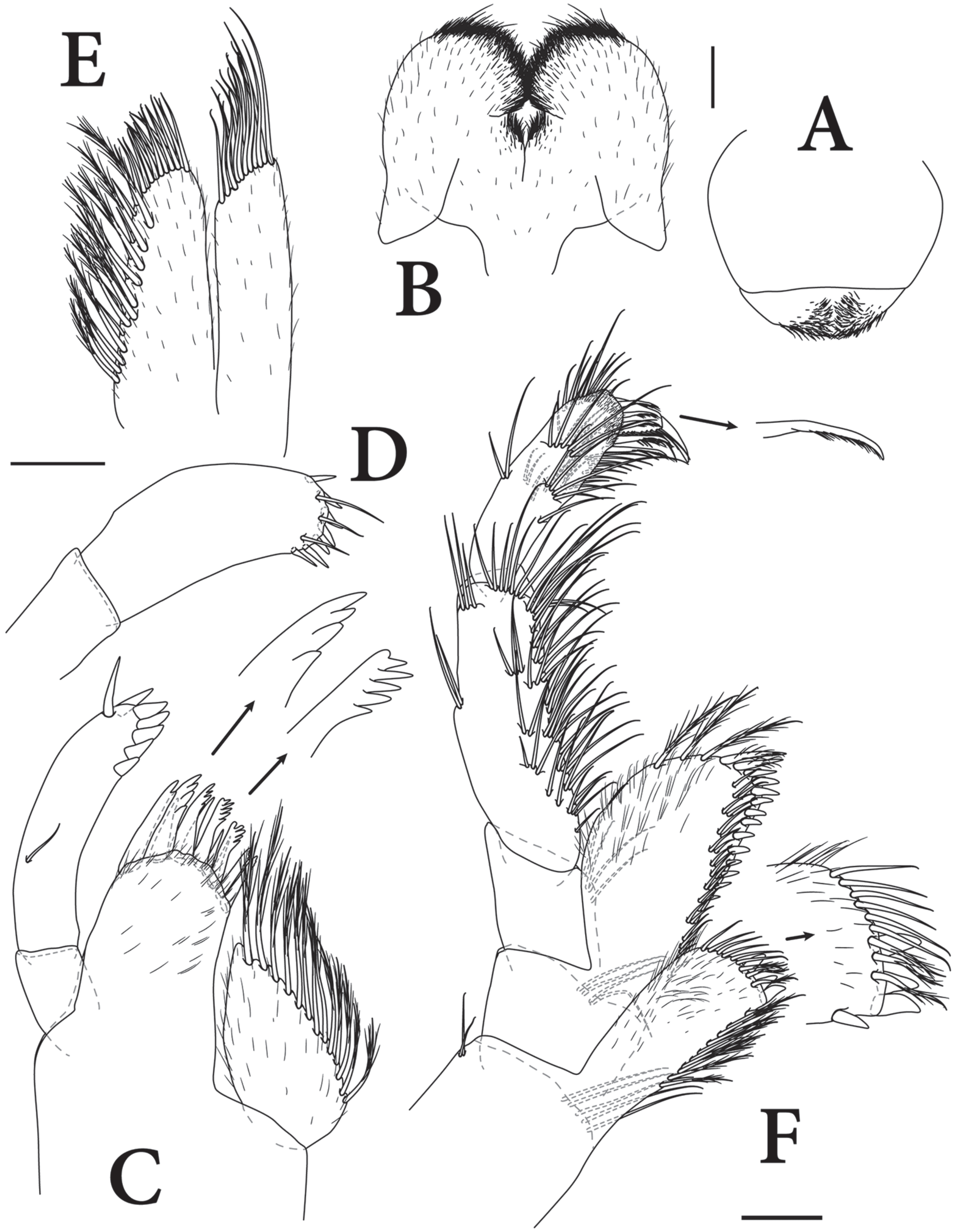

Figure 4. *Gammarus fasciatus* topotype male, 13.12 mm (USNM 1694137), Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA. **A**. Upper lip; **B.** Lower lip; **C.** Maxilla 1, left (outer plate apical serrate setae enlarged); **D.** Maxilla 1, right palp; **E.** Maxilla 2; **F.** Maxilliped (apical margin of inner plate enlarged). Scale bars: 0.1 mm.

basis with numerous long setae inserted along anterior and posterior margins, robust, serrate setae on posterodistal corner; ischium with tuft and pubescent setules along posterior margin; merus with 2 anterior setae, setae lining posterior and distal margins, posterior surface of segment with weak pubescent setules; carpus approximately

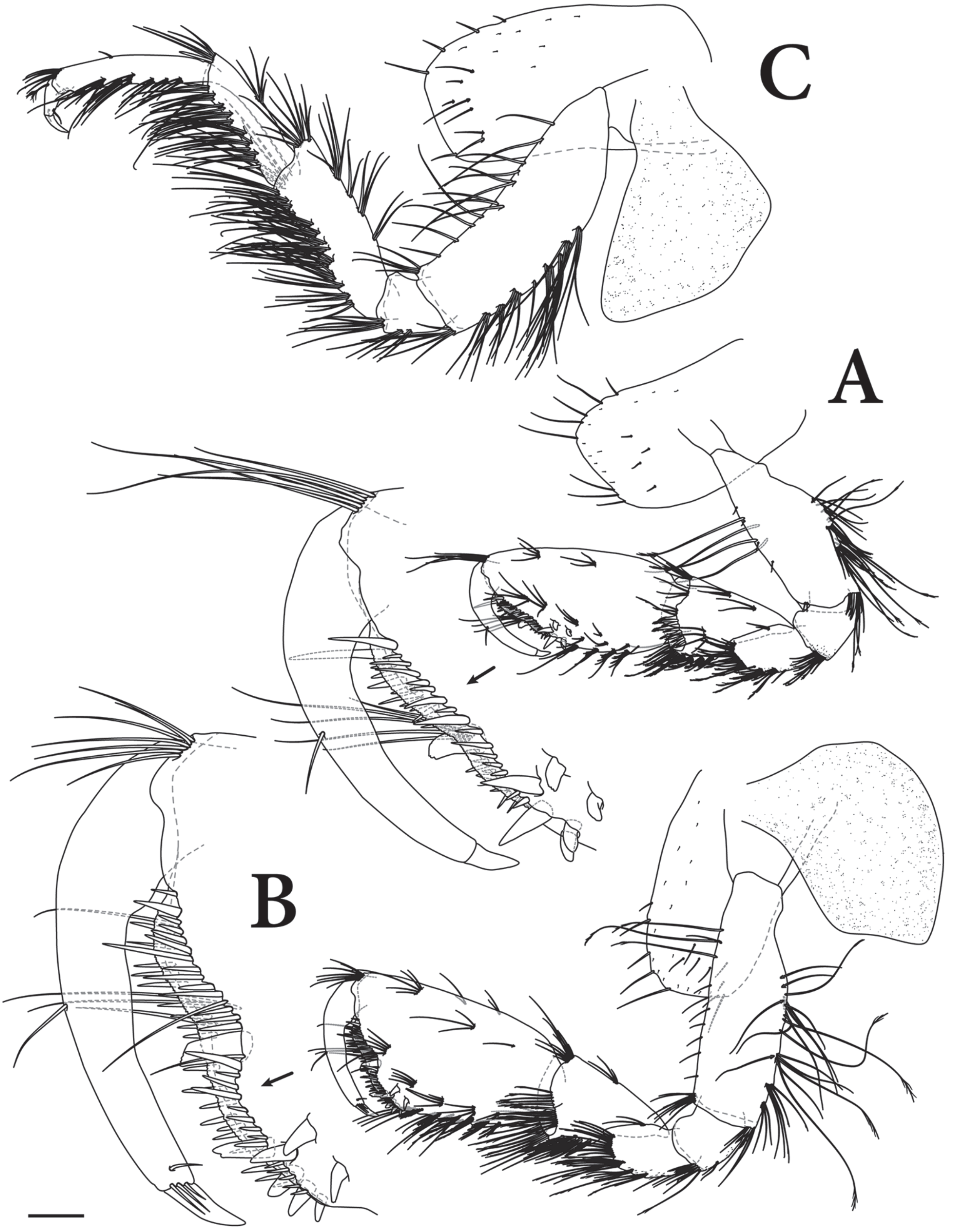

Figure 5. *Gammarus fasciatus* topotype male, 13.12 mm (USNM 1694137), Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA. **A**. Gnathopod 1 (palmar margin and dactylus enlarged); **B.** Gnathopod 2 (palmar margin and dactylus enlarged; **C.** Pereopod 3. Scale bar: 0.25 mm.

55% length of propodus with 3 tufts of setae along anterior margin, posterior margin with numerous setae and 4 submarginal setae directed distally, distal margin with robust, plumose setae; propodus 2× longer than broad, with 2 tufts of superior medial setae, 5 anterodistal setae, 7 tufts of inferior medial setae, proximal most singly or doubly inserted, and 4 tufts of posterior setae; palm oblique, highly concave with large, robust setae placed medially along margin and numerous inner/outer marginal setae; defining angle with 6 robust setae; dactylus with outer seta, inner setae lacking. Gnathopod 2 (Fig. 6B): coxal plate with 5 anterior setae, 3 posterior setae, 6 facial setae, and additional smaller, submarginal setae lining margin of plate; basis with numerous long setae inserted along posterior margin, anterior margin with long setae proximally and shorter setae distally; ischium with 2 tufts of posterior setae and sparse pubescent setules along posterior margin; merus with 2 anterior setae, setae lining posterior and distal margins, posterior surface of segment with weak pubescent setules; carpus approximately 50% length of propodus with 2 tufts of setae along anterior margin, posterior margin with numerous setae, distal margin with plumose setae; propodus 2× longer than broad, with 3 tufts of anterior setae, 7 anterodistal setae, 2 tufts of superior medial setae with proximal-most singly inserted, 4 tufts of inferior medial setae, and 6 tufts of posterior setae; palm oblique, concave with large, robust setae placed medially along margin and numerous inner/outer marginal setae; defining angle with 7 robust setae; dactylus with outer seta and 4 inner setae.

Pereopods. Pereopod 3 (Fig. 5C): coxal plate with 4 anterior setae, 1 posterior seta, 7 facial setae, and additional smaller, submarginal setae line margin of plate; basis with numerous long setae inserted along anterior and posterior margins; merus approximately 1.5× longer than carpus, posterior margin heavily setose; carpus approximately 75% length of propodus, posterior margin heavily setose; dactylus approximately 45% length of propodus, with plumose seta proximally on anterior margin, stout seta placed distally along posterior margin, proximal to nail, and an additional seta placed medial near base of nail. Pereopod 4 (Fig. 6A): approximately 90% of the length of pereopod 3, coxal plate with 4 anterior setae, 7 posterior setae, 11 facial setae, and additional smaller submarginal setae line plate, plate with distinct posteroproximal excavation; basis with numerous long setae inserted along anterior and posterior margins; merus approximately 1.5× longer than carpus, posterior margin heavily setose; carpus approximately 70% length of propodus, posterior margin heavily setose; dactylus approximately 50% length of propodus, setation as in pereopod 3. Pereopod 5 (Fig. 6B): coxal plate large, bilobate with distinct anterior and posterior lobes, anterior lobe with a seta, posterior lobe with 3 setae; basis posterior margin convex with 11 serrations bearing setae, posterior face of segment with 5 long setae, distoposterior margin without free lobe and nearly fused to ischium, anterior margin with 6 groups of robust setae and 7 anteroproximal setae, anterior face of segment with 8 long setae; merus subequal in length to carpus; carpus approximately 80% length of propodus; dactylus approximately 30% length of propodus, setation as in other pereopods. Pereopod 6 (Fig. 6C): coxal plate weakly bilobate, posterior lobe with 3 setae, increasing in length distally; basis posterior margin weakly convex with 13 shallow serrations bearing setae, posterior face of segment with 9 long setae, distoposterior margin without free lobe and nearly fused to ischium, junction with robust seta and 2 setae, anterior margin with 5 groups of robust setae and 7 anteroproximal setae; merus subequal in length to carpus, densely setose; carpus subequal in length to propodus, densely setose, some setae slightly curled; dactylus approximately 25% length of propodus, setation as in other pereopods. Pereopod 7 (Fig. 6D): coxal plate small, lobes indistinct, with 5 posterior setae; basis posterior margin convex, with 15 shallow serrations bearing long setae, seta length increasing distally, longest posterior seta 40% width of segment, face of segment with 28 long setae, distoposterior margin without free lobe, nearly fused to ischium, junction with robust seta and a tuft of setae, anterior margin with 7 robust setae, and 4 anteroproximal setae; merus approximately 80% length of carpus, densely setose; carpus subequal in length to propodus, densely setose, some setae slightly curled; dactylus approximately 25% length of propodus, setation as in other pereopods.

Gills (Figs 5B, C, 6A–D): coxal gills on somites 2–6, somite 7 with pereopod 7 gill.

Pleon. Pleopod 1 (Fig. 6E): peduncle with 3 outer setae, 7 inner setae, and 3 coupling hooks; outer, inner rami with 19 and 16 segments, respectively. Pleopod 2 (Fig. 6F): peduncle with 14 outer setae, 18 inner setae, and 3 coupling hooks; outer, inner rami with 17 and 14 segments, respectively. Pleopod 3 (Fig. 6G): peduncle with numerous setae and 3–4 coupling hooks; outer, inner rami with 16 and 12 segments, respectively. Epimera (Fig. 7A): first epimeron anterior margin with 6 setae, ventral margin straight, distoposterior corner not produced, posterior margin with 4 short setae; second epimeron anterior margin with 4 setae, ventral margin with 4 setae, 10 additional setae are inserted facially in an oblique row, distoposterior corner produced, posterior margin with 4 setae; third epimeron anterior margin with 8 setae, ventral margin with 7 setae, distoposterior corner produced, posterior margin with 4 setae.

Urosome. Uronites (Fig. 7B): first and second uronites strongly humped dorsally; dorsomedial surface of first and second uronites with 3–4 robust setae and 3–6 setae; dorsolateral margins of first and second uronites with a pair of 2–3 robust setae and 2–3 setae; third uronite weakly humped, with 3 robust setae and 1 seta dorsomedially, and 2–3 robust setae and 2–3 setae dorsolaterally. Uropod 1 (Fig. 7C): peduncle 2.4× rami in length with 8 outer robust setae, 5 inner robust setae, and 2 anteroproximal robust setae; outer ramus subequal in length to inner, with 3 marginal robust setae, 4 apical robust setae, and 1 anteroproximal seta; inner ramus with 2 outer robust setae, 3 inner robust setae, and 5 apical robust setae. Uropod 2 (Fig. 7D): peduncle approximately 85% length of outer ramus, with 2 outer robust setae and 4 inner robust setae; outer ramus approximately 1.3× inner ramus in length, with 3 outer robust setae and 5 apical robust setae; inner ramus with 1 inner robust seta, 1 outer robust seta,

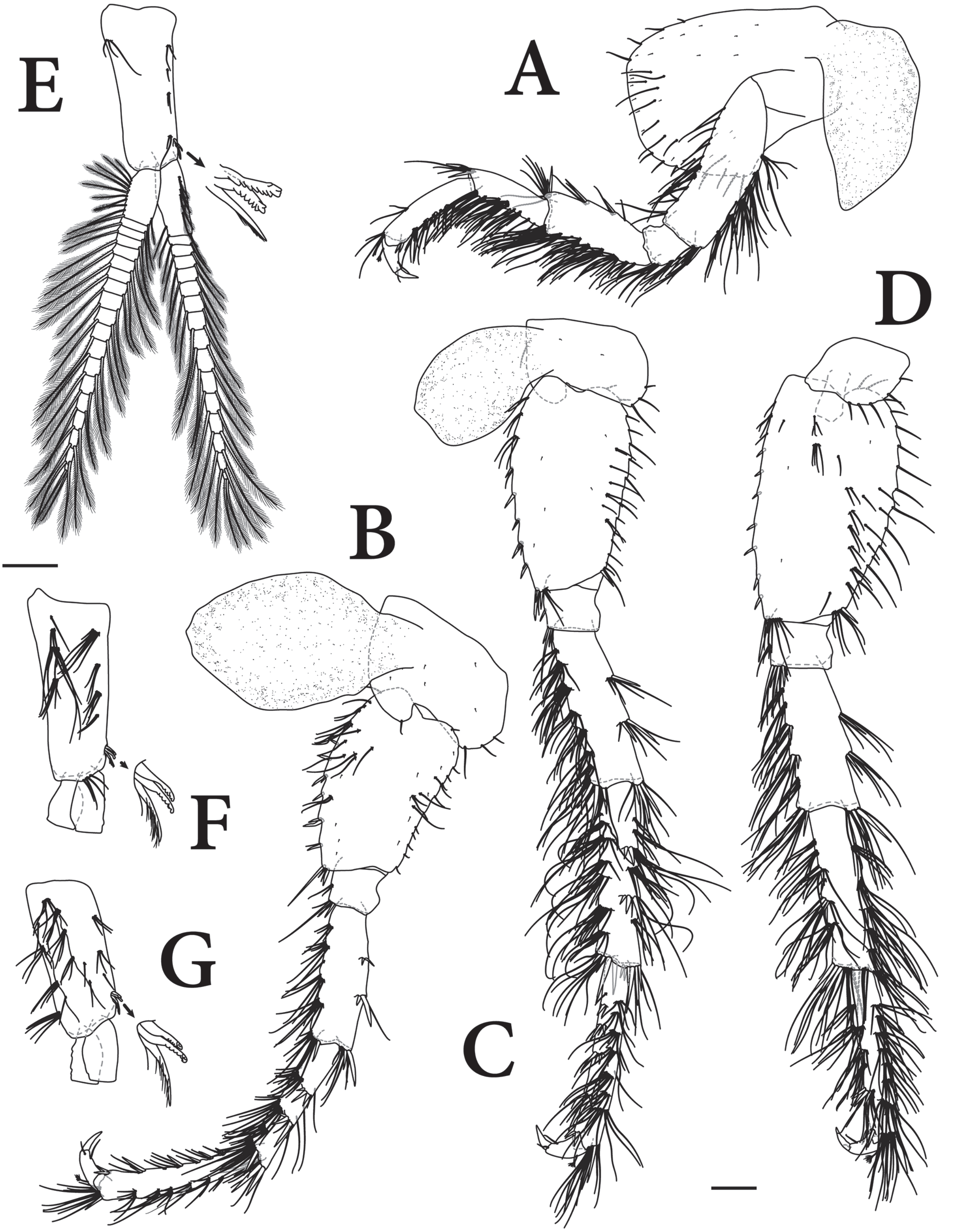

Figure 6. *Gammarus fasciatus* topotype male, 13.12 mm (USNM 1694137), Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA. **A**. Pereopod 4; **B.** Pereopod 5; **C.** Pereopod 6; **D.** Pereopod 7; **E.** Pleopod 1 (coupling spines enlarged); **F.** Pleopod 2 (coupling spines enlarged, rami omitted); **G.** Pleopod 3 (coupling spines enlarged, rami omitted). Scale bar: 0.25 mm.

and 5 apical robust setae. Uropod 3 (Fig. 7E): elongate, biramous; peduncle approximately 40% length of outer ramus with 4 marginal robust setae; outer ramus 2-segmented, 1st segment approximately 7× length of 2nd segment, with 4 groups of robust setae on outer margin paired with setae and/or plumose setae, 3 additional robust setae

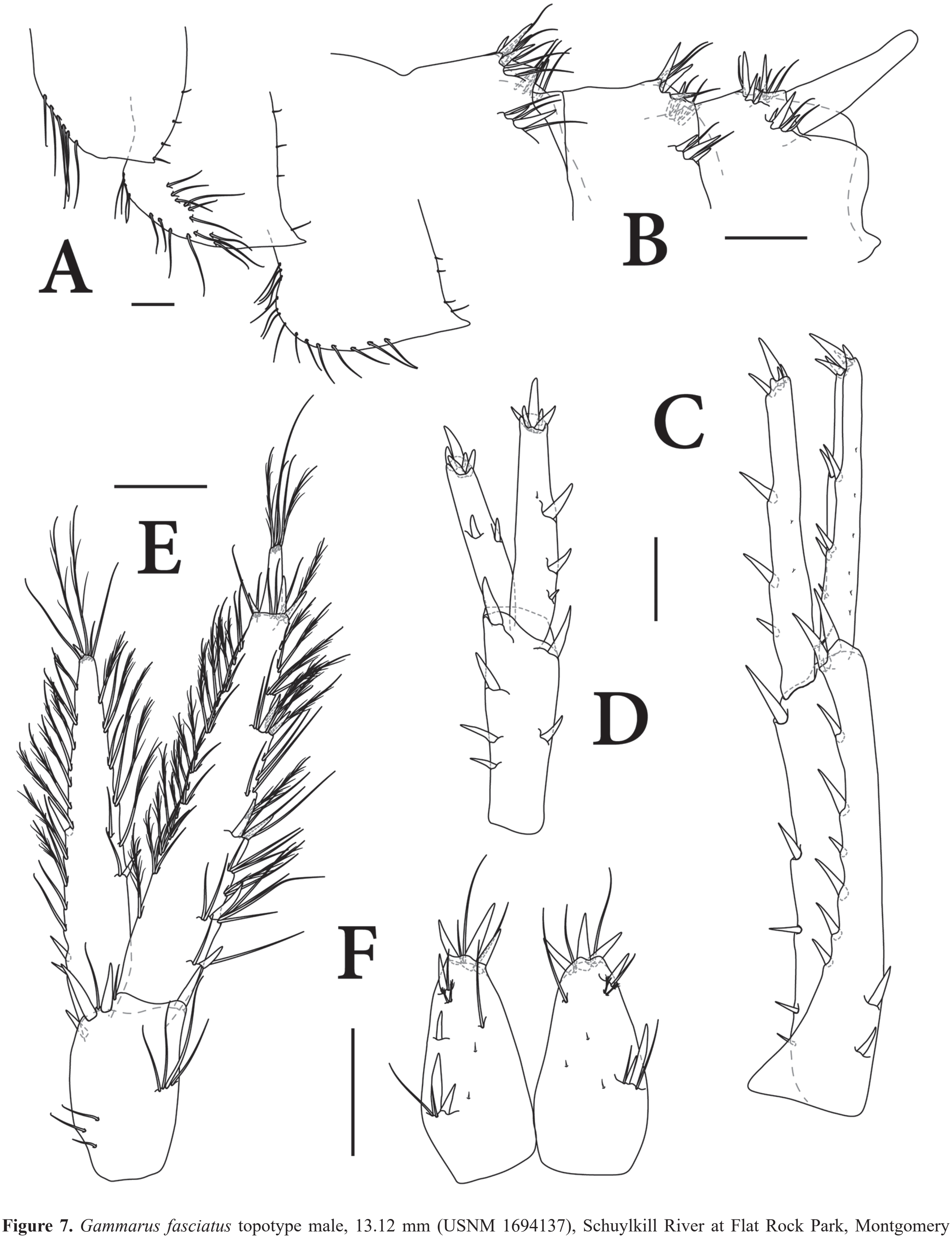

Figure 7. *Gammarus fasciatus* topotype male, 13.12 mm (USNM 1694137), Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA. **A.** Epimera; **B.** Uronites; **C.** Uropod 1; **D.** Uropod 2; **E.** Uropod 3; **F.** Telson. Scale bars: 0.25 mm.

line apical margin; 2nd segment with apical group of setae and plumose setae; inner ramus 75% length of outer ramus, 1-segmented, with 1 robust seta on both outer and inner margins, apical margin with robust seta and setae/plumose setae. Telson (Fig. 7F): cleft to base, apices each with 3 robust setae and 2–3 setae; outer margins with 2–4 robust setae and 3 setae, 2 additional plumose setae arise distally; facial/inner surfaces with a seta.

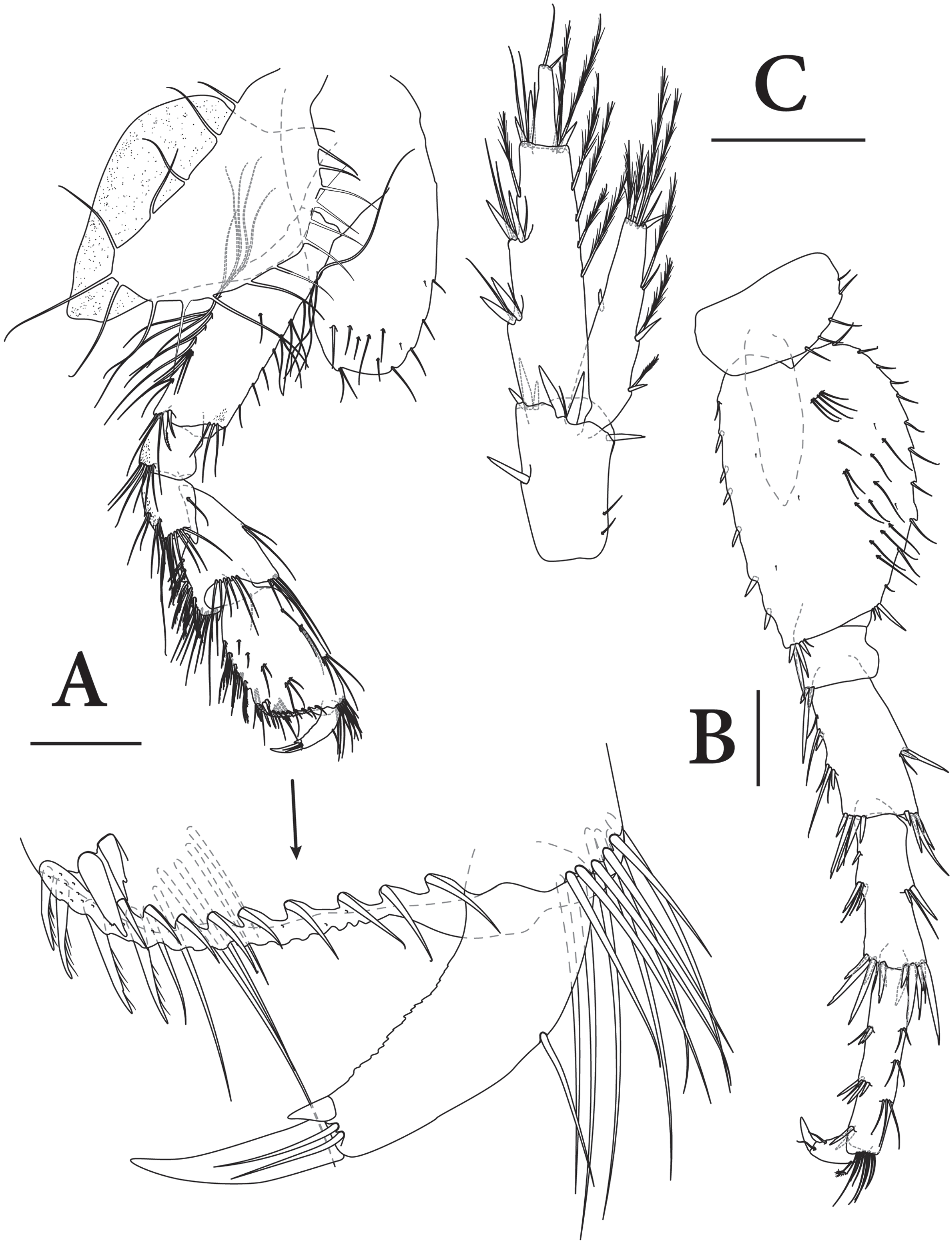

Figure 8. *Gammarus fasciatus* topotype female, 5.91 mm (USNM 1694138), Schuylkill River at Flat Rock Park, Montgomery County, Pennsylvania, USA. **A.** Gnathopod 1 (palmar margin and dactylus enlarged); **B.** Pereopod 7; **C.** Uropod 3. Scale bars: 0.25 mm.

Female: (Figs 2B, 8): 5.91 mm in length. Differing from male in smaller body size, gnathopod 2 shape/setation, pereopod shape/setation, and uropod 3 size/setation. Structures not described below are as in male.

Gnathopods. Gnathopod 2 (Fig. 8A): coxal plate with 4 anterior setae, 2 posteroventral setae, 6 facial setae, and additional smaller, submarginal setae lining margin of plate; basis with numerous long setae inserted along posterior margin, anterior margin with long setae proximally and shorter setae distally, posterodistal margin with 2 serrate robust setae; ischium with 1 tuft of posterior setae and pubescent setules along posterior margin; merus with 1 anterior seta and setae lining the posterior and distal margins, posterior surface of segment with weak pubescent setules; carpus approximately 80% length of propodus with 2 tufts of setae along anterior margin, posterior margin with numerous setae, distal margin with 6 setae; propodus approximately 2.5× longer than broad, with 1 tuft of anterior setae, 12 anterodistal setae, 3 tufts of superior medial setae with proximal-most singly inserted, 4 tufts of inferior medial setae with proximal-most singly inserted, and 5 tufts of posterior setae; palm transverse, straight, with 8 setae along inner margin and 6 long setae along outer margin; defining angle with 2 robust setae, a seta on inner margin, and 4 serrate setae on outer margin; dactylus with outer seta, margin at base of nail with 2 seta, and a distinct tooth.

Brood plates (Fig. 8A). Broad, expanding apically with medium-length setae, present on somites 2–5.

Pereopods. Pereopod 7 (Fig. 8B): coxal plate small, lobes indistinct, with 5 posterior setae; basis posterior margin convex, with 11 shallow serrations bearing setae, seta length increasing slightly distally, longest posterior seta 15% width of segment, face of segment with 17 long setae, distoposterior margin without free lobe, nearly fused to ischium, junction with robust seta and a tuft of setae, anterior margin with 6 robust setae and an anteroproximal seta; merus subequal in length to carpus; carpus approximately 75% length of propodus; dactylus approximately 30% length of propodus, setation as in male.

Urosome. Uropod 3 (Fig. 8C): less elongate than in male, biramous; peduncle approximately 40% length of outer ramus with 1 outer robust seta and 6 marginal robust setae; outer ramus 2-segmented, 1st segment approximately 3× length of 2nd segment, with 2 groups of robust setae on outer margin paired with setae, 3 additional robust setae line apical margin; 2nd segment with apical group of setae; inner ramus approximately 66% length of outer ramus, 1-segmented, with 1 robust seta on both outer and inner margins, apical margin with robust seta and setae/plumose setae.

Variation. Individuals examined were shown to vary notably in several morphological characteristics (Tables 2, 3; Fig. 9).

Distribution and ecology. While *Gammarus fasciatus* has historically had an uncertain distribution, the core portion of its range is found on the northern Atlantic coastal plain of the United States and southern Canada (Fig. 1). Additional records putatively identified as *G. fasciatus* are known as far south as South Carolina (Fig. 1). However, critical examination of these populations may prove them to be either similar species, such as *G. tigrinus/daiberi*, or undescribed species closely related to *G. fasciatus* sens. str. Furthermore, *G. fasciatus* has also been recorded from the Laurentian Great Lakes and the Mississippi River drainages; evidence suggests recent dispersal into these regions, either naturally or due to anthropogenic factors (see Discussion).

Table 2. Notable morphological variation observed among individuals of *Gammarus fasciatus*.

Character	Variability
Antenna 1 primary flagellum segments	12–23
Antenna 1 accessory flagellum segments	4–5
Antenna 1 peduncle segment 1 posterior setal groups	0–1
Antenna 1 peduncle segment 2 posterior setal groups	0–3
Antenna 2 flagellum segments	6–10
Antenna 2 peduncle segment 4 posterior setal groups	4–6
Antenna 2 peduncle segment 5 posterior setal groups	4–6
Antenna 1 to antenna 2 length	0.9–1.5×
Mandibular palp A-setae	4–8
Mandibular palp B-setae	2–3
Mandibular palp C-setae	2–4
Mandibular palp C-setae to D-setae length	2–2.5×
Gnathopod 1 propodus superior medial seta groups	2–3
Pereopod 7 posterior setae length to basis width	35–40%
Second/third epimeron robust setae	present/absent
Second/third epimeron posterior setae length	short to medium
Uropod 3 inner ramus to outer ramus length	75–85%

Table 3. PERMANOVA summary statistics based on morphometric data for *Gammarus fasciatus* populations. Bolded population pairs indicate those for which no statistical significance was detected.

Population pairs	F Model	P-value	P-corrected
Lake Champlain - Hudson River	4.333	0.01526	0.2289
Lake Champlain - Grand Rapids	5.024	0.008029	0.1204
Lake Champlain - St. Lawrence River	3.39	0.008114	0.1217
Lake Champlain - Lake Superior	**2.352**	**0.1285**	**1**
Lake Champlain - Potomac River	4.403	0.007771	0.1166
Hudson River - St. Lawrence River	3.751	0.023	0.345
Hudson River - Grand Rapids	7.121	0.007971	0.1196
Hudson River - Lake Superior	4.865	0.03097	0.4646
Hudson River - Potomac River	**3.917**	**0.06327**	**0.9491**
St. Lawrence River - Grand Rapids	3.389	0.02203	0.3304
St. Lawrence River - Lake Superior	**2.698**	**0.08697**	**1**
St. Lawrence River - Potomac River	4.652	0.007857	0.1179
Grand Rapids - Lake Superior	3.87	0.007629	0.1144
Grand Rapids - Potomac River	8.537	0.007843	0.1176
Lake Superior - Potomac River	4.844	0.02367	0.3551

Gammarus fasciatus has been recorded from a range of habitats, including lakes, rivers, and estuaries, where it appears to prefer slower-moving, turbid water. The species is also known from lower-order habitats such as small streams and even spring runs; this appears to be especially true in the southern portions of its range (Holsinger 1972). Ovigerous females have been collected from February to September, with populations in the southern portion of the range appearing ovigerous earlier in the year (Bousfield 1958; Holsinger 1972). Life history information is relatively limited, but two generations have been reported per year, with a life cycle of less than a year in duration (Bousfield 1958).

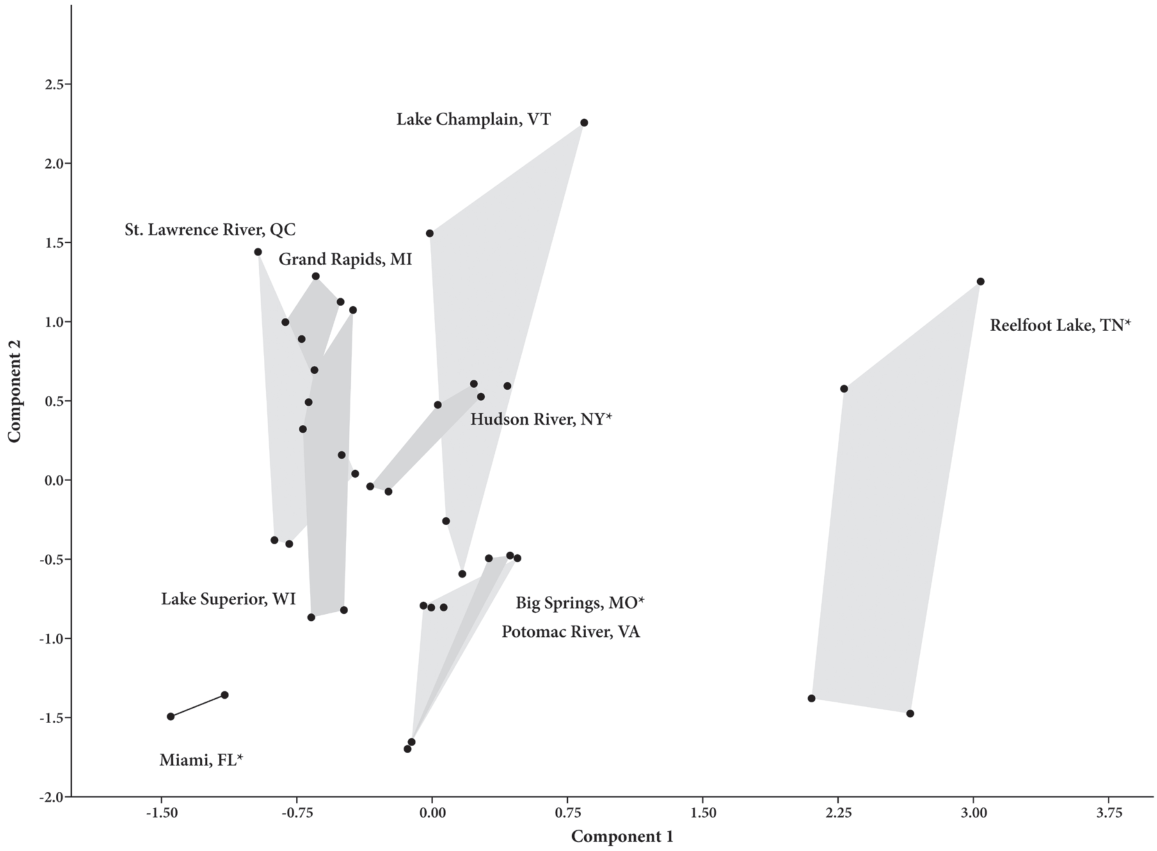

Figure 9. Principal component analysis (PCA) scatterplot based on 19 morphological variables collected from populations of *G. fasciatus* sens. lat. Principal component 1 (Epimera 2 ventral setae) and principal component 2 (Antenna 2 peduncular segment 4 posterior setae) explain 58% and 21% of variation, respectively. Asterisks indicate populations not conspecific with *G. fasciatus* sens. str. Convex hulls indicate individuals from the same population; convex hull shades vary to provide contrast for overlapping populations.

Results

Molecular phylogenetic analyses

Both maximum-likelihood and Bayesian inference recovered all sequenced individuals of *Gammarus fasciatus* as monophyletic, forming a clade sister to *G. tigrinus*/*G. daiberi* (Figs 10, 11). A majority of *G. fasciatus* individuals examined as a part of this project displayed high levels of similarity, falling under a single clade with very short branch lengths (Figs 10, 11). The sole exception to this was an individual from Washington, DC, mined from GenBank, which was recovered as basally derived relative to all other individuals (Figs 10–12). Results of the BEAST analysis place the most recent common ancestor of *Gammarus fasciatus* and its closest congeners in the Miocene (~18–10 Ma; Fig. 11). Within *G. fasciatus*, populations were observed to have diverged later during the Pliocene/Pleistocene (~7.2–0.1 Ma; Fig. 11).

Morphological analyses

A principal component analysis (PCA) based on 19 characters (Suppl. material 2) showed minor separation for a majority of individuals/populations analyzed (Fig. 9). The first principal component (PC1 on the x-axis of Fig. 9) explained 58% of the variation with an eigenvalue of 7.895 and separated individuals primarily based on epimeron 2 ventral setae count. The second principal component (PC2 on the y-axis of Fig. 9) explained 21% of the variation with an eigenvalue of 2.906 and separated individuals primarily based on antenna 2 peduncle segment 5 posterior setal groups. Exceptions to this were observed in populations at the edges of the species' range, with individuals initially identified as *G. fasciatus* from Tennessee, Missouri and Florida displaying separation, suggesting they may not be conspecific with *G. fasciatus* sensu stricto (Fig. 9). Results of the PERMANOVA analysis recovered statistically significant differences for most of the *G. fasciatus* populations examined (Table 3).

Discussion

Amphipod crustaceans have long been noted for their challenging taxonomy, due in part to their high degree of morphological variability and the lack of taxonomically relevant reproductive structures, which are often used to diagnose species in other crustacean taxa such as the Isopoda and Decapoda. As a result, use of modern taxonomic tools has revealed a staggering number of cryptic or pseudocryptic species of amphipods (Delić et al. 2017; Beermann et al. 2018; Wattier et al. 2020; Sisco and Sawicki 2023). Such species are often observed in taxa that at one point were thought to possess large distributions, such as *Hyalella azteca* (Saussure, 1858) in the Nearctic and Neotropics (Witt et al. 2006) and *Gammarus fossarum* Koch, 1836 in the Palearctic (Wattier et al. 2020). Taxonomy for such taxa often lags behind phylogenetic understanding – the so-called "Linnean Shortfall" – due in part to a lack of taxonomic expertise as well as the complicated histories these species often possess. Many amphipod species were described in the 19th or early 20th century; they often have inadequate original descriptions with unclear or undefined type localities, making proper identification and placement difficult, especially when cryptic species are presumed to be present.

Among the Nearctic amphipod fauna, members of the genus *Gammarus* present an ideal model system for highlighting these shortfalls, as species within the genus are morphologically similar and cryptic species appear to be common (Walters et al. 2021; Smith et al. 2024; Cannizzaro and Berg 2024). These trends have been well documented within a clade of species that includes both estuarine taxa (such as *G. tigrinus*) and members of the *G. pecos* complex, freshwater species occupying springs in the Chihuahuan Desert (Smith et al. 2024; Cannizzaro and Berg 2024). The only member of this clade that has yet to be examined phylogenetically and taxonomically is *G. fasciatus*. This is curious, as the species is common, possesses the largest freshwater range within the clade, and is also of interest due to its presumed introduction into parts of the Laurentian Great Lakes and Ohio River basins (Holsinger 1972; Beckett et al. 1998; Dermott et al. 1998; Mills et al. 1993; Grigorovich et al. 2003). As *G. fasciatus* currently remains poorly understood taxonomically, we sought to rectify this by redescribing the species from its type locality and providing information on its status and evolutionary history.

As currently understood, *Gammarus fasciatus* occupies a range extending throughout the Atlantic coast and coastal plain of the United States and Canada into the Great Lakes and Mississippi River basins (Fig. 1). The species is presumed native in the Atlantic drainage portions of its range (extending from Quebec to South Carolina), but in both the Great Lakes and Mississippi River drainages it has been referred to as both native and introduced by various authorities (Holsinger 1972; Mills et al. 1993; Beckett et al. 1998; Van Overdijk et al. 2003; Grigorovich et al. 2003). Gaining a clearer understanding of this has been difficult due to the small number of records of the species from the 19th and early 20th centuries. The first known record appears in the original description by Say (1818). The species was collected in the Schuylkill River, with only vague references to a larger distribution, as Say noted it to be a "common inhabitant of our rivers." By the late 19th century, Smith (1874) reported the species as occurring "throughout the Northern States," presenting records from Michigan, Wisconsin, and possibly Illinois. In the early 20th century, Weckel (1907) noted that the species occurred as far south as Tennessee and Florida, although these records are likely misidentifications (Hubricht and Mackin 1940; Fig. 9). In the 21st century, Grigorovich et al. (2003), when reviewing records from the Great Lakes, noted that the species was found throughout the region with the exception of north-central Lake Superior, from which it was only recently observed, suggesting a very recent expansion. Many records of *Gammarus* from the region in the 19th and early 20th centuries are of dubious species-level identification – especially those presented by Weckel (1907), as noted by Hubricht and Mackin (1940) – due to confusion with other species, namely *"Gammarus limnaeus"* Smith, 1874. However, given that Smith described *"G. limnaeus"* in the same publication, it seems unlikely that he would mistake *G. fasciatus* for that species. Based on its occurrence in both Wisconsin and Michigan (Smith 1874), *G. fasciatus* was likely present in the Great Lakes in the mid-to-late 19th century at the latest; however, given the lack of positive/negative records prior to this, it is still difficult to discern whether the species is truly native to the region. Conversely, in the Mississippi River basin, the first records of the species (identified as *Gammarus* nr. *fasciatus*) appear in 1973 from the Ohio River, with amphipod surveys in the region prior to this date recording only *Crangonyx* spp. (Beckett et al. 1998). Additional introduced records of the species are known from Nevada (1941–1962) and Colorado (2009); however, the persistence of these populations and their specific identities are unclear (La Rivers 1962; United States Environmental Protection Agency, 2017).

Molecular investigations into the metapopulation of *Gammarus fasciatus* have the potential to lend further insight into its status in the Great Lakes and Mississippi River basins. Initial analyses using allozymes (Hogg et al. 2000) suggested a recent introduction or range expansion due to relatively low differentiation within and among populations collected in the Great Lakes and the St. Lawrence River, with slightly higher differentiation in the latter. Our results corroborate this, with individuals sequenced from both Pennsylvania and the Ohio River displaying high similarity at all loci examined (Figs 10–12). Our sequences were also observed to be nearly identical to those from Ontario and Minnesota mined from GenBank (Figs 10–12). Higher differentiation was observed in a mined sequence from Washington, DC, which was also recovered as basally derived in all of our phylogenies, suggesting an Atlantic coastal plain origin for the species. Given the low diversity observed both within and outside the Great Lakes and Mississippi River basins, it is likely that populations of *G. fasciatus* in these regions represent introductions or recent range expansions (Hogg et al. 2000). While it remains difficult to discern whether this expansion was due to anthropogenic activity, the fact that *G. fasciatus* was only first recorded in the Ohio River in the 1970s, likely through dispersal from the Great

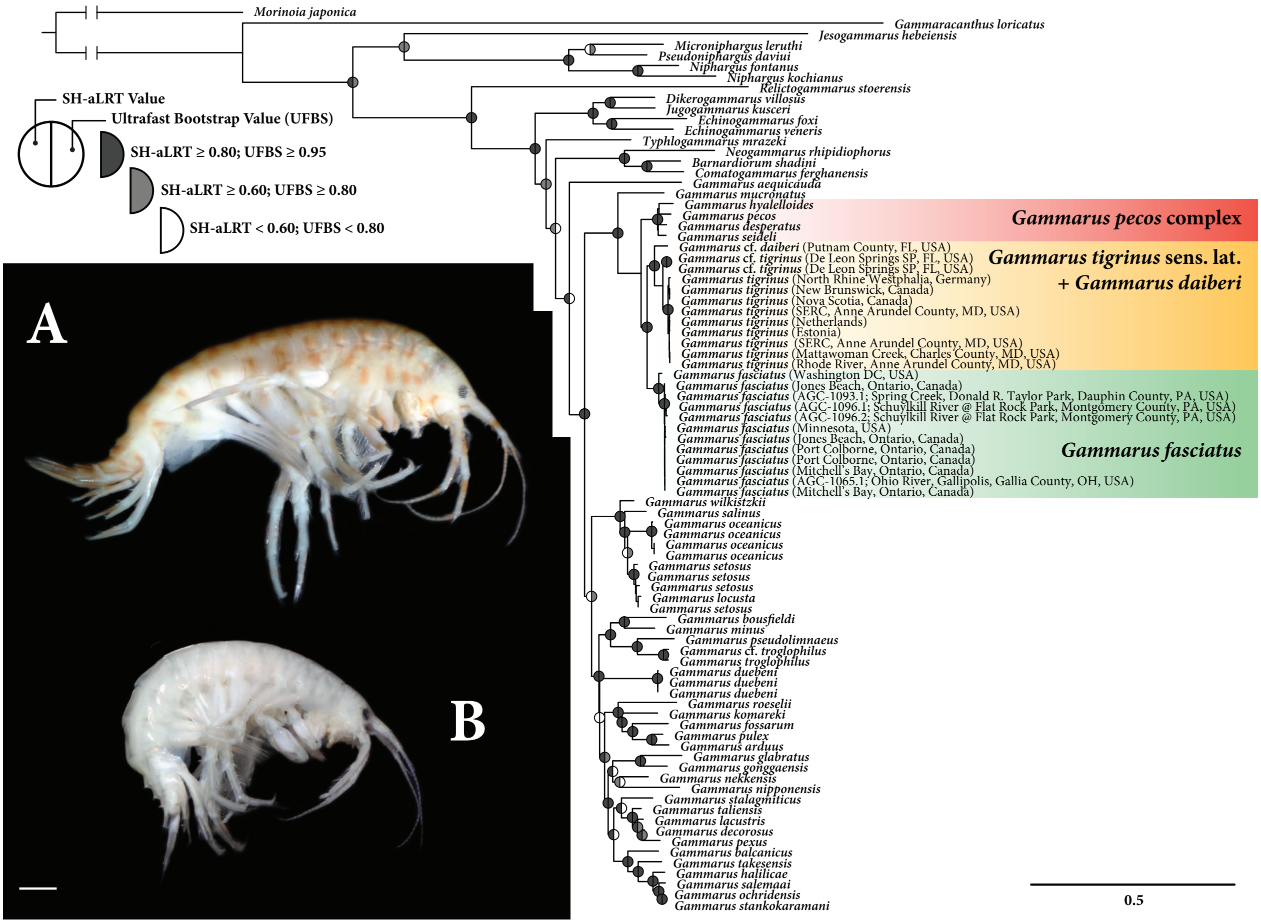

Figure 10. Maximum-likelihood phylogeny reconstructed from a concatenated dataset (18S rDNA, 28S rDNA, 16S rDNA, COI) using IQTREE. Node support for the two support methodologies used (UBFS, ultrafast bootstrap, and SHaLRT, Shimodara-Hasegawa approximate likelihood ratio test) is indicated by shaded circles placed on nodes. **A.** *Gammarus fasciatus*, male, 12.7 mm, Ohio River, Gallipolis, Gallia County, Ohio, USA; **B.** *Gammarus fasciatus*, male, 11.4 mm, Spring Creek, Donald R. Taylor Park, Dauphin County, Pennsylvania, USA. The scale bar represents 1 mm. Root of tree (*Morinoia japonica*) shortened to preserve readability.

Lakes drainage, lends more credence to an anthropogenic hypothesis than to a natural one. It is also plausible that the species did occur naturally in the lower Great Lakes and St. Lawrence River before expanding into the Mississippi River basin and the upper Great Lakes due to the creation of navigation structures such as canals and other anthropogenic activities.

Beyond its complex distributional history, little is known regarding the evolutionary history of *Gammarus fasciatus* other than its close association with *G. tigrinus*/*G. daiberi* and members of the *G. pecos* complex. Results of our BEAST analysis suggest that *G. fasciatus* split from its closest congeners (*G. tigrinus*/*G. daiberi*) during the Middle-to-Late Miocene (~18–10 Ma; Fig. 11). The timing of this split corresponds with others observed in the clade; most members of the *G. pecos* complex are thought to have split during the Miocene/Pliocene (~14–3 Ma) based on estimates by Cannizzaro and Berg (2024). Similarly, *Gammarus daiberi* and *G. tigrinus* were demonstrated to have split ~14–7 Ma (Smith et al. 2024), with comparable dates being recovered by us (Fig. 11). Taken together, these results strongly suggest that the Middle-to-Late Miocene was an important period for speciation within the lineage, with at least four independent transitions from marine to freshwater environments being hypothesized. Factors promoting such transitions have been linked to climate and geography, as the region that would become the Chihuahuan Desert experienced significant stream capture and rearrangement due to volcanism, aridification, and expansion of the paleo-Rio Grande (Adams et al. 2018; Cannizzaro and Berg 2024). In addition, the formation of the Labrador Current, which occurred during the Late Miocene, is thought to account for the clades observed within *Gammarus tigrinus* (Kelly et al. 2006; Smith et al. 2024).

Similar factors may have been at play for *Gammarus fasciatus*. The species likely derived from an estuarine ancestor similar to modern *G. tigrinus*/*G. daiberi*, which may have occupied what is now the Atlantic coastal plain, probably within or near what would become modern-day Chesapeake Bay. During the Miocene, the Earth experienced a transition to colder temperatures than those observed in the Eocene, with accompanying sea level decreases (Miller et al. 2005; Kotthoff et al. 2014). While

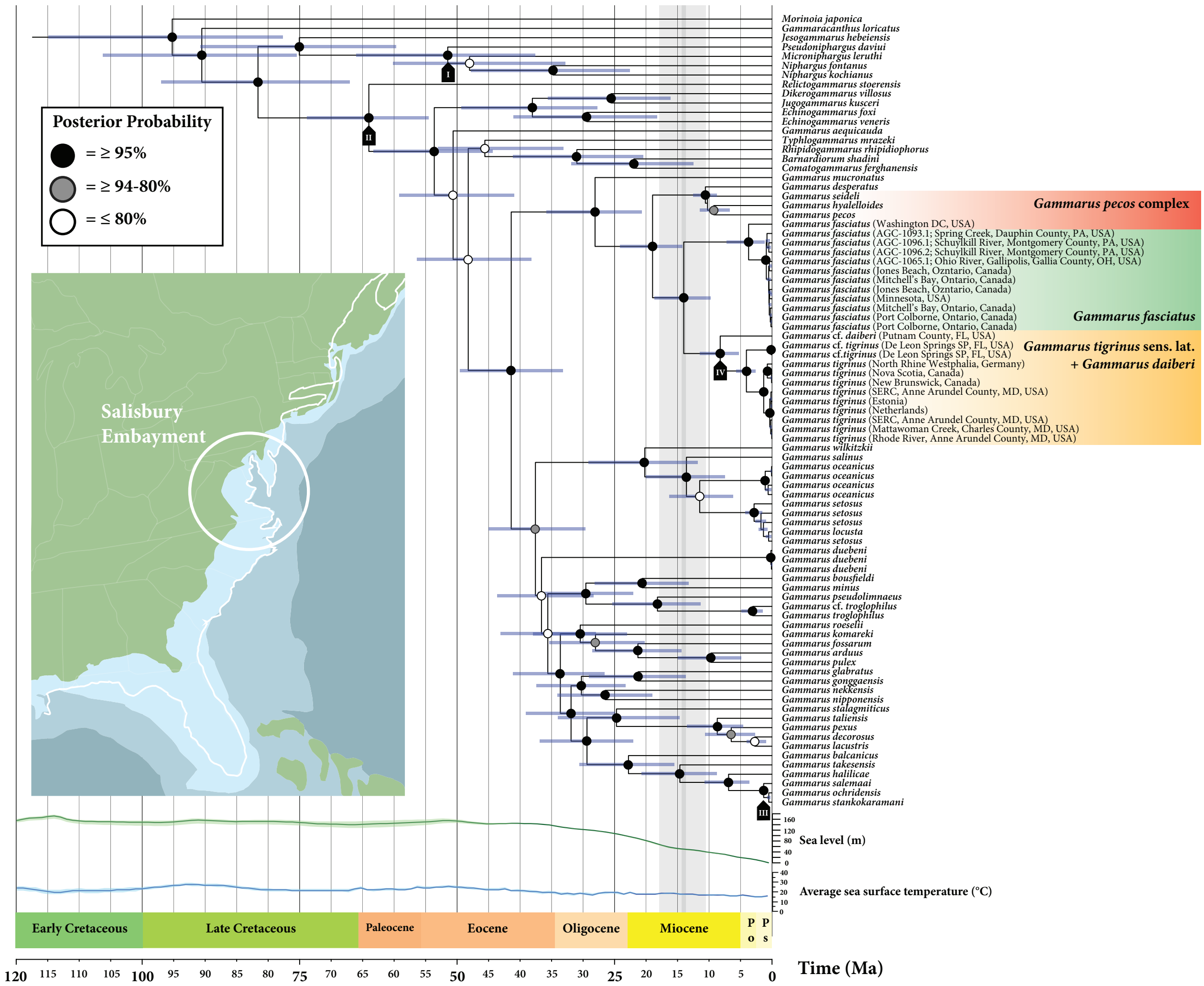

Figure 11. Time-calibrated multilocus phylogeny reconstructed using BEAST. Posterior probabilities are indicated by circles placed at nodes; node bars indicate the 95% HPD intervals of clade ages. Approximate sea level (in meters) and average sea surface temperature (in degrees Celsius) through time are plotted at the base of the tree using estimates generated by van der Meer et al. (2022). Inset: map of the eastern United States during the Middle Miocene, based on reconstructions from Ron Blakely and Colorado Plateau Geosystems, Inc.; approximate modern sea level is outlined in white; the approximate location of the Salisbury Embayment is also indicated.

this was the general trend, it was not consistent; several periods of warmer and colder fluctuations occurred, with associated effects on sea levels (Zachos et al. 2001). In eastern North America during the Middle Miocene, large portions of what is now the Atlantic coastal plain were covered by shallow arms of the Atlantic Ocean. One of the most prominent of these was the Salisbury Embayment, an extension of the Baltimore Canyon trough, which covered an area roughly equivalent to the modern-day Chesapeake Bay. Stretching from New Jersey to Virginia, it persisted from the early Paleogene to the Late Miocene (Ward and Powars 2004). Throughout this interval and into the Pliocene, sea level and temperature fluctuated considerably until conditions returned to those resembling the modern day after the Pleistocene (Ward and Powars 2004). The eustatic conditions present in the embayment likely would have been ideal for promoting a transition from marine to freshwater or estuarine habitats, and it is plausible that the ancestor of *G. fasciatus* utilized such a pathway to access interior freshwaters on the continent and disperse further through riverine systems to achieve its current distribution. Such a scenario would mirror that proposed for related members of the *G. pecos* complex (Adams et al. 2018; Cannizzaro and Berg 2024).

While it appears that individuals of *Gammarus fasciatus* from the northern portion of the range are relatively homogenous, both morphologically and molecularly, individuals from other portions of its range show significant variation (Figs 9–11). Morphological analyses of populations initially identified as *G. fasciatus* from New York, Tennessee, Florida, and Missouri suggest they are likely not conspecific with *G. fasciatus* sensu stricto (Fig. 9). Misidentifications of *G. fasciatus* are not uncommon, given the close morphological association and similarity in coloration between *G. fasciatus* and its sister species (*G. tigrinus*/*G. daiberi*). This has led to uncertainty regarding the former's range (Hubricht and Mackin 1949; Grigorovich et al. 2005). This trend dates back as far as

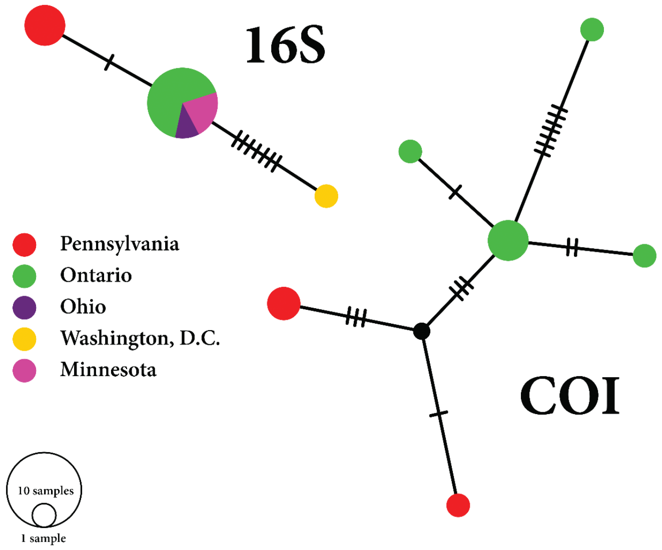

Figure 12. Median joining haplotype networks for mitochondrial loci (16S, COI) generated in PopART V.1.7. Hash marks indicate missing haplotypes.

the original species description, where Say (1818) noted the presence of a "saltwater form" that he was unable to separate and considered to be conspecific with *G. fasciatus*. This form was later described as *G. tigrinus* (Sexton and Cooper 1939). While these species are similar in many aspects of morphology, they are best separated by examination of the epimera, where *G. tigrinus* lacks the dense ventral/facial setation on epimera 2 and 3 and possesses more robust setae, which are rare in *G. fasciatus* (Fig. 7A; Cole 1970; Grigorovich et al. 2005). While populations of *G. fasciatus* have been misidentified as other described species, it is also plausible that cryptic species are present within the taxon, a hypothesis reiterated by previous investigators who have proposed that the species may represent a complex, especially in the southern portion of its presumed range (Clemens 1950; Holsinger 1972). Further collections and analyses in this region and at other edges of the range may indeed reveal the existence of such a species complex. Given that members of the clade to which *G. fasciatus* belongs have independently transitioned to freshwater environments on at least four different occasions, it is possible that the process by which *G. fasciatus* likely originated could have been replicated, especially given the large number of embayments and marine transgressions and regressions that occurred along the Atlantic seaboard of North America during the Miocene (Ward and Powars 2004). Future studies targeting regions such as the southeast and west Georgia embayments, the Charleston Embayment, and others would shed much-needed light on the status of a potential *G. fasciatus* species complex.

A clear taxonomy is fundamental to understanding the evolutionary placement of a species. Without proper identification, aspects of ecology, natural history, and conservation can easily become confused. *Gammarus fasciatus* presents an ideal case study of both this phenomenon and the utility of redescribing species, as it is morphologically similar to its closest congeners and has a complex history of uncertainty regarding its distribution and potential introductions (Mills et al. 1993; Beckett et al. 1998; Dermott et al. 1998; Van Overdijk et al. 2003; Grigorovich et al. 2003). In the Laurentian Great Lakes, the species has been regarded as native, introduced, or potentially a mix of both (Mills et al. 1993; Van Overdijk et al. 2003; Grigorovich et al. 2003). This confusion has greatly hampered efforts to understand the species. For example, it was once considered to be a candidate for conservation in an area where it may potentially be an introduced species (Dermott et al. 1998). In cases such as these, natural history can be better understood by clarifying taxonomy based on comparison to type material and/or a redescription of the target taxon. *Gammarus* spp. in the Nearctic have slowly been revealed to be more diverse than previously thought, with many taxa possessing cryptic species or lineages (Walters et al. 2021; Smith et al. 2024; Cannizzaro and Berg 2024). Further investigations into *G. fasciatus* have the potential to reveal similar trends; our results can act as a foundation from which future studies investigating the species and its metapopulation can proceed. In addition, results presented here further highlight the utility a redescription can provide. Not only did we address the taxonomy of *G. fasciatus*, but also its natural history and conservation as well. *Gammarus fasciatus* is certainly not the only species that possesses a limited original description and faces taxonomic, phylogenetic, and biogeographic uncertainties; this is true both within the genus and among eukaryotic organisms as a whole. Further attention granted to traditionally "mundane" analyses such as redescriptions has great potential to increase our understanding of these species well beyond their taxonomy and to better resolve the evolutionary history of biogeographic regions.

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Supplementary material 1

Accession and collection info for museum specimens of *G. fasciatus* sens. lat. examined as a part of this project

Authors: Andrew G. Cannizzaro, David J. Berg
Data type: xlsx

Link: https://doi.org/10.3897/zse.101.161784.suppl1

Supplementary material 2

Morphometric characters collected from museum specimens of *Gammarus fasciatus* sens. lat.

Authors: Andrew G. Cannizzaro, David J. Berg
Data type: xlsx

Link: https://doi.org/10.3897/zse.101.161784.suppl2

Supplementary material 3

GenBank accession numbers of sequences utilized in this study

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