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A nearly cryptic Scorpionfly, *Panorpa cryptica* n. sp. (Mecoptera: Panorpidae) from North America

WESLEY BICHA[1], NATHAN SCHIFF[2], AARON LANCASTER[2] & BRIAN SCHEFFLER[3]

[1]*121 Old Batley Road, Oliver Springs, TN 37840 USA. E-mail: mecoptera@live.com*
[2]*United States Department of Agriculture Forest Service, Center for Bottomland Hardwoods Research, P.O. Box 227, Stoneville, MS USA 38776 USA. E-mail: nschiff@fs.fed.us*
[3]*USDA Agricultural Research Service, USDA-ARS-CGRU, MSA Genomics Laboratory, 141 Experiment Station Road, Stoneville, MS 38776 USA. E-mail: brian.scheffler@ars.usda.gov*

Abstract

The first nearly cryptic species of scorpionfly from the United States, ***Panorpa cryptica* Bicha and Schiff, n. sp.**, is described from northern Georgia, southwestern North Carolina and northwestern South Carolina. This insect was initially differentiated from the very similar *Panorpa nebulosa* Westwood by its unique cytochrome oxidase subunit I (COI) mitochondrial DNA. Habitat details, distribution, and biology are described.

Key words: COI, DNA, Georgia, new species, North Carolina, *Panorpa*, *Panorpa acuta*, *Panorpa flexa*, *Panorpa nebulosa*, scorpionfly, South Carolina

Introduction

Mecoptera is an ancient, small, holometabolous order of insects with approximately 650 described extant species assigned to nine families. Two-thirds of the Mecoptera species are in the family Panorpidae, of which the United States has approximately 54 species in one genus, *Panorpa* Linnaeus, 1758. The males of Panorpidae possess a genital bulb recurved upwards, resembling the sting of a scorpion, hence, the common name, scorpionfly. Scorpionflies are widely distributed throughout the Holarctic and Oriental regions (Penny & Byers 1979) and are often the most abundant conspicuous insects in the appropriate habitat.

Panorpa nebulosa Westwood, 1841 (Byers 1962, Somma 2011) is one of the most widespread and abundant scorpionflies in the spring in the eastern United States (Byers 1954). The insect generally has a boreal distribution, extending south along the Appalachian Mountains at moderate altitudes. *Panorpa nebulosa* is replaced by *Panorpa flexa* Carpenter, 1935, at the higher elevations of the Great Smoky Mountains National Park and the mountains associated with the Blue Ridge Parkway. *Panorpa nebulosa* shows some variation of the male ventral parameres throughout the range and is most pronounced in mountainous areas of Virginia. Dr. Oliver Flint, of the National Museum of Natural History (USNM), Washington, DC, has expressed interest and has questioned the significance of these forms (Flint 2013).

We sequenced the COI mitochondrial DNA region (the "DNA barcode") (Hebert *et al.* 2003) of multiple examples, often at extremes of the geographical range, of each of the 54 currently recognized species of Panorpidae from the United States, as well as 55 additional species from Mexico, Asia, and Europe (unpublished data). It was anticipated that molecular differences between the Virginia mountain forms of *P. nebulosa* might be revealed during our COI study. In contrast, we found that the COI results were similar throughout that portion of the range, but differed at the far southern extent of the range, on the south side of the Appalachian Mountains in northern Georgia. For several years we thought this to be a cryptic species, but careful study has revealed several subtle, but consistent, morphological differences between *Panorpa nebulosa* and the new northern Georgia species. This is possibly the insect that Byers observed from the middle elevations in eastern Tennessee and western North

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Carolina that he regarded as an intermediate between *P. flexa* and *P. nebulosa*. He felt that the similar morphological structures and the occurrence of apparent intergrades suggested a recent separation of *P. flexa* from *P. nebulosa* that was not yet complete (Byers 1969).

Material and methods

Specimens were collected across as much of the known range for each species as possible and killed in 80%–100% ethanol. The specimens were identified by inspection and sent to the Center for Bottomland Hardwoods Research in Stoneville, Mississippi, for DNA extraction and sequencing. DNA was extracted, amplified and sequenced according to the methods described in Schiff et al. (2012) except that additional primers as listed below were used for specimens that did not amplify easily using primers LCO1490 (5'GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994). Primer WES1 (5'-GGC TTT TCT CTA CTA ATC ATA AGG ATA TTG G-3'), only a slight modification from LCO1490, was often paired with HCO2198, RENEE1 (5'-GAC AGC TAA ACT TCA GGG TGA C-3') was paired with RENEE2 (5'-GCG GTC AAC AAA TCA TAA AGA TAT TGG-3'); and RONII SAWFLY (5'-GGA GCT CCA GAT ATA GCA TTC CC-3') was paired with NANCY (5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3') (Simon *et al.* 1994). RON II SAWFLY is a new name for RONII of MacQuairie et al. (2007). They used the name RONII even though the name was already in use for another oligo (Simon *et al.* 1994). After consulting with MacQuarrie, we decided to rename the oligo RONII SAWFLY (C. MacQuarrie, personal communication). Templates were sequenced in both directions and the corresponding sequences were combined into individual specimen contigs using Lasergene Seqman by DNAStar. Specimen contigs were aligned using Clustal V, built into neighbor-joining trees (Saitou and Nei 1987 using Kimura distances) and bootstrap values calculated using the DNAStar program Megalign.

The taxonomic terminologies of Esben-Petersen (1921), Carpenter (1931), Byers (1958), and Thornhill *et al.* (1974) were followed.

Results

As part of a larger study of the Panorpidae, DNA barcodes were generated for 50 specimens thought to represent 3 closely related but morphologically distinct species, *P. flexa*, *P. acuta* and *P. nebulosa*. All sequences were complete (658bp) except for *P. nebulosa* sample 1251, which was 444bp in length. When the sequences were used to construct a neighbor-joining tree (Fig. 1), 28 specimens formed three well-supported clades (bootstrap values above 95 with 1,000 trials) representing the three known species but 22 specimens that appeared to be *P. nebulosa* morphologically fell between *P. acuta* and *P. flexa* in two poorly supported clades (bootstrap values below 40 with 1,000 trials). As mitochondrial DNA is maternally inherited, these specimens cannot be hybrids because their barcodes would place them with the mother's species, so we concluded they must represent one or more new, essentially cryptic taxa. Close morphological inspection revealed that all male specimens (19 of the 22) shared the defining morphological characters in the new species description, namely, that the tufts of setae on the apices of male basistyles were not borne on pronounced caudally directed tubercles or lobes as in *P. nebulosa*. Also, in contrast to *P. nebulosa*, the male ventral parameres were thin and nearly sigmoidal, typically curving corkscrew-like ventrally out of the genital bulb. The combination of novel barcode sequences, subtle but definitive morphological characters and geographical isolation without overlap between the new species and *P. nebulosa* all support designation of a new species.

Although we are confident that the 22 specimens are not *P flexa*, *P. acuta* or *P. nebulosa*, the tree suggests that there might be two new species present. As the bootstrap values supporting the two clades are low and we are unable to differentiate between the two clades morphologically even though there appears to be some geographical isolation by counties (Gilmer, Murray, Chattooga, Floyd, Catoosa and Pickens counties versus Lumpkin, White, Dawson and Stephens counties (Table 1, Fig. 2), we have conservatively chosen to designate a single new species until more cogent data become available. It is possible that we are observing incipient speciation due to geographic isolation that has not yet manifested itself with observable morphological characters. The description of this insect follows.

TABLE 1. Sequenced specimen collection localities and GENBANK accession numbers. A complete set of sequences (FASA format) can be obtained at http://www.srs.fs.usda.gov/cbhr/products/downloads/2013_nms_PcrypticaFASA.txt.

Species	State	County	Longitude	Latitude	CBHR#	Genbank Accession #
acuta	VA	Wythe	37.045° N	81.229° W	1240	KF887247
acuta	TN	Johnson	36.498° N	81.886° W	1726	KF887255
acuta	MA	Middlesex	42.627° N	71.594° W	1991	KF887256
acuta	TN	Johnson	36.498° N	81.886° W	1997	KF887258
acuta	VA	Giles	37.400° N	80.516° W	2037	KF887260
acuta	MA	Hampshire	42.514° N	73.080° W	2044	KF887262
acuta	NC	McDowell	35.927° N	81.954° W	2308	KF887268
acuta	GA	Stephens	34.594° N	83.362° W	2612	KF887276
acuta	GA	White	34.574° N	83.643° W	2623	KF887283
acuta	GA	White	34.574° N	83.643° W	2624	KF887284
cryptica	GA	Murray	34.760° N	84.732° W	2248	KF887266
cryptica	GA	Murray	34.760° N	84.732° W	2249	KF887267
cryptica	GA	Murray	34.760° N	84.732° W	2610	KF887275
cryptica	GA	Chattooga	34.579° N	85.203° W	2662	KF887285
cryptica	GA	Gilmer	34.764° N	84.574° W	2701	KF887286
cryptica	GA	Gilmer	34.686° N	84.599° W	2702	KF887287
cryptica	GA	Pickens	34.477° N	84.542° W	2703	KF887288
cryptica	GA	Catoosa	34.579° N	85.203° W	3104	KF887291
cryptica	GA	Floyd	34.457° N	85.221° W	3106	KF887293
cryptica	GA	Lumpkin	34.532° N	84.019° W	1225	KF887245
cryptica	GA	Dawson	34.550° N	84.283° W	2049	KF887264
cryptica	GA	Lumpkin	34.517° N	84.667° W	2050	KF887265
cryptica	GA	Lumpkin	35.519° N	84.056° W	2607	KF887272
cryptica	GA	Lumpkin	34.519° N	84.056° W	2608	KF887273
cryptica	GA	Stephens	34.594° N	83.362° W	2609	KF887274
cryptica	GA	White	34.574° N	83.643° W	2613	KF887277
cryptica	GA	Lumpkin	35.519° N	84.056° W	2618	KF887278
cryptica	GA	Lumpkin	35.519° N	84.056° W	2619	KF887279
cryptica	GA	Lumpkin	35.519° N	84.056° W	2620	KF887280
cryptica	GA	Lumpkin	34.519° N	84.056° W	2621	KF887281
cryptica	GA	Lumpkin	34.519° N	84.056° W	2622	KF887282
cryptica	GA	Stephens	35.594° N	83.362° W	3105	KF887292
flexa	NC	Swain	35.53°9 N	83.494° W	1257	KF887249
flexa	NC	Transylvania	35.301° N	82.900° W	2309	KF887269
flexa	NC	Haywood	35.573° N	83.180° W	2704	KF887289
flexa	NC	Graham	35.358° N	83.718° W	3114	KF887294

.....continued on the next page

TABLE 1. (Cotninued)

Species	State	County	Longitude	Latitude	CBHR#	Genbank Accession #
nebulosa	VA	Tazwell	37.042° N	81.519° W	1236	KF887246
nebulosa	PA	Schulkyll	40.535° N	76.450° W	1251	KF887248
nebulosa	NC	Ashe	36.404° N	81.454° W	1396	KF887250
nebulosa	PA	Washington	40.042° N	80.281° W	1399	KF887251
nebulosa	WV	Randolph	38.696° N	79.539° W	1434	KF887252
nebulosa	WV	Randolph	38.696° N	79.539° W	1435	KF887253
nebulosa	MI	Hillsdale	42.077° N	84.661° W	1719	KF887254
nebulosa	MA	Berkshire	42.248° N	73.332° W	1993	KF887257
nebulosa	VA	Giles	37.400° N	80.516° W	2035	KF887259
nebulosa	MA	Hampshire	42.514° N	73.080° W	2043	KF887261
nebulosa	MA	Berkshire	42.248° N	73.332° W	2045	KF887263
nebulosa	NC	McDowell	35.812° N	82.148° W	2310	KF887270
nebulosa	NC	McDowell	35.927° N	81.954° W	2311	KF887271
nebulosa	NC	Smyth	36.628° N	81.587° W	2820	KF887290

***Panorpa cryptica* Bicha & Schiff, new species**
Figs. 1–10

Diagnosis. *Panorpa cryptica* can be differentiated from the similar *P. nebulosa* by the tufts of setae on the apices of the male basistyles not borne on pronounced caudally directed tubercles or lobes as in *P. nebulosa*. Further, the male ventral parameres are thin and nearly sigmoidal, typically curving corkscrew-like ventrally out of the genital bulb.

Type material examined. Holotype male, allotype female, and 2 males, 1 female paratypes: GEORGIA, Murray Co. 29 April 2011. Additional paratypes: GEORGIA, 9 males, Bartow Co. 34.3485ºN 84.6647ºW, 260 m, 3 May 2014; 7 males, 6 females, Catoosa Co. 34.57899ºN 85.20343ºW, elev. 275 m, 27 May 2013; 1 male, Chatooga Co. 34.57912ºN 85.20348ºW, 271 m, 5 May 2012; 9 males, 1 female, Cherokee Co. 34.24047ºN 84.41557ºW, 370 m, 3 May 2014; 7 males, 1 female, Dawson Co. 34.35167ºN 84.11645ºW, 330 m, 12 May 2014; 1 male, 1 female, Floyd Co. 34.45695ºN 85.22108ºW, 300 m, 27 May 2013; 5 males, 4 females, Forsyth Co. 34.30142ºN 84.22302ºW, 333 m, 11 May 2014; 1 male, Gilmer Co., 25 May 1996; 4 males, Gilmer Co. 34.68612ºN 84.59940ºW, 431 m, 16 June 2012; 4 males, 14 females, Gilmer Co. 34.7636ºN 84.57363ºW, 458 m, 1 July 2012; 1 male, Gilmer Co., Chattahoochee National Forest, 31 May 1990, C. L. Smith & R. M. Duffield (GMNH); 1 male, Lumpkin Co., 15 mi. NW Dahlonega, 2 June 1971, F. Sherberger (GMNH); 1 male, 15 mi NW Dalonega, 16 June 1973, M.R. Humphery (GMNH); 9 males, 1 female, Lumpkin Co. 34.51929ºN 84.05619ºW, 448 m, 28 April 2012; 1 male, Lumpkin Co. 34º31'56"N 84º1'7"W, 26 May 2007; 10 males, 1 female, Murray Co. 34.75957ºN 84.73206ºW, 387 m, 28 April 2012; 9 males, 2 females, same locality, 29 April 2011; 4 males, 2 females, Pickens Co. 34.47702ºN 84.54227ºW, 394 m, 16 June 2012; 1 male, Stephens Co., Toccoa College 34.59409ºN 83.36178ºW, 14 April 2012; 6 males, 6 females, same locality, 18 May 2013; 1 male, Union Co., 25 June 1973, R. J. Beshear (GMNH); 1 male, Union Co., Brasstown Bald View, off Jack's Knob Trail, 1 June 1986, K. Morgan, C. L. Smith, & S. P. Smith (GMNH); 1 male, Neel Gap, 22 May 1946, W.F. Fattig (GMNH); 1 male, Union Co., Neel Gap, 22 May 1946, P.W. Fattig, det. as *P. nebulosa* by A. B. Gurney 1947 (CUAC); 1 male, Union Co., 17 June 1984, G. Storey (GMNH); 2 males, White Co., 28 May 2007; 1 female, White Co. 34.57371ºN 83.64269ºW, 415 m, 28 April 2012; 1 male, White Co., Anna Ruby Falls, 2 June 1974, H. O. Lund (GMNH); NORTH CAROLINA, 1 male, Henderson Co., 2 miles SE Edneyville off road 1717, 21 July 1984, K. J. Smart at light trap (CUAC); 1 male, Macon Co., Coweeta Hydrologic Lab, 3 July 1983, R. Turnbow (GMNH); 2 males, same locality, 1 June 1986, K. Morgan, C. L. Smith, & S. P. Smith (GMNH), 2 males, same locality, 4–18 September 1979, C. L. Smith, in

malaise trap (GMNH); 1 male, same locality, 18 June 1985, A. D. Buryn & R. Morris, at black light (GMNH); 1 male same locality, 11–24 July 1979, C. L. Smith, in malaise trap (GMNH); 2 males, Macon Co., Nantahala N.F. Standing Indian Campground, 17 June 1978, J. Morse (CUAC); Macon Co., Wayah Crest, 8–9 June 1984, J. Jamison, det. as *P. nebulosa* by Morse 1984; (CUAC); SOUTH CAROLINA, 1 male, Pickens Co., Laurel Creek, 12 June 1976, J. W. Chapin (CUAC). Unless otherwise noted, specimens at GMNH and CUAC were previously determined by G. W. Byers as *P. nebulosa*. Holotype, allotype, and paratypes are deposited in the NMNH; additional paratypes are deposited in the Georgia Museum of Natural History (GMNH), Athens, Georgia; Clemson University Arthropod Collection (CUAC), Clemson, South Carolina; California Academy of Sciences (CAS), San Francisco, California; Florida State Collection of Arthropods (FSCA), Gainesville, Florida; and collection of the author (WB).

Description. Head dorsum yellowish brown; area surrounding ocelli dark brown. Rostrum yellowish brown; genae yellowish brown. Labrum and maxillary palps brown. Antennal scape yellowish brown; pedicel dark brown; flagellum black, with approximately 40 flagellomeres.

Pronotum yellowish brown, anterior margin with one spot on each side of mid-line; 7–11 stout black setae at each side on anterior margin and 2–4 less stout black setae on posterior margin. Mesonotum and metanotum yellowish brown with brown spot at wing bases, with numerous short hairs directed caudally. Scutellum brown. Pleural surfaces, coxae and mera sordid white with numerous short, golden setae on anterior coxa and on mesothoraxic coxa and episternum; sparse, pale, fine setae on metathorax, most numerous on coxa; small black spot at each end of mesepimeron and metaepimeron. Femora and tibiae sordid white to yellowish brown with numerous short apically directed golden hairs and a lesser number of black setae. Tibiae with two brown spines. Tarsi yellow-brown with numerous apically directed golden hairs.

Wings (Fig. 3) faintly tinged with brown, markings brown, veins brown, stigma indistinct, thyridium at first fork of M hyaline, humeral spots (Byers 1993) absent, first basal spot (Esben-Petersen, 1921) present, second basal spot absent, basal band reduced to one spot at OR_S and one spot between $1cu_1$-$2cu_2$ and $2cu_1$-$2cu_2$, marginal spot (Esben-Petersen, 1921) reduced to two spots between termination of Sc and thyridium, pterostigmal band includes both basal and apical branches but reduced to variable series of spots extending from C to M_4, and from C to Cu_1, apical band reduced to numerous spots between R_1 and M_4 termini.

Male terga 2–5 yellowish brown, sometimes with patches of brown; sterna 2–5 yellowish brown; pleural areas sordid white; tergum 3 posterior margin with notal organ slightly pronounced above; tergum 4 anterior margin with small protuberance; segment 6 yellowish orange, long, narrowing caudally with length twice anterior diameter, posterior truncate without anal horn; segments 7–8 yellowish orange, long, diameter expanding caudally, length five times caudal diameter.

Genital bulb elliptic (Fig. 4). Basistyles fused one-third of length ventrally, apices bearing tuft of 4–9 long, dark brown to black setae. Hypovalves of sternum 9, slender, parallel, extending two-thirds distance between ventral fusion of basistyles and bases of dististyles, mesal margins bearing 8–15 long, black setae. Tergum 9 (Fig. 5) yellowish brown, narrowing apically, extending additional one-fifth beyond cerci to just beyond base of dististyles, deeply emarginate. Cerci yellowish brown. Dististyles falcate tips, thin, 37–40% of length; outer margin slightly concave until acute tip; with large, cuplike basal lobe prolonged ventromesally; dorsomesal edge of basal cup with triangular tooth. Ventral parameres of aedeagus long, sigmoidal or cork-screw-like, slender, single-branched, extending one half length beyond base of dististyles and typically curving ventrally out of genital bulb; apices accuminate; ventral parameres apically narrow, flattened, glossy orange-brown, with few fine setae on mesal margins; basal mesal margins with numerous longer, fine golden setae. Dorsal parameres of aedeagus broad basally, narrowing apically, dorsally flattened, extending just beyond bases of dististyles. Dorsal and ventral valves small, cone-shaped. No aedeagal hamulus (Byers, 1993).

Female terga 2–8 brown; sterna yellowish brown; pleural areas sordid white; female subgenital plate yellowish brown, shield-shaped, yellow to yellowish brown, pale medially, with numerous fine setae on caudal margin; genital plate (Fig. 6) with well developed distal plate, basal plate, accessory plate; distal plate deeply emarginate, spermathecal apodemes (Thornhill et. al. 1974) narrow, long, divergent.

Measurements. Length of male approximately 10.1 mm; length of female approximately 9.0 mm.

Etymology. The specific name *cryptica* or "hidden" refers to the morphologically cryptic nature of this species.

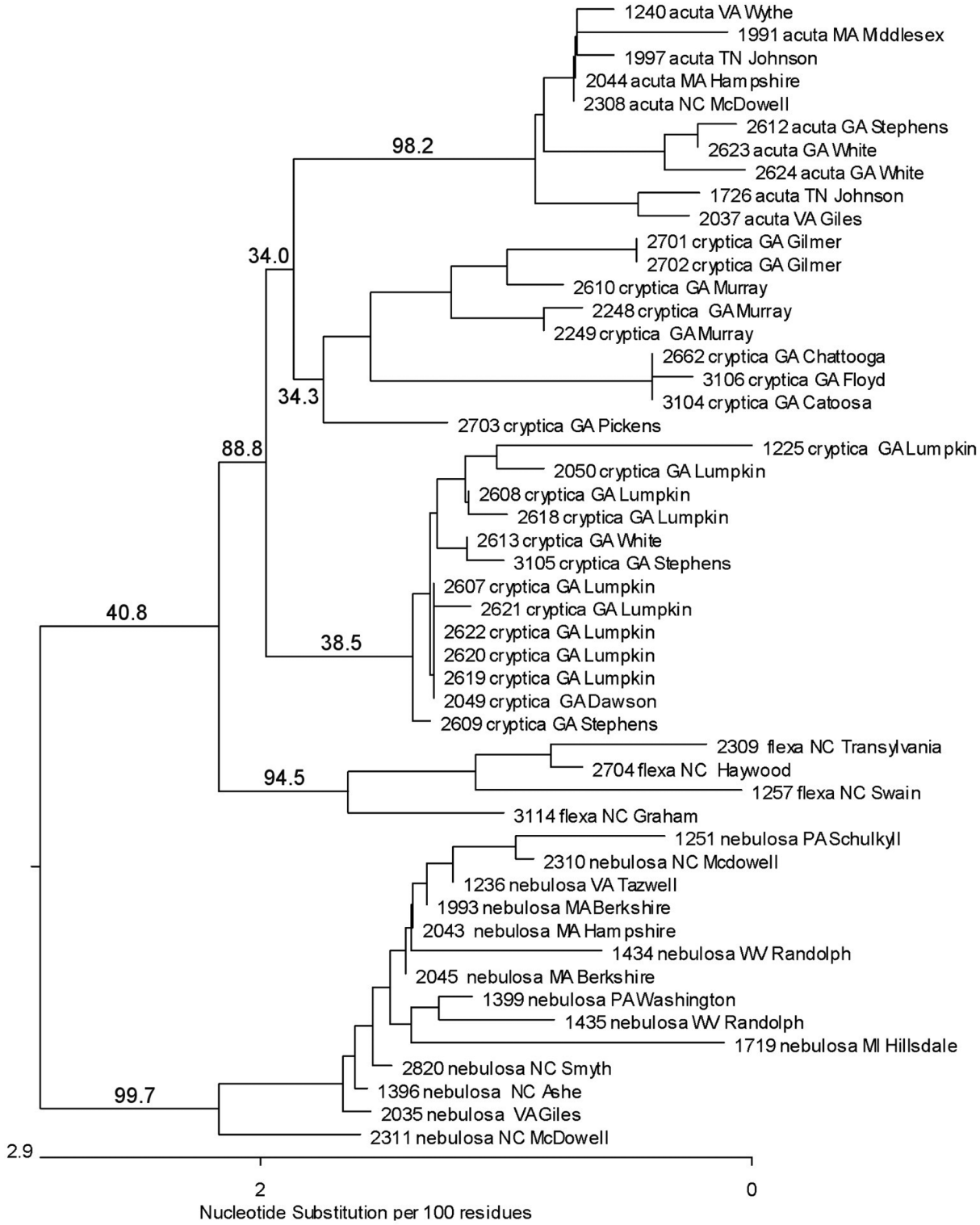


FIGURE 1. Neighbor-joining tree of 50 specimens of *Panorpa acuta*, *P. cryptica* **n. sp.**, *P. flexa*, and *P. nebulosa*. Bootstrap values based on 1,000 trials are included for the major nodes. Scale indicates percent difference.

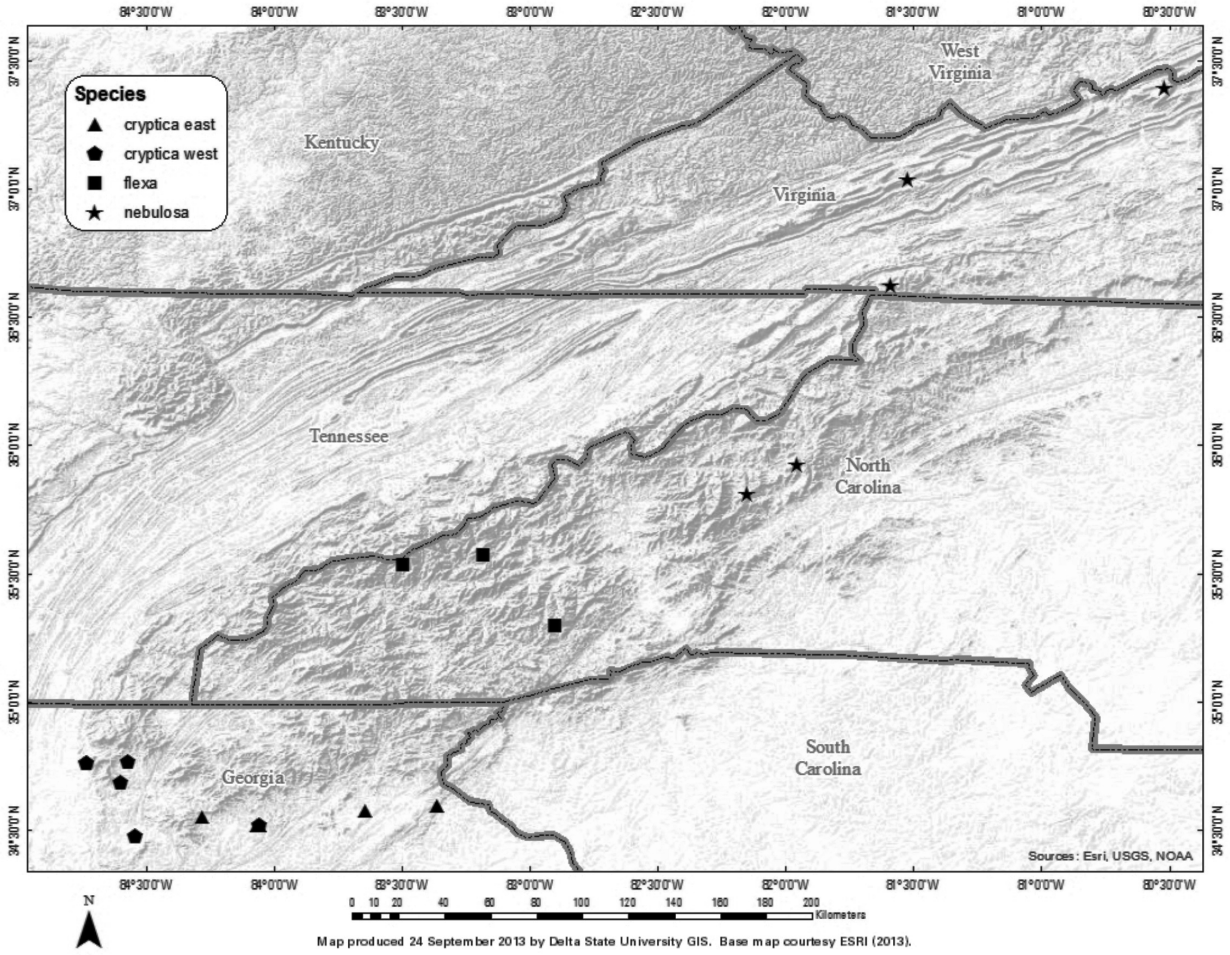


FIGURE 2. Map showing collection localities of sequenced *Panorpa cryptica* **n. sp.**, *flexa*, and *nebulosa* specimens.

Taxonomic remarks. *Panorpa cryptica* appears very similar to *P. nebulosa* and somewhat similar to *P. flexa*, but the males of *P. cryptica* differ in having thin, nearly sigmoidally curved ventral parameres, which typically curve corkscrew-like ventrally out of the genital bulb. The ventral parameres of *P. nebulosa* are slightly thicker, are either straight or curve slightly mesad, and remain within the genital bulb. Further, the tufts of long, black setae on the apices of the basistyles of *P. cryptica* (Fig. 7) are not borne by pronounced caudally directed tubercles or lobes, whereas those of *P. nebulosa* (Fig. 8) are pronounced in all specimens examined from Wisconsin to Canada and New England to Tennessee. The basistyle apices of *P. flexa* (Fig. 9) are somewhat similar to those of *P. cryptica*, but the dististyles have shorter and thicker falcate tips than *P. cryptica* and *P. nebulosa*. The ventral parameres of *P. flexa* curve strongly mesad, and the apices are broad, bare, and do not curve ventrally out of the genital bulb. *Panorpa acuta* may be readily differentiated from *P. cryptica* and all other North American *Panorpa* by its unique truncate tergum 9 and uniquely shaped dorsal parameres and hypovalves.

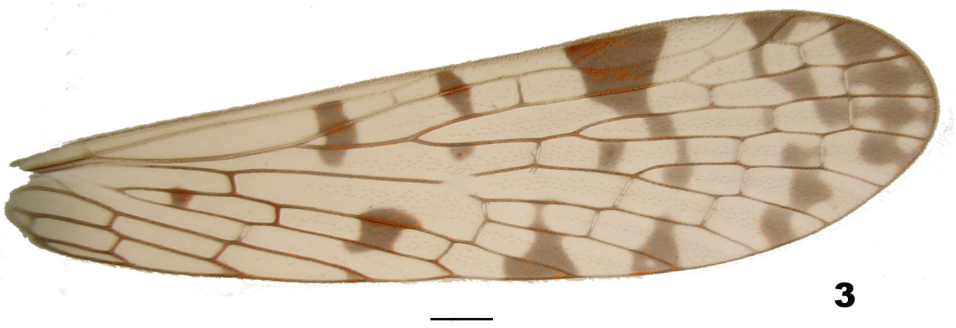
In the field, *P. acuta* appears comparatively larger and males have a reddish orange abdomen (segment 6). *Panorpa flexa* typically has a black abdomen (segments 2–5) and unmarked or faintly marked wings. *Panorpa cryptica* is the smallest of the four species and the males have a paler genital bulb than *P. nebulosa*. *Panorpa cryptica* flies earlier in the year than *P. nebulosa*.

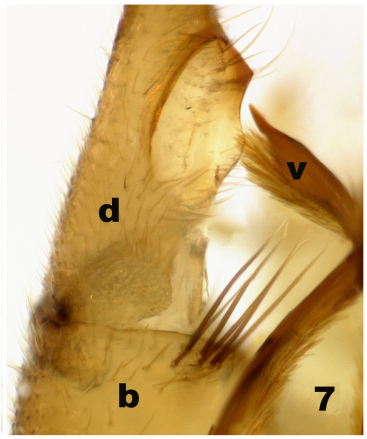
COI analysis suggests that *P. cryptica* is closely related to *P. acuta*, *P. flexa*, and *P. nebulosa* but most closely related to *P. acuta* (Fig. 1). Yet, morphologically *P. acuta* is more different from the other three species than they are from each other, and the range of *P. acuta* overlaps that of each of the other three species.

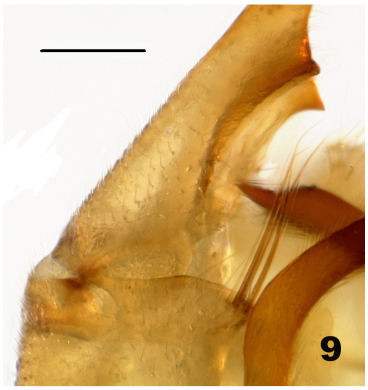
Biology and ecology. Individuals of *P. cryptica* were observed from mid-April through until early July sitting horizontally on the top surfaces of vegetation in partially broken to deep shade. Other scorpionflies, such as *Panorpa isolata* Carpenter, 1931, tended to be more abundant in areas that offered slightly greater sunlight, while

P. cryptica tended to be more abundant deeper into the forest shade. Males of *P. cryptica* were observed on the top surfaces of vegetation (Fig. 10) offering dead insects to females. Males were not observed offering salivary masses to females, although it is possible that males do when dead insects are scarce. Ma *et al.* (2011) presented internal structural evidence of why some species of *Panorpa* do not offer salivary masses as nuptial gifts. We have not attempted to dissect males of *Panorpa cryptica* to determine the extent of development of the male salivary glands.

Distribution. Carpenter (1935) recorded *P. flexa*, which is likely *P. cryptica* **n. sp.** from Yonah Mountain, Georgia. *Panorpa cryptica* **n. sp.** appears to be limited to mid-elevation areas south of the Appalachian Mountains of northern Georgia, western North Carolina, and northwestern South Carolina. *Panorpa cryptica* **n. sp.** is active earlier in the spring than *P. nebulosa* and *P. flexa* and sometimes flies in mixed populations with *P. acuta*.







FIGURES 3–6. *Panorpa cryptica* **n. sp.**, topotypes. 3) Male right forewing, scale bar = 2 mm. 4) Male genital bulb, ventral aspect. 5) Male genital bulb, dorsal aspect, scale bar = 0.5 mm. 6) Female genital plate. Scale bar = 0.5 mm.
FIGURES 7–9. Apical margin, left male basistyle, ventral aspect. 7) *Panorpa cryptica* **n. sp.**, topotype: b, basistyle; d, dististyle; v, ventral paramere. 8) *Panorpa nebulosa*, specimen from Berkshire Co., Massachusetts. 9) *Panorpa flexa*, specimen from Swain Co., North Carolina. Scale bar = 0.1 mm.

FIGURE 10. *Panorpa cryptica* **n. sp.**, male at type locality in Murray Co., Georgia.

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