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A taxonomic reassessment of the *Drunella lata* (Morgan) species complex (Ephemeroptera:Ephemerellidae) in northeastern North America

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Abstract. For many years the *Drunella lata* complex of eastern North America consisted of 4 recognized species: 3 described from the Northeast, *Drunella lata* (Morgan), *Drunella cornuta* (Morgan), and *Drunella cornutella* (McDunnough), and 1, *Drunella longicornis* (Traver), from the southern Appalachian Mountains region. Recently, all 4 were synonymized to a single species (*D. lata* [Morgan], by priority) because morphological variation was perceived to be continuous and historical characterizations of species were viewed as arbitrary. We used genetic, morphometric, and life-history data to reevaluate *D. lata*, *D. cornuta*, and *D. cornutella* in northeastern North America, where all 3 morphotypes coexist in certain catchments and often in the same reach of stream. Pairwise genetic comparisons of coexisting morphotypes revealed fixed allelic differences at 6 to 10 of 19 allozyme loci, showing reproductive isolation among *D. cornuta, D. cornutella*, and *D. lata* even within the same reach of stream. Morphometric studies confirmed their distinction by uncovering significant differences in a suite of characters, some with nonoverlapping ranges (i.e., diagnostic). The 3 taxa also were shown to be segregated ecologically by consistent differences in seasonality, thermal requirements, and spatial distribution. We conclude that *D. cornuta*, *D. cornutella*, and *D. lata* are all worthy of full species status, and so reinstate *D. cornuta* and *D. cornutella*. Diagnostic descriptions are provided and existing keys emended.

Key words: mayfly, species boundary, allozymes, morphometrics, life history.

Ephemerellid mayflies are a common and conspicuous component of the stream insect fauna in North America, and the ability to determine the presence or absence of taxa in a given stream can be important for assessing water quality. Unfortunately, this task can be difficult and sometimes is confounded by the large amount of morphological variation found within the group and its interpretation by systematists over the years. In the early 20th century, authors of some species failed to appreciate the extent of intraspecific variation, resulting in very narrow species concepts and an inflated number of available names. Some of these were reduced to synonymy as part of Allen and Edmunds' comprehensive revision of the North American fauna (Allen and Edmunds 1959, 1961a, b, 1962a, b, 1963a, b, 1965), which provided the first really workable keys for identifying species in this group. Following Allen and Edmunds' revision, species-level taxonomy remained relatively stable for many years. Recently, many additional taxa were synonymized in 3 of the largest genera (*Ephemerella* and *Serratella* [McCafferty 2001, Jacobus and McCafferty 2003] and *Drunella* [McCafferty 1993, Jacobus and McCafferty 2004]) which reduced the number of recognized North American species in *Serratella* and *Drunella* by ⅓ and in *Ephemerella* by ½.

Such reductions are convenient because they make it easier to apply names to specimens, but they can jeopardize our understanding of benthic community structure and weaken the foundation of water-quality monitoring if the reductions are unwarranted. We reexamine one of the objects of this synonymization, the *Drunella lata* complex of eastern North America. Specifically, we test Jacobus and McCafferty's (2004) conclusion that character states traditionally attributed to each of the 4 species in that complex were arbitrary and specimens matching published descriptions of particular species represented points along a continuum and, thus, were actually manifestations of a single, highly variable species, *Drunella lata* (Morgan). Our

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test is based largely (but not solely) on data and specimens from the headwater tributaries of the Delaware River, where populations of the previously recognized morphotypes of northeastern *D. lata* complex (i.e., *Drunella lata*, *Drunella cornuta*, and *Drunella cornutella*) had been reported to coexist (Sweeney 1993). Our null hypothesis for the study was that if the synonymization of the *D. lata* complex were valid, then morphological characters of the study populations would represent an uninterrupted continuum and evidence would exist for uninterrupted gene flow among the populations and a continuum of life-history characteristics among the morphotypes.

Based on extensive morphometric, genetic, and life-history evidence, we reject our null hypothesis and conclude that *D. lata*, *D. cornuta*, and *D. cornutella* are distinct taxa in the Delaware River and elsewhere. Thus, we reinstate full species status for *D. cornuta* and *D. cornutella*, provide diagnostic characters for larvae and adult males, and emend the taxonomic keys of Allen and Edmunds (1962a).

Methods

Taxonomic history of the Drunella lata *complex*

The *Drunella lata* complex of mayflies in eastern North America originally consisted of 6 species described between 1911 and 1932: *D. lata* (Morgan 1911), *D. cornuta* (Morgan 1911), *Ephemerella inflata* McDunnough 1926, *Ephemerella depressa* Ide 1930, *D. cornutella* (McDunnough 1931a), and *Drunella longicornis* (Traver 1932). Following the revision of Allen and Edmunds (1962a), the group was left with 4 described species: *D. lata* (Morgan 1911), *D. cornuta* (Morgan 1911), *D. cornutella* (McDunnough 1931a), and *D. longicornis* (Traver 1932), distinguished from each other mainly by the shape and relative degree of development of the frontoclypeal projection and median ocellar tubercle in the larvae and by differences in the shape of the genital forceps in adult males (McDunnough 1931b, Allen and Edmunds 1962a). Recently, Jacobus and McCafferty (2004) examined specimens from much of the range of this group in eastern North America and concluded that the *D. lata* complex consisted of a single, highly variable species, *Drunella lata* (Morgan) (by precedence).

Study sites/populations

Table 1 lists the collection sites used in this study and the types of observations or data collected from each. Most of the genetic and morphometric data came from material collected at 8 sites in the Delaware River drainage in New York and Pennsylvania (sites 12–19 in Table 1; Fig. 1). These sites were established during a study done in 1982 to 1984 to investigate thermal effects of reservoirs on mayfly biology (Sweeney et al. 1986, Sweeney 1993). Included were 2 upstream control sites (WBC5 and EBM5), 2 sites impacted thermally by hypolimnetic release from the Cannonsville and Pepacton reservoirs (WBD6 and EBD5, respectively), 2 partial recovery sites (WBH7 and EBH5), an unimpounded control site (BVK5), and a full recovery site (DEL8). Together, these sites present an array of thermal regimes that strongly affect the distribution and life-history patterns of the native fauna. For our study, the relative abundance of *D. cornuta*, *D. cornutella*, and *D. lata* morphotypes at these sites was determined by calculating the proportion of each in the total number of specimens collected during that study. *Drunella tuberculata* and *Drunella walkeri* also were found at those sites and were included here to help put genetic differences among members of the *D. lata* complex into perspective.

Analysis of genetic structure

Enzyme electrophoresis was done on adult tissues, following the techniques described in Funk et al. (1988). Adults were obtained by rearing field-collected full-grown or nearly full-grown larvae in the laboratory. Preliminary analysis of larval gut contents and indirect evidence (the raptorial nature of the forelegs) indicated that members of the *D. lata* complex are predatory. Thus, larvae were fed fresh algae (a mixed-diatom community) cultured on acrylic plates (Sweeney and Vannote 1984) supplemented with live animal material (cultured larvae of the baetid mayfly *Centroptilum triangulifer* and a parthenogenetic midge, *Paratanytarsus* sp.). Emerging subimagos were reared to the imago, and thoracic tissues were stored at −80°C prior to enzyme electrophoresis. Larval exuviae and imaginal heads, abdomens, and legs were preserved in 95% ethanol and referenced individually to frozen thoracic tissues. Genetic data were analyzed using BIOSYS-1 (Swofford and Selander 1981).

Morphometrics

Morphometric data were taken either directly with a calibrated reticle on a Leica MZ16 (Leica Microsystems, Wetzlar, Germany) stereomicroscope (wing length) or from digital photographs taken through a Wild M5 (Wild Heerbrugg AG, Heerbrugg, Switzerland) stereomicroscope (all other measures). Linear measurements from photos were made in Adobe PhotoShop® 7.0 (Adobe Systems, Inc., San Jose, California) and area was determined with IP Lab Spectrum 3.1.2a (Scanalytics, Inc., Fairfax, Virginia).

TABLE 1. Collection site information and type of data collected for *Drunella* species. Under the morphometric (M) subheading, M followed by a number indicates the number of individuals measured; E indicates specimens were examined but not included in our statistical analysis. Under the phenological (P) subheading, X indicates that regular samplings of population mass structure were made for ≥1 y. Under the genetic (G) subheading, numbers indicate number of individuals electrophoresed. PQ = Province de Québec, ME = Maine, VT = Vermont, NY = New York, PA = Pennsylvania, VA = Virginia. Trib. = tributary. Blanks indicate data not collected.

Site no.	Latitude (°N)	Longitude (°W)	State/ province	Site name	Site code	*cornuta*			*cornutella*			*lata*			*walkeri*		*tuberculata*	
						M	P	G	M	P	G	M	P	G	P	G	P	G
1	50.28250	−65.64190	PQ	River Pigou	PIG6	M 6	X											
2	50.27890	−65.72000	PQ	Rive aux Loups Marins	LOU6	M 3	X											
3	46.05361	−68.44333	ME	Crystal Brook	CRY3	E	X		E	X								
4	45.90056	−69.03944	ME	Nesowadnehunk Stream	WAD5	E	X		E	X								
5	45.86389	−68.52306	ME	Swift Brook	SWI4	E	X		E	X								
6	45.24167	−69.54528	ME	Piscataquis River	PIS7	E	X		E	X		E	X					
7	43.24139	−73.10806	VT	Gilbert Brook	BAT2	E	X											
8	43.22972	−73.11972	VT	South Fork Goodman Brook	BAT1	M 12	X											
9	43.21944	−73.07167	VT	West Branch Batten Kill River	BAT3				E	X					X			
10	43.10111	−73.24194	VT	Batten Kill River	BAT5	E	X		E	X		E	X		X			
11	43.09778	−73.14194	VT	Batten Kill River	BAT4	E	X		E	X		E	X		X			
12	42.17530	−75.01720	NY	West Branch Delaware River	WBC5	M 2		2										
13	42.15500	−74.62500	NY	East Branch Delaware River	EBM5	M 4		3										
14	42.07722	−75.40583	NY	West Branch Delaware River	WBD6	E	X		E	X								
15	42.07194	−75.00694	NY	East Branch Delaware River	EBD5	M 2	X	9	M 8	X								
16	42.02500	−75.12056	NY	East Branch Delaware River	EBH5	M 31	X	26	E	X		E	X		X	8	X	
17	42.00278	−75.38417	NY	West Branch Delaware River	WBH7	M 3	X	3	E	X								
18	41.97167	−75.03972	NY	Beaver Kill	BVK5	E	X		M 24	X	20	M 10	X	10	X	4	X	
19	41.86722	−75.26389	PA	Delaware River	DEL8	E	X		E	X		M 30	X	10	X		X	6
20	41.81972	−75.93333	PA	Trib. of Wyalusing Creek	MES2				E	X								
21	41.78982	−75.58628	PA	Tunkhannock Creek	TUN4	E			M 5		5							
22	41.75750	−75.74611	PA	Trib. of Partners Creek	MES1				E	X								
23	41.73694	−75.86000	PA	Trib. to Meshoppen Creek	MES3				E	X								
24	41.71722	−75.87139	PA	Meshoppen Creek	MES4	E	X		E	X								
25	41.61250	−76.01611	PA	Meshoppen Creek	MES5	E	X		E	X								
26	40.10250	−75.57750	PA	Pickering Creek	WCC4				E	X					X			
27	38.79389	−78.11778	VA	Hittles Mill Stream	JOR3				E	X								
28	38.78528	−78.14833	VA	Bearwillow Creek	JOR2				E	X					X		X	
29	38.76417	−78.03444	VA	Jordan River	JOR4				E	X					X			
30	38.62917	−78.06667	VA	Thornton River	JOR5				E	X					X		X	
31	37.38944	−79.55139	VA	Big Otter River	BIG4				M 2	X								
32	37.37056	−79.42056	VA	Big Otter River	BIG5				M 2	X					X			

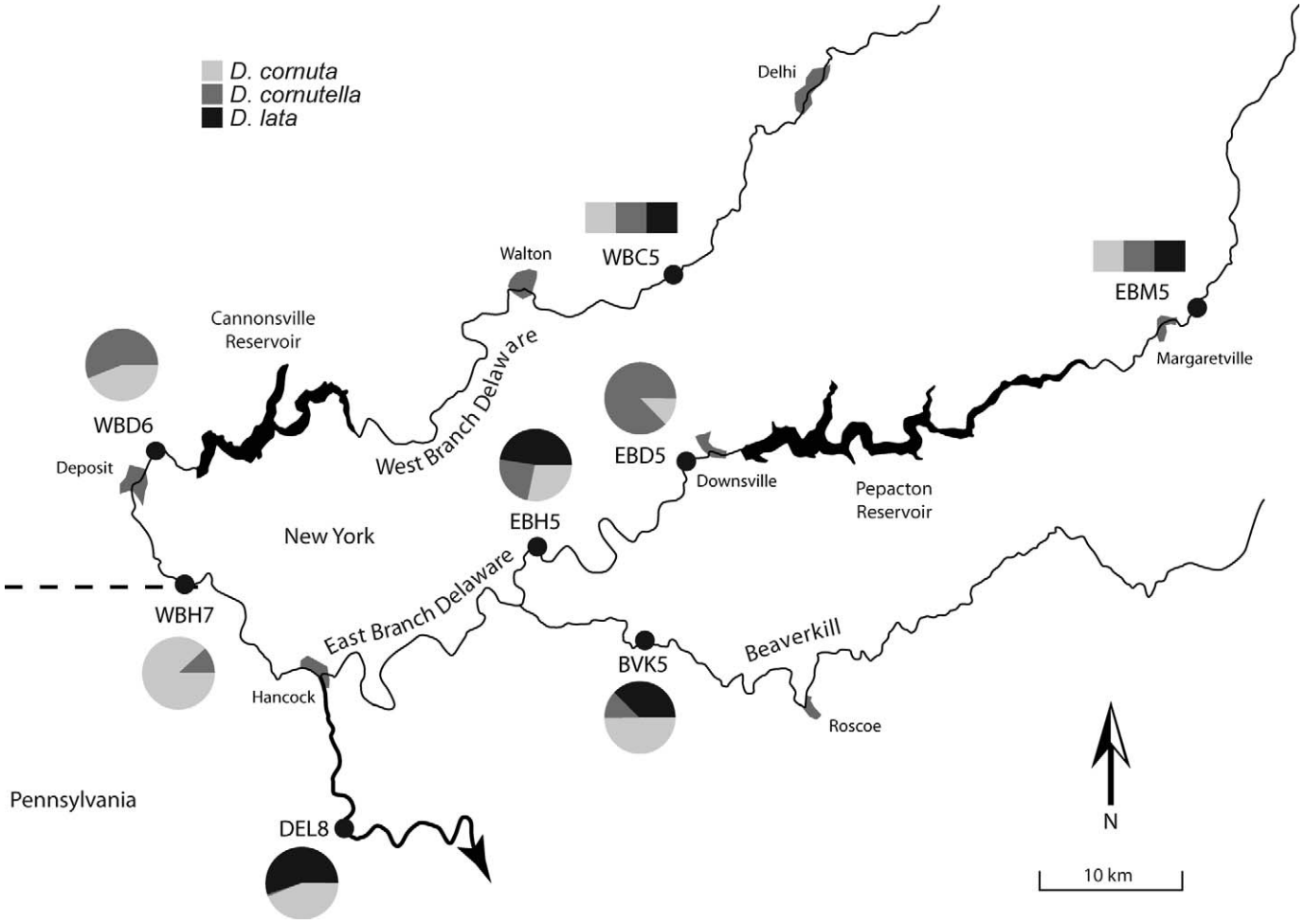

FIG. 1. Collection sites on the upper Delaware River drainage in New York and Pennsylvania. The West and East branches of the Delaware (with reservoirs) and the Beaverkill River are shown (site abbreviations are in Table 1). Pie charts show the relative abundance of *D. lata* complex species at the sampling sites. All 3 species were present at sites WBC and EBM, but relative abundances were not determined.

Data were analyzed using DataDesk® 6.2.1 (Data Description, Inc., Ithaca, New York). In most cases, larval measurements were made on final-instar larval exuviae from reared specimens. When no reared material was available, measurements were made on full-grown larvae (as indicated by darkened wing-pads). All specimens used in our study are deposited at the Stroud Water Research Center, Avondale, Pennsylvania (US).

For 1- or 2-dimensional quantitative characters to be used effectively in identifications of 3-dimensional animals, consistency in measurement technique is essential. Figure 2 illustrates how measurements were taken for our study. The following descriptions refer to that figure. For larval characters, measure *a*, the protrusion of the median ocellar tubercle, was taken from a dorsal perspective, viewed tangentially across the surface of the frontoclypeal sclerite. One line was drawn across the peaks of the low tubercles of the lateral ocelli and a parallel line was drawn across the peak of the median ocellar tubercle, with *a* representing the distance between those lines. Measures *b–d* were taken from a facial view; *b* is the frontoclypeal width, and *c* and *d* represent the length and width of the frontoclypeal projection, respectively. Measures *e–i* were made from a dorsal view of the foreleg. Measure *e* represents the greatest width of the femur (exclusive of the spines on the anterior margin), perpendicular to its length, *f*. Measure *g* is the length of the fore tibia exclusive of its distal spine, *h*. Measure *i* is the length of the fore tarsus. Area of the fore femur was taken on the entire silhouette of the femur from a dorsal view, including spines (but not setae). Lengths of the tibia and femur for the middle and hind legs were measured in dorsal view, *j* and *k* in the figure. Body length was measured in dorsal view from the front of

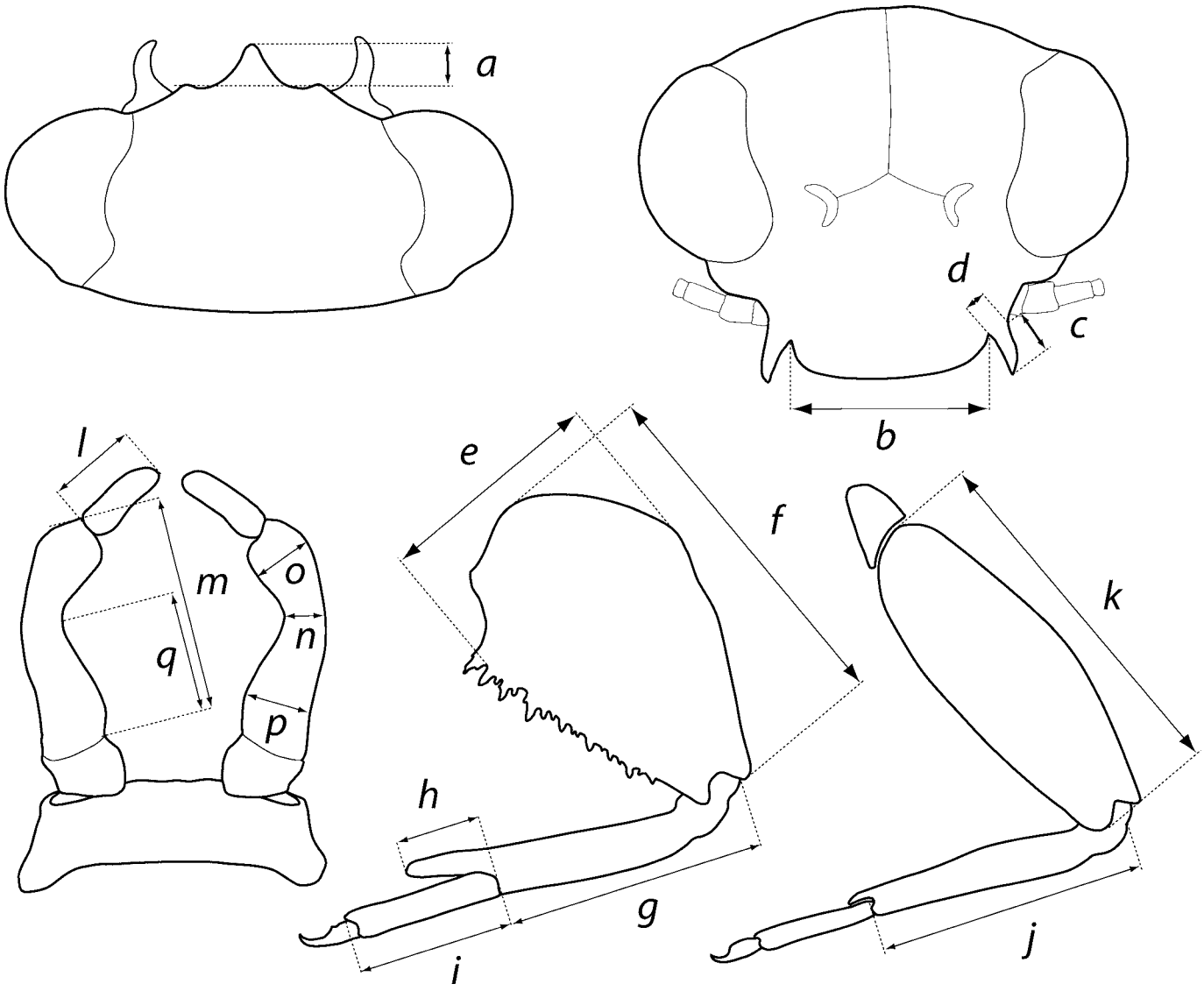

FIG. 2. Larval measurements: *a–k*.—Measure *a* (head of *Drunella cornuta*) was taken from a dorsal view, tangential to the surface of the frontoclypeal sclerite. Measures *b–d* (head of *Drunella cornutella*) were taken from a frontal view. Measures *e–i* (foreleg of *D. lata*) were made from a dorsal view of the foreleg, and *j* and *k* (hind leg of *Drunella lata* is shown, middle leg is similar) were made from a dorsal view of the middle and hind legs. Adult measurements: *l–q* (genital forceps of *D. lata*).—Measures of the male genital forceps were taken in ventral view. See text for detailed explanations.

the head (exclusive of antennae) to the tip of the 10th tergite (exclusive of caudal filaments). In imagos, measures of the male genital forceps were taken in ventral view. Measures *l* and *m* represent the lengths of the 3rd and 2nd segments, respectively. The 2nd forceps segment in the *D. lata* complex is narrowest somewhere near or just beyond the middle and dilated near the base and near the distal end. Measure *q* is the distance from the base of segment 2 to the center of the narrowest region. Measure *n* is the width of segment 2 at the center of its narrowest region, *o* is the width at the broadest spot on the distal dilation, and *p* the widest spot on the basal dilation. Wing length was measured from the notch at the base of the costal vein to the tip of the forewing on male and female imagos.

Life history and ecology

Sites used in life-history studies were visited periodically throughout the year. Estimates of the size structure of larvae on a given sampling date were made by measuring individual dry mass of 30 to 50 specimens. Collections during the major growth season for larvae were made at intervals of $\leq$14 d. Dates for the onset of adult emergence were deter-

TABLE 2. Allele frequencies for *Drunella* species. (Loci MDH2, TPI, and G3PDH were monomorphic.) *n* refers to the total number of individuals sampled at a particular locus.

Locus	Allele	*cornuta*	*cornutella*	*lata*	*tuberculata*	*walkeri*
MDH1	*n*	34	34	20	6	12
	A	–	0.62	–	–	–
	B	0.99	0.38	1.00	1.00	–
	C	0.02	–	–	–	1.00
ME	*n*	34	34	20	6	12
	A	–	–	1.00	–	–
	B	1.00	1.00	–	1.00	1.00
PK	*n*	16	20	16	2	2
	A	1.00	–	–	–	–
	B	–	–	1.00	1.00	1.00
	C	–	1.00	–	–	–
ADK	*n*	20	25	20	6	12
	A	1.00	1.00	1.00	–	–
	B	–	–	–	1.00	1.00
SOD	*n*	34	34	20	6	12
	A	1.00	–	–	–	–
	B	–	1.00	1.00	1.00	–
	C	–	–	–	–	1.00
GPI	*n*	34	34	20	6	12
	A	–	0.16	–	–	–
	B	1.00	–	–	–	–
	C	–	0.81	1.00	–	–
	D	–	0.03	–	–	0.04
	E	–	–	–	1.00	0.92
	F	–	–	–	–	0.04
PGM	*n*	34	34	20	6	12
	A	–	–	–	0.92	1.00
	B	–	–	–	0.08	–
	C	–	–	1.00	–	–
	D	–	1.00	–	–	–
	E	1.00	–	–	–	–
MPI	*n*	34	34	20	6	12
	A	–	–	–	–	0.08
	B	–	–	–	1.00	0.79
	C	0.94	0.62	–	–	0.13
	D	0.06	0.38	–	–	–
	E	–	–	0.98	–	–
	F	–	–	0.03	–	–
aGPDH	*n*	34	34	20	6	12
	A	0.02	–	–	–	–
	B	0.99	1.00	1.00	1.00	1.00
HEX	*n*	34	34	20	6	12
	A	–	0.62	–	–	–
	B	1.00	0.38	0.88	–	1.00
	C	–	–	–	1.00	–
	D	–	–	0.13	–	–
ISDH2	*n*	34	34	20	6	12
	A	–	0.85	1.00	–	–
	B	–	0.15	–	–	–
	C	–	–	–	1.00	1.00
	D	1.00	–	–	–	–
6PGD	*n*	34	34	20	6	12
	A	–	1.00	–	–	–
	B	1.00	–	1.00	1.00	1.00
TRI	*n*	20	25	20	6	12
	Y	0.03	–	–	–	–
	Z	–	0.96	–	–	–
	A	0.63	–	–	–	0.21
	B	0.33	0.04	–	–	–

Table 2. Continued.

Locus	Allele	*cornuta*	*cornutella*	*lata*	*tuberculata*	*walkeri*
TRI	C	–	–	0.93	–	–
	D	0.03	–	–	0.92	0.75
	E	–	–	–	0.08	0.04
	F	–	–	0.08	–	–
DIP2	*n*	20	25	20	6	12
	Z	–	0.02	–	–	–
	A	–	0.08	–	–	–
	B	1.00	0.90	–	–	–
	C	–	–	–	–	1.00
	D	–	–	1.00	–	–
	E	–	–	–	1.00	–
AAT1	*n*	34	34	20	6	12
	Z	0.02	–	–	–	–
	A	0.99	0.02	–	–	–
	B	–	0.99	–	0.08	0.17
	C	–	–	1.00	0.92	–
	D	–	–	–	–	0.83
AAT2	*n*	34	34	20	6	12
	A	1.00	1.00	1.00	0.08	0.96
	B	–	–	–	0.92	0.04

mined either by direct observation of adults in the field, rearing of adults in the laboratory from field-collected larvae held $\leq$1 wk at temperatures simulating field conditions, or by the presence of mature larvae (with darkened wingpads) in samples taken for population mass structure. Water temperature was recorded with Ryan Instruments Model J thermographs (Ryan Instruments, Redmond, Washington) throughout the study period. Degree-days for larval development were calculated as the sum of the daily mean water temperature from 1 January until the beginning of adult emergence. The masses of the 5 largest individuals collected at the onset of emergence from any given site/year combination were averaged to characterize maximum male and female masses. Summary statistics for degree-days and maximum masses include only those site/year combinations for which data were complete enough to yield a robust estimate of the date for onset of emergence.

Fertilized eggs of *D. lata*, *D. cornutella*, and *D. walkeri* were obtained by placing field-collected female imagos (that had been preparing to oviposit when collected, as evidenced by extruded egg masses) on the surface of water in a 30-mL museum jar, at which time they released their eggs into the water. Eggs were reared in the same jar filled with filtered (0.45-μm-pore size) sterilized stream water (Sweeney and Vannote 1987, Funk et al. 2006) at ambient White Clay Creek (Pennsylvania) temperature and photoperiod in the wet laboratory at Stroud Water Research Center. For *D. cornuta*, copulation was induced between male and female imagos reared in the laboratory as described by Huff and McCafferty (1974). Eggs were dissected from the females 10 min after copulation and placed in rearing jars as above. Eggs were monitored 3 times/wk (on average), and hatchlings were removed and counted.

Results and Discussion

Genetics

Nineteen allozyme loci, 16 of which were polymorphic (Table 2), were scored. Only 1 significant departure from Hardy–Weinberg expectations in genotype fre-

Table 3. Genetic distances among *Drunella* species. Values for Nei's *D* are shown above the diagonal; values for Roger's *D* are shown below the diagonal.

Species	*cornuta*	*cornutella*	*lata*	*tuberculata*	*walkeri*
cornuta	–	0.633	0.734	0.951	0.782
cornutella	0.491	–	0.630	0.983	0.959
lata	0.523	0.487	–	0.718	0.839
tuberculata	0.613	0.626	0.517	–	0.369
walkeri	0.545	0.616	0.569	0.331	–

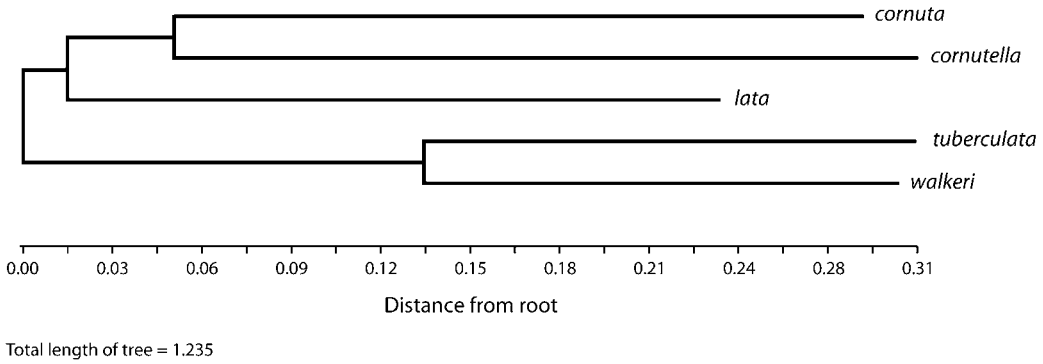

FIG. 3. Wagner tree produced from Rogers (1972), showing distances among *Drunella* species.

quencies was detected: locus HEX in *D. cornutella* from the Beaverkill showed evidence of heterozygote deficiency. Frequencies (Table 2) were averaged by species because no significant differences in allele frequencies were found among local populations of *D. lata*, *D. cornuta*, or *D. cornutella* morphotypes. Pairwise comparisons of species revealed fixed allelic differences at numerous loci. For example, *D. cornuta* shared no alleles with *D. cornutella* at 6 loci or with *D. lata* at 10 loci, and *D. cornutella* and *D. lata* shared no alleles at 8 loci. These data indicate a lack of recent gene flow despite the clear opportunity afforded by sympatry.

Genetic distances among *D. cornuta*, *D. cornutella*, and *D. lata* were high (Table 3). Nei's (1978) *D* ranged from 0.630 to 0.734, distinctly higher than the value observed between *Drunella tuberculata* and *D. walkeri* (0.369), a pair that Jacobus and McCafferty (2004) considered good species. These values indicate that members of the *D. lata* complex share only ~½ of their genes.

An unrooted Wagner tree generated from Rogers (1972) distances (Table 3) depicts the 3 species of the *D. lata* group as sister to *D. tuberculata* + *D. walkeri*, and within the *D. lata* group, *D. cornuta* + *D. cornutella*

TABLE 4. Summary of morphometric and life-history data for *Drunella lata*-group species. Measures 1–11 are larval, 12–18 are imaginal. See **Methods** for detailed descriptions of measurement techniques. *n*-values for morphometrics are number of individuals measured and for life-history data are number of population/year combinations. Entries under the Fig. 2 heading refer to labels in the figure.

Measure no.	Measure	Fig. 2	*D. cornuta*				
			Mean	*n*	SE	Min	Max
1	Body length (mm)		9.466	54	0.123	7.835	11.424
2	Fore femur width/length	*e/f*	0.518	54	0.004	0.457	0.576
3	Fore femur area/body length		0.177	53	0.002	0.146	0.219
4	Median ocellar tubercle protrusion (mm)	*a*	0.146	55	0.003	0.104	0.216
5	Median ocellar tubercle protrusion/frontoclypeal width	*a/b*	0.205	55	0.004	0.153	0.275
6	Frontoclypeal projection length/width	*c/d*	1.980	55	0.030	1.636	2.564
7	Frontoclypeal projection length/frontoclypeal width	*c/b*	0.274	55	0.005	0.199	0.361
8	Middle tibia length/middle femur length	*j/k*	0.908	48	0.006	0.829	1.006
9	Hind tibia length/hind femur length	*j/k*	0.810	51	0.007	0.719	0.923
10	Middle tibia length (mm)	*j*	1.914	48	0.021	1.648	2.202
11	Hind tibia length (mm)	*j*	1.873	51	0.023	1.596	2.235
12	Male wing length (mm)		9.560	7	0.183	9.010	10.330
13	Female wing length (mm)		10.931	19	0.126	9.940	12.000
14	Length of forceps segment 3 (mm)	*l*	0.205	14	0.004	0.180	0.232
15	Width of forceps segment 2 at constriction (mm)	*n*	0.084	14	0.002	0.068	0.092
16	Length of forceps segment 3/length forceps segment 2	*l/m*	0.497	14	0.008	0.437	0.541
17	Forceps segment 2 constriction/ length of segment 2	*q/m*	0.578	14	0.007	0.545	0.635
18	Forceps segment 2 distal dilation width/length segment 2	*o/m*	0.277	14	0.006	0.225	0.301
	Degree-days to emergence		783	18	21	561	918
	Average temperature (°C) at emergence		16	18	1	10	21
	Maximum female mass (mg)		7.8	17	0.5	5.0	10.7
	Maximum male mass (mg)		4.9	17	0.3	3.0	7.1

appear as a sister group to *D. lata* (Fig. 3). This topology is further supported by morphological evidence (see *Morphometrics* below). Thus, a clear lack of recent gene flow and large genetic distances between populations strongly suggest that *D. lata*, *D. cornuta*, and *D. cornutella* are distinct species.

Morphometrics

Morphometric data were collected from 144 specimens: 40 *D. lata*, 63 *D. cornuta*, and 41 *D. cornutella* (Table 1). Twenty-two measures were taken (Fig. 2). Eighteen characters derived from these measures showed significant variation among species and, thus, were useful in distinguishing species (Table 4). A principal components analysis was done using the 6 measures that most clearly differentiated species (characters 1, 2, 5, 6, 8, and 9; Table 4). The first 2 principal components together explained 80% of the variance in the morphometric data (Fig. 4A). The points fall into 3 distinct clouds corresponding to the 3 species, with no overlap.

Some of the morphological measures that differed most markedly among the study species were structures that have been used to distinguish them in the past. For example, the length of the frontoclypeal projections (relative to either their own width or the width of the frontoclypeus, measures 6 and 7; Table 4) clearly distinguished *D. lata* from the other 2 species. This character was used by both McDunnough (1931b) and Allen and Edmunds (1962a) separate *D. lata* from *D. cornuta* and *D. cornutella* in their keys. Similarly, both used body length of full-grown larvae as their primary character to distinguish *D. cornuta* from *D. cornutella*. Our body length data agree reasonably well with their figures: McDunnough used 10 and 6 to 7 mm, respectively; Allen and Edmunds used 9 to 11 and 6 to 8; we measured ~7.8 to ~11.4 and ~6.4 to ~8.5 (Table 4). Allen and Edmunds (1962a) used the relative sharpness of the median ocellar tubercle as a secondary character to distinguish *D. cornuta* from *D. cornutella*. The perception of sharpness is difficult to quantify, but one component of sharpness is the relative degree of protrusion of the tubercle, which was quantified in our study. Measures 4 and 5 (Table 4) represent this protrusion in millimeters and normalized for body size (as indicated by the width of the frontoclypeus). These measures were very effective in separating *D. cornuta* from *D. cornutella* (although there was a small amount of overlap in measure 5). McDunnough also used the relatively short middle and hind tibiae in *D. cornutella* to distinguish it from *D. cornuta*. The lengths of the middle and hind tibiae were

Table 4. Extended.

D. cornutella					*D. lata*				
Mean	*n*	SE	Min	Max	Mean	*n*	SE	Min	Max
7.418	40	0.087	6.435	8.482	7.372	40	0.066	6.600	8.741
0.578	41	0.004	0.528	0.653	0.626	40	0.004	0.567	0.684
0.140	40	0.002	0.121	0.167	0.142	40	0.002	0.102	0.163
0.059	41	0.003	0.016	0.098	0.046	40	0.002	0.025	0.079
0.105	41	0.004	0.032	0.165	0.076	40	0.003	0.037	0.128
2.054	41	0.039	1.545	2.519	1.020	40	0.017	0.778	1.286
0.289	41	0.006	0.198	0.416	0.129	40	0.003	0.095	0.175
0.806	41	0.005	0.725	0.857	0.854	40	0.005	0.729	0.925
0.701	41	0.003	0.654	0.748	0.746	40	0.004	0.695	0.806
1.220	41	0.014	1.068	1.415	1.304	40	0.012	1.178	1.522
1.253	41	0.014	1.112	1.454	1.325	40	0.014	1.077	1.577
7.304	26	0.054	6.550	7.900	7.417	19	0.064	6.720	7.730
7.981	49	0.054	7.230	8.740	8.114	21	0.072	7.560	8.740
0.124	14	0.003	0.099	0.141	0.143	16	0.003	0.123	0.164
0.058	14	0.001	0.052	0.062	0.064	16	0.001	0.055	0.072
0.375	14	0.006	0.336	0.420	0.414	16	0.010	0.338	0.486
0.521	14	0.006	0.477	0.561	0.538	16	0.005	0.504	0.568
0.239	14	0.003	0.220	0.266	0.281	16	0.004	0.259	0.318
1306	16	31	1092	1443	1598	7	52	1409	1765
16	16	1	7	24	22	7	1	19	24
4.4	15	0.3	2.6	6.4	4.6	7	0.4	2.4	5.8
3.0	15	0.2	1.9	4.2	3.0	7	0.2	1.8	3.5

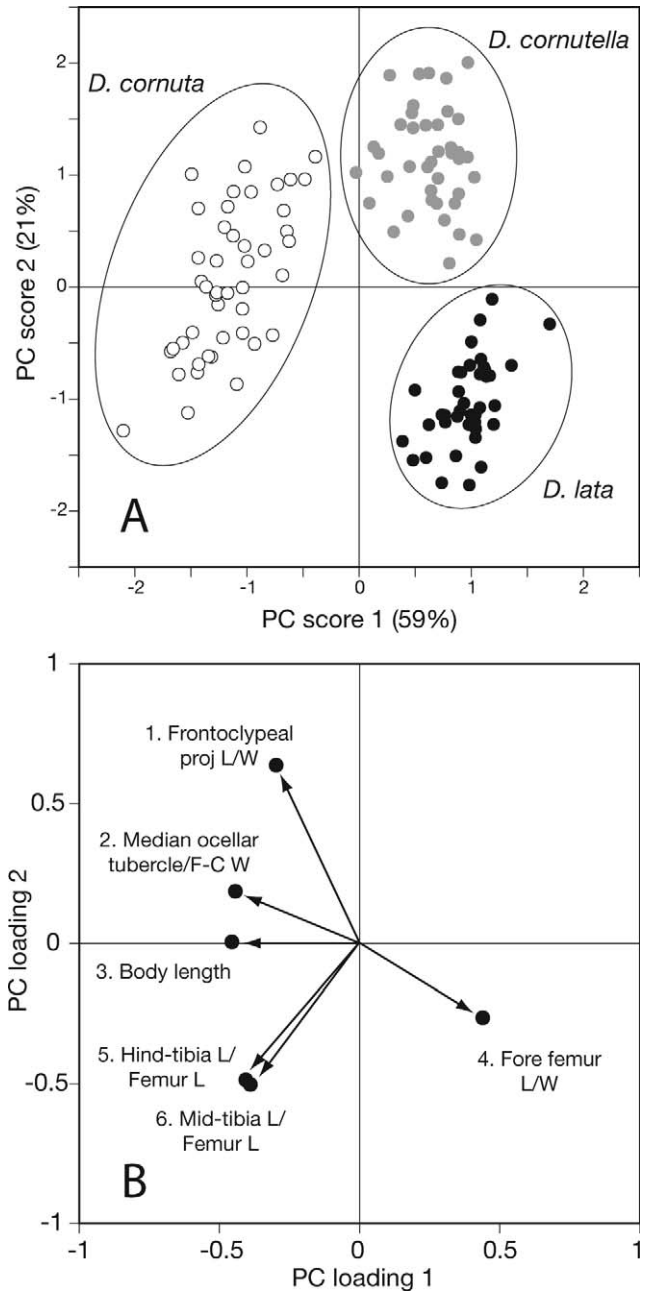

FIG. 4. Principal components analysis results for 6 morphometrics (measures 1, 2, 5, 6, 8 and 9; Table 4) in *Drunella* species, including individual scores for 130 specimens measured (A), and input variable loadings (B) for the first 2 principal components (PCs). Variability explained by each of the first 2 PCs is given in parentheses in the axes labels (A). Arrows in the bottom plot indicate direction and relative magnitude of input variables. See Table 4 and Fig. 2 for details of variable measurement. Proj = projection, L = length, W = width, F-C W = frontoclypeal width.

quantified both as absolute measures and as proportions of the lengths of their respective femora (measures 8–11; Table 4). Our data confirmed that the tibiae are distinctly shorter, on average, in *D. cornutella*.

The relationships among species derived from allozyme data (Fig. 3) are supported by morphological evidence, in particular the size and shape of the larval frontoclypeal projection and median ocellar tubercle. These 2 characters distinguish this complex from other eastern North American *Drunella* (Jacobus and McCafferty 2004). In *D. lata*, the frontoclypeal projection is short, flat, and does not protrude anteriorly from the surface of the frontoclypeus. In *D. cornuta* and *D. cornutella*, this structure is much longer (measures 6 and 7; Table 4), conical, and protrudes anteriorly (best observed in lateral view). The median ocellar tubercle is small in *D. lata*, larger in *D. cornutella*, and larger still in *D. cornuta* (measures 4 and 5; Table 4). Thus, the morphometric data corroborate the genetic analysis and further suggest that *D. cornuta, D. cornutella*, and *D. lata* are distinct species.

Life history and ecology

Life-history patterns in species of the *D. lata* complex were characterized by studies of the seasonal pattern of larval growth (mass), the onset of adult emergence/oviposition, the duration of the egg stage, and the number of degree-days (after 1 January) required to complete larval development.

Seasonal growth of each *Drunella* species was based on samples taken regularly for $\geq$1 y for 49 site/species combinations from the Delaware River and elsewhere in their geographic range (Table 1). At most of these sites (sites 3–35), *D. cornuta, D. cornutella*, and *D. lata* were each represented by a single, normally distributed mass class on any given sampling date. The only exceptions were at the 2 northernmost sites in Quebec (sites 1 and 2), where only *D. cornuta* was found. Our data are incomplete at these sites, but samples from the summer months appeared to consist of 2 mass classes. Figure 5 is a box plot depicting 2 y of population mass structure data for the Beaverkill in the upper Delaware River drainage. This site was chosen for illustration because *D. lata, D. cornutella*, and *D. cornuta* were all common, the thermal regime was natural (i.e., no reservoirs or other conspicuous human alterations), the data represent 2 y of extensive sampling, and the patterns observed there were typical. At the Beaverkill, *D. cornuta* emerged as adults 5 to 6 wk earlier than either *D. cornutella* or *D. lata* and averaged nearly twice the dry mass at maturity. The same pattern of seasonality and size was evident at all other sites where *D. cornuta* occurred with *D. lata* or *D. cornutella*.

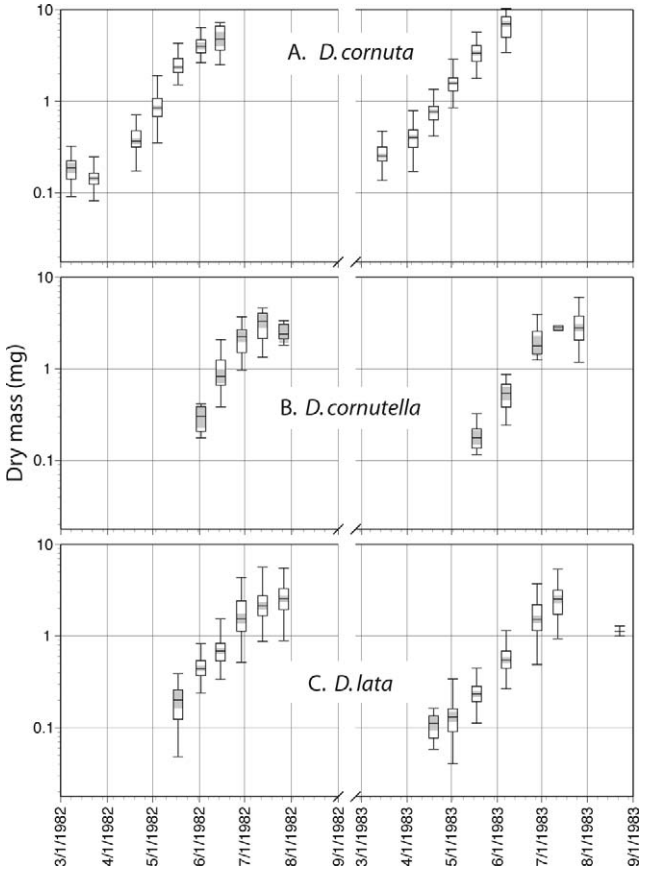

FIG. 5. Box plots showing statistics for individual larval dry mass for *Drunella cornuta* (A), *Drunella cornutella* (B), and *Drunella lata* (C) of the *D. lata* complex over 2 consecutive years in the Beaverkill River, New York. Boxes depict the data between the 25th and 75th percentiles, a horizontal line marks the median, whiskers delimit the range, and the shaded area marks the 95% confidence intervals for the median. Dates are formatted month/day/year.

Neither the adult emergence seasons nor the ranges of larval dry mass overlapped on any given collection date between *D. cornuta* and either *D. cornutella* or *D. lata* at any of the study sites. In contrast, the adult emergence seasons overlapped considerably between *D. cornutella* and *D. lata*, although *D. cornutella* emergence began slightly earlier, and the average dry masses of *D. cornutella* and *D. lata* at emergence were nearly identical (Table 4).

Eggs were obtained from 1 female each of *D. lata* and *cornutella* and 2 *D. walkeri* all captured as they hovered over a riffle preparing to oviposit on the evening of 3 July 2007 at site BVK5. Species determinations were confirmed genetically using allozymes. Eggs from 2 laboratory-mated female *D. cornuta* were obtained on 31 June 2007. *Drunella lata* eggs began hatching on 7 September and all others began hatching 8 October 2007. Hatching continued for $\geq$1 mo for all clutches. Thus, it appears that eggs enter a quiescence during the summer and that development in autumn might be triggered by shorter days or falling temperatures. Larvae at Delaware River sites did not reach a field-collectable size ($\sim$0.05 mg dry mass) until late winter or spring the following year. Very small larvae of *D. cornuta* were found occasionally as early as mid-January, but they were not common at these sites until about mid-March. The earliest collections of *D. cornutella* and *D. lata* were 4 to 6 wk later, in mid- to late April.

Taken collectively, the data for egg development and seasonal pattern of larval growth and adult emergence show that *D. cornuta, D. cornutella*, and *D. lata* were univoltine at all of our study sites from Maine to Virginia (sites 3–32; Table 1) (although preliminary data suggest *D. cornuta* might be semivoltine at the 2 northernmost sites, 1 and 2). Larval recruitment in all 3 species occurs around the same time in autumn, but the season of adult emergence varied predictably by species. Thus, the number of degree-days (measured from 1 January) required to complete larval development differed significantly among species: *D. cornuta* required an average of 783 degree-days, *D. cornutella* 1306, and *D. lata* 1598. Differences among species were significant (*t*-tests, $p < 0.001$) in all pairwise comparisons.

Patterns in the distribution of *D. lata* complex species were consistent within watersheds of eastern North America. *Drunella cornuta* seemed to have the greatest range in terms of stream size, and was found in sites that ranged in size from 1st-order spring streams to our largest site on the mainstem Delaware River (drainage area = 4118 km^2, mean annual discharge = 102 m^3/s). *Drunella cornutella* was usually absent from the smallest streams and was more commonly found in the 3rd- to 5th-order streams. *Drunella lata* seemed to prefer larger streams (never collected in streams $<\sim$4th-order), and it was consistently the most abundant member of the *D. lata* complex in the largest streams.

The geographic distribution of *D. lata* complex species appeared to be determined at least partly by thermal requirements. At our study sites, *D. cornuta* emergence occurred in late spring or early summer, when mean daily water temperatures were still rising (daily mean of 16°C on average; range 10–21°C). In contrast, emergence of both *D. cornutella* and *D. lata* generally coincided with the summer maximum temperatures. At sites where summer temperatures were artificially depressed by hypolimnetic release from reservoirs on the Delaware River (EBD5, WBD6, and WBH7; Fig. 1), the 1300+ degree-day requirement

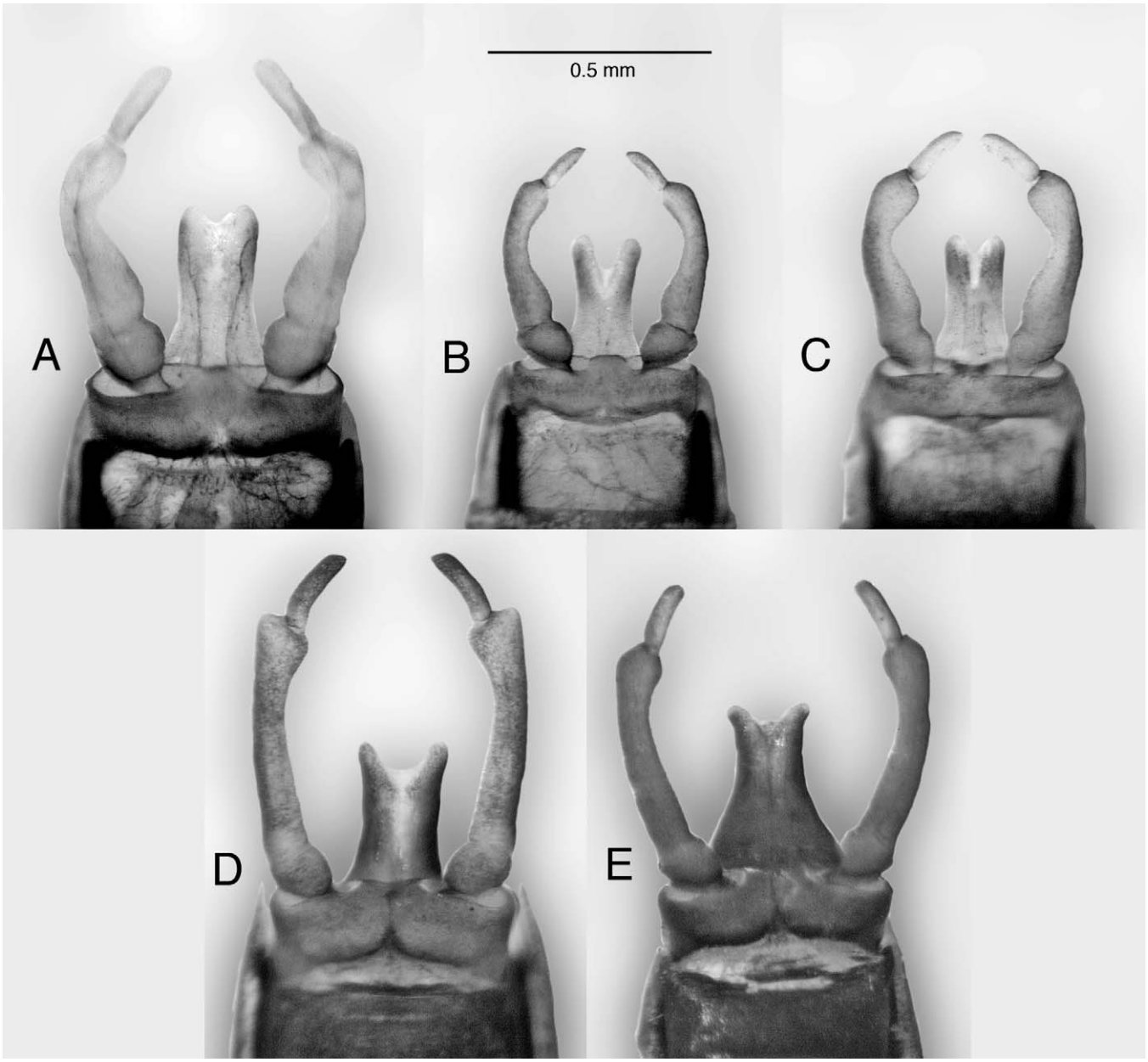

Fig. 6. Ventral view of male genital forceps and penes in *Drunella* species. A.—*D. cornuta*. B.—*D. cornutella*. C.—*D. lata*. D.—*D. tuberculata*. E.—*D. walkeri*.

for *D. cornutella* pushed its emergence into late summer (well beyond the summer peak) when daily mean temperatures were as low as 7°C (site EBD5). *Drunella lata*, common at our other Delaware River sites, was absent from these coldwater sites (Fig. 1). Because *D. lata* are now found on the East and West branches of the Delaware above their respective reservoirs and at the recovery sites below them, it seems likely the species was present throughout both reaches prior to dam construction. The 1577 degree-day requirement for *D. lata* (as measured at our other Delaware River sites) suggests that (were larval populations to exist there still): 1) adult emergence at sites EBD5 or WBD6 would not begin until late September, when water temperatures averaged 7 and 10°C, respectively; and 2) adult emergence at site WBH7 would not begin until the 2nd wk of August when water temperatures averaged 13°C. Data from laboratory rearings indicate that, unlike *D. cornuta* and *D. cornutella*, whose larvae were able to transform at water temperatures at least as low as 10°C, *D. lata* larvae required temperatures of $\geq$17°C for successful

emergence. Thus, it appears that low water temperature associated with hypolimnetic release has eliminated *D. lata* from reaches of greatest thermal impact.

These data show consistent and significant differences in seasonal pattern of larval growth, timing of emergence, degree-day requirements, and adult size, and are consistent with the genetic and morphometric data that suggest that *D. cornuta, D. cornutella*, and *D. lata* are distinct species.

Taxonomic conclusions

Jacobus and McCafferty (2004) concluded that the morphological variation observed for the *D. lata* complex over a broad geographic range was indicative of a single, highly variable species. In contrast, our study shows unequivocally that *D. cornuta, D. cornutella*, and *D. lata* are distinct genetically, morphologically, and ecologically in northeastern North America. Is it possible that these 3 species are reproductively isolated in the Northeast, but that species boundaries break down in other parts of their range? There are cases in which animals appear to be good species in one region while interbreeding in another. Perhaps the best known examples are ring species, such as the plethodontid salamander *Ensatina eschscholtzi* (Wake and Yanev 1986). Ring species consist of a linear arrangement of locally differentiated populations arrayed around a geographic barrier. Local populations (or subspecies) interbreed with their immediate neighbors, but at the ends of the distribution where terminal populations meet, they do not interbreed. Could the *D. lata* complex be an example of a ring species? At present, no evidence exists to suggest so (i.e., no biogeographic data indicating a ring-shaped distribution and no genetic, behavioral, or quantitative morphologic evidence to suggest that members of the *D. lata* complex interbreed anywhere within their ranges). Thus, in the absence of any direct evidence to the contrary, we conservatively conclude that *D. cornuta, D. cornutella*, and *D. lata* are good species throughout their range.

Prior to Jacobus and McCafferty's (2004) synonymization, application of species names to larvae of the *D. lata* complex had been inconsistent and problematic. Jacobus and McCafferty's solution, although convenient (e.g., from the standpoint of easily assigning species names to specimens from benthic samples), is not consistent with observed patterns of morphology, gene flow, or life history. We suggest that the difficulties encountered in morphological identification of *Drunella* larvae from some regions (e.g., southern Appalachians) are probably the result of 2 factors. First, local populations in those regions might be much older, resulting in a greater degree of local population differentiation than is seen in the northeastern part of their range, where local populations are relatively recent ($\leq$12,000 y since the last glaciation of those areas). Second, additional, but currently unrecognized, species might occur in the southern Appalachians. Obviously, these species would not be expected to fall neatly into the concepts of *D. cornuta, D. cornutella*, or *D. lata* that we present here. The application of a more rigorous analysis using genetic or morphometric techniques might eventually help to resolve species of *Drunella* (and other ephemerellids) in Appalachia. Regardless, our study shows the value of ancillary genetic and ecological data to understanding the taxonomy of North American Ephemerellidae and suggests that other recent revisions within the family should be regarded as tentative until confirmed in similar fashion.

Our study does not settle all taxonomic issues in the *D. lata* complex. In particular, the status of *D. longicornis* (Traver) in the southern Appalachian region (see discussion under *D. cornuta* in Appendix 1) remains unclear. However, our data clearly document the existence of 3 distinct species within Jacobus and McCafferty's (2004) concept of *D. lata* in northeastern North America. Thus, we hereby remove *D. cornuta* (Morgan) and *D. cornutella* (McDunnough) from synonymy with *D. lata* (Morgan), restoring species status for each. We provide reliable morphological means of distinguishing the three and emend the keys of Allen and Edmunds (1962a) in Appendices 1 and 2.

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APPENDIX 1. Species diagnosis.

For a nomenclatural history of North American *Drunella*, see Allen and Edmunds (1962a) and Jacobus and McCafferty (2004). Synonymies below include original descriptions and specific status changes only. As larvae, members of the *Drunella lata* complex can be distinguished from other North American *Drunella* by

the following combination of characters: 1) the presence of conspicuous tubercles on the anterior margin of the fore femora; 2) the presence of distinct frontoclypeal projections; 3) the presence of a relatively well-developed median ocellar tubercle and low, lateral ocellar tubercles; 4) a lack of genal projections; 5) a lack of submedian tubercles protruding from the posterior margins of the terga; and 6) a lack of small, knob-like protuberances on the dorsal surface of the femora. Male imagos can be distinguished by the presence of basal and distal dilations and a constriction near or slightly beyond middle of the second segment of genital forceps, resulting in an inward "bow" (Fig. 6A–D).

Drunella lata (Morgan 1911)

Figs 2*e–q*, 6C

Ephemerella lata Morgan 1911: 112 (orig. comb.).
Ephemerella inflata McDunnough 1926: 187 (syn. by McDunnough 1931b: 211).
Drunella lata (Morgan 1911), Jacobus and McCafferty 2004: 132 (in part).

Larval diagnosis.—The frontoclypeal projections in full-grown *D. lata* are qualitatively and quantitatively distinct from *D. cornuta* or *D. cornutella*. In *D. lata* the projections are about as long as they are wide at their base, and appear as flattened outgrowths of the frontoclypeus which protrude downward from, but in the same plane as, the remainder of the frontoclypeal sclerite. In contrast, the frontoclypeal projections in *D. cornuta* and *D. cornutella* are 1.5 to 2.5× as long as they are wide at their base, conical and usually incurved, and always project anteriorly away from the plane of the frontoclypeal sclerite. This character alone will separate full-grown *D. lata* from *D. cornuta* or *D. cornutella*, but in very young specimens of the latter two the projections are relatively smaller, with the result that younger (<⅓ grown) *D. cornuta* or *D. cornutella* could be confused with *D. lata* if only the frontoclypeal projection character is used.

The general body color of *D. lata* is brownish or gray, often with sharply contrasting pale bands at the anterior ⅓ and posterior margin of the pronotum, the posterior half of tergite 8, or along the posterior margins of the femora. Pale areas might have bright red maculae within them or superimposed. In contrast, both *D. cornuta* and *D. cornutella* larvae are brown or pale brown with slightly paler mottling. These color differences are usually evident even in larvae that are small enough that the frontoclypeal character might be ambiguous. In fact, the coloration of *D. lata* is so different that, where larvae of *D. lata* and *D. cornutella* coexist, they can be easily sorted that way in the field. Similarly, coexisting *D. lata* and *D. cornuta* can be separated on the basis of coloration as well as a large size difference—at the time *D. cornuta* emerges, *D. lata* is only ~½ grown (Fig. 5A, C).

In addition to color differences, *D. lata* larvae generally appear glabrous in comparison to *D. cornuta* and *D. cornutella*. In particular, both *D. cornuta* and *D. cornutella* have dense rows of long, fine setae on the hind margin of the middle and hind (and, to a lesser degree, the fore) femora, as well as on all tibiae. On the tibiae, these setae are distinctly longer than the width of the tibiae. In contrast, in *D. lata* the hind margins of the femora are essentially bare and, whereas the tibiae usually have a row of fine setae on the hind margin, these are shorter than in *D. cornuta* and *D. cornutella*, their length being less than or subequal to the width of the tibiae.

Another feature of *D. lata* larvae that separates them from *D. cornuta* and *D. cornutella* is the shape of the fore femora: in *D. lata* these appear wider than in *D. cornuta* or *D. cornutella*. Measure 2 (Table 4) is the width of the fore femur as a proportion of its length. Although the mean is significantly higher in *D. lata*, there is some overlap between *D. lata* and *D. cornutella*.

Adult diagnosis.—The forewing length of males averages 7.4 mm, range 6.7 to 7.7. Females average 8.1 mm, range 7.6 to 8.7 (Table 4). There is no overlap between these ranges and those of *D. cornuta*. However, they are nearly the same as those of *D. cornutella*. Male *D. lata* and *D. cornutella* can be distinguished by the shape of the genital forceps: in *D. lata* these have a more "bowed" appearance, with the degree of dilation distally being distinctly greater in *D. lata* (measure 18; Table 4, Fig. 6C). Also, the lobes of the penes diverge less and have a shallower median notch in *D. lata* (Fig. 6C vs 6B). Imagos of both male and female *D. lata* often have red streaks on the dorsal surface of the femora. This condition has not been observed in either *D. cornuta* or *D. cornutella*.

Drunella cornuta (Morgan), new status

Figs 2*a–k*, 6A

Ephemerella cornuta Morgan 1911: 114 (orig. comb.).
Ephemerella depressa Ide 1930 (syn. by Allen and Edmunds 1962a: 153).
Drunella lata (Morgan 1911), Jacobus and McCafferty 2004: 132 (in part).

Larval diagnosis.—*Drunella cornuta* larvae can be easily distinguished from *D. lata* by the characters discussed under that species. Distinguishing *D. cornuta* from *D. cornutella* can be more difficult. Although discounted by Jacobus and McCafferty (2004), observations by McDunnough (1931a, b) that *D. cornuta* is

an early season species relative to *D. cornutella* were confirmed at all of our sites where they co-occurred. When both are present at a site (which is frequently the case in the Northeast), and collections are made throughout the season, the difference in size and seasonality is obvious, and the 2 species often can be distinguished on that basis alone. There are, however, consistent morphological differences in full-grown larvae (Table 4) that, taken in combination (Fig. 4A, B), corroborate the large genetic differences revealed by allozyme data.

Three measures in particular enable the distinction of *D. cornuta* from *D. cornutella* in most situations. On average, *D. cornuta* larvae are distinctly larger at maturity than *D. cornutella*: although the largest specimens of *D. cornutella* are larger than the smallest *D. cornuta*, any specimens >9 mm in length are likely to be *D. cornuta*. The middle and hind tibiae in *D. cornuta* are longer than in *D. cornutella* both in absolute terms (measures 10 and 11; Table 4) and normalized for the length of their respective femora (measures 8 and 9; Table 4), although there is some overlap in the latter. The protrusion of the median ocellar tubercle is consistently greater in full-grown *D. cornuta* than in *D. cornutella* (measure 4; Table 4) (but see discussion of *D. longicornis* [Traver] below). When normalized for overall size (as indicated by frontoclypeal width, measure 5; Table 4), there is almost no overlap between *D. cornuta* and *D. cornutella*.

Two secondary characters used previously to help distinguish *D. cornuta* from *D. cornutella,* but that we found ineffective, are the relative curvature of the frontoclypeal projections (Allen and Edmunds 1962a) and the presence (in *D. cornuta*) or near absence (in *D. cornutella*) of a very small tubercle on the lateral edge of the pronotum (McDunnough 1931b). Though we did not attempt to quantify it, the curvature of the frontoclypeal projection clearly exhibits wide and overlapping variation in both species and its use has probably led to misidentifications in the past. The pronotal tubercle difference, though not quantified here, appeared negligible to us.

We have not seen Traver's (Traver 1932) types of *D. longicornis* (which Jacobus and McCafferty [2004] synonomized with *D. lata*), but from her descriptions (Traver 1932, 1935) as well as that of Allen and Edmunds (1962a), it seems *D. longicornis* might be either a southern variant of *D. cornuta* or, as Traver indicated, a distinct species. We have collected larvae from 3 sites in the headwaters of the Big Otter River in Virginia that, based on their relatively long, straight frontoclypeal projections, seem to fit Traver's *D. longicornis*. We do not include these in our concept of *D. cornuta* for the present treatment because: 1) we have no genetic data for these populations; 2) 2 y of regular sampling for population mass structure showed that 1317 degree-days (range: 1210–1392) were required to complete larval development, a figure considerably higher than we have observed for any *D. cornuta* population sampled from the Northeast (Table 4); and 3) whereas morphometric data for most characters fell within the ranges we report for *D. cornuta*, the protrusion of the median ocellar tubercle in the Virginia populations fell outside the range (less than) we have observed in *D. cornuta* from the Northeast. The differences in degree-day requirements and morphometric measures appear significant in light of the fact that data for *D. cornutella* collected just downriver on the Big Otter (sites 31 and 32; Table 1) fit squarely within the ranges we have observed in northeastern populations of that species.

Adult diagnosis.—The forewing length in males averages 9.6 mm, range 9.0 to 10.3. Females average 10.9 mm, range 9.9 to 12.0 (Table 4). These figures are outside of the range (larger) observed for either *D. cornutella* or *D. lata*. The genital forceps in male *D. cornuta* have a "bowed" appearance (Fig. 6A) owing to an inward bend in combination with wide basal and distal dilations and a narrow constriction just beyond middle of segment 2. This bowed appearance also is found in *D. lata* and, to a lesser degree, in *D. cornutella*. *Drunella cornuta* can be distinguished from *D. lata* and *D. cornutella* by the long forceps segment 3 (relative to segment 2, measured as *l*/*m*; Fig. 2, Table 4).

Drunella cornutella (McDunnough), new status

Figs 2*b*–*d*, 6B

Ephemerella cornutella McDunnough 1931a: 82 (orig. comb.).

Drunella lata (Morgan 1911), Jacobus and McCafferty 2004: 132 (in part).

Larval diagnosis.—Although *D. cornutella* larvae are often found in the same streams at the same time and at about the same size as *D. lata*, *D. cornutella* larvae can be easily distinguished from *D. lata* by the length (~2× as long as broad at base) and shape (conical, incurved, and projecting anteriorly) of the frontoclypeal projections (measures 6 and 7; Table 4), as well as their mottled brown coloration (see description of *D. lata* for comparison), long, dense setation along the posterior edge of the femora, and relatively narrow fore femora.

Compared to *D. cornuta, D. cornutella* is smaller at maturity, 6.4 to 8.5 mm vs 7.8 to 11.4 mm for *D. cornuta*, has shorter middle and hind tibiae (relative to their respective femora), and a shorter median ocellar tubercle (Table 4).

Adult diagnosis.—The forewing length of *D. cornutella* males averages 7.3 mm, range 6.6 to 7.9. Females average 8.0 mm, range 7.2 to 8.7 (Table 4). These values are smaller than *D. cornuta*, but comparable to *D. lata*. Segment 3 of the genital forceps in males is shorter than that of *D. cornuta* (measures 14 and 16; Table 4), and segment 2 is straighter with less pronounced basal and distal dilations than either *D. cornuta* or *D. lata* (measure 18; Table 4, Fig. 6B). Red maculae on the femora, common in *D. lata* imagos, are never present in *D. cornutella*.

Appendix 2. Emendation of keys.

Allen and Edmunds (1962a) provided the most recent (and effective) keys to North American *Drunella* species. We provide the following emendations based primarily on morphometric data from our study.

Key to full-grown larvae of the *Drunella lata* complex from northeastern North America (substitute for couplets 8–10 in Allen and Edmunds' [1962a] larval key). See discussion of the status of *D. longicornis* (Traver) under *D. cornuta* heading in Appendix 1.

8. Frontoclypeal projections consisting of flattened extensions of the frontoclypeus, about as long as wide (*c*/*d* in Fig. 2; mean = 1.02, range 0.78–1.29) (fig. 21 in Allen and Edmunds [1962a]), with little or no protrusion anterior to the major plane of the frontoclypeus; femora with few or no long, fine setae on the posterior margin ..... *lata*

8′. Frontoclypeal projections conical and usually incurved, about twice as long as wide (*c*/*d* in Fig. 2; range 1.5–2.5) (figs 22–23 in Allen and Edmunds [1962a]), protruding distinctly anterior to the major plane of the remainder of the frontoclypeus; femora with dense row of long, fine setae on the posterior margin 9

9(8). Full-grown body length larger, usually >8 mm (mean = 9.5, range 7.8–11.4); middle and hind tibiae longer relative to the length of their respective femora (average length of middle tibia = 1.9 mm, range 1.6–2.2; ratio of middle tibia to middle femur usually >0.85; average length of hind tibia = 1.9 mm, range 1.6–2.2; ratio of hind tibia to hind femur usually >0.74); median ocellar tubercle longer, projecting anteriorly >0.1 mm beyond the apices of the lateral ocellar tubercles (*a* in Fig. 2)..... *cornuta*

9′. Full-grown body length smaller, usually <8 mm (mean = 7.4, range 6.4–8.5); middle and hind tibiae shorter (average length of middle tibia = 1.2 mm, range 1.1–1.4, ratio of middle tibia to middle femur usually <0.85; average length of hind tibia = 1.3 mm, range 1.1–1.5; ratio of hind tibia to hind femur usually <0.74); median ocellar tubercle shorter, projecting anteriorly <0.1 mm beyond the apices of the lateral ocellar tubercles ..... *cornutella*

Key to known male imagos of eastern North American *Drunella* species (substitute for couplets 8–11 in Allen and Edmunds' [1962a] key to male imagos).

8. Second segment of genital forceps with basal and distal dilations and a constriction near or beyond middle (Fig. 6A–C); width (in ventral view) of segment 2 at basal dilation >0.25× length of segment (*p*/*m* in Fig. 2)..... 9

8′. Second segment of genital forceps without basal dilation or distinct constriction near middle (Fig. 6D, E); width of segment at widest portion near base (basal dilation) <0.25× length of segment (*p*/*m* in Fig. 2).....11

9(8). Forewing length ≥9 mm; forceps segment 3 long (0.18–0.23 mm; Fig. 6A), usually >0.45× length of segment 2 (*l*/*m* in Fig. 2) ..... *cornuta*

9′. Forewing length ≤8 mm; forceps segment 3 shorter (<0.17 mm; Fig. 6B, C), usually <0.45× length of segment 2..... 10

10(9′). Forceps segment 2 relatively slender and straight (Fig. 6B), distal dilation narrower relative to length of segment (*o*/*m* in Fig. 2 <0.26); femora never with longitudinal red streak ..... *cornutella*

10′. Forceps segment 2 having a more "bowed" appearance (Fig. 6C), distal dilation wider relative to length of segment (*o*/*m* in Fig. 2 >0.26); femora often with longitudinal red streak on dorsal surface..... *lata*

11(8′). Abdominal sterna mostly dark brown ...*walkeri*

11′. Abdominal sterna mostly white, each with a pair of small submedian dots, larger sublateral dashes, and even larger lateral dashes, all dark brown 12

12(11′). Middle and hind femora with numerous blackish dots on a pale brown background..... *tuberculata*

12′. Middle and hind femora without numerous blackish dots ..... *allegheniensis*