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TAXONOMY AND POLYTENE CHROMOSOMES OF *SIMULIUM PARNASSUM* MALLOCH (DIPTERA: SIMULIIDAE)

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Abstract.—The taxonomic status of *Simulium parnassum* Malloch was investigated cytologically and morphologically. Specimens were collected at 44 localities from the Gaspé Peninsula of Quebec to northern Alabama. A previously unreported pupal form of *S. parnassum* from two streams in South Carolina and one stream each in North Carolina and Massachusetts lacked the typical rugose sculpturing on the dorsum of the head and thorax. No additional structural characters in any life stage differentiated the two forms. A standard map of the silk-gland chromosomes was constructed and compared with the standard map for the subgenus *Simulium*. *Simulium parnassum* differed from the subgeneric standard by six inversions in IL, one in IIL, three in IIS, and an unresolved number in IIIL. Placement of *S. parnassum* in a separate species group, the *S. parnassum* species group, in the subgenus *Simulium* is suggested on the basis of cytological evidence. No fixed rearrangements were found among populations of *S. parnassum* from Quebec to Alabama nor between populations of smooth and rugose pupal forms. A Y-linked polymorphism was observed in the centromere region of chromosome I in some males, but it was not associated with either pupal form. Present evidence indicates that *S. parnassum* is a single species throughout its range.

Key Words: *Simulium parnassum,* black flies, aquatic insects, polytene chromosomes

Simulium parnassum is one of the most distinctive members of the subgenus *Simulium* in the Nearctic Region (Stone 1964). The female is shiny black and each claw is long and nearly sigmoidal, with a small tooth near its midlength. The pupal gill of six filaments and the simple, slipper-shaped cocoon are similar to those of the *S. tuberosum* and *S. venustum* species groups, but the rugose head and thorax are unique. The deeply incised, triangular postgenal cleft of the larva is distinctive. Despite the unique attributes of *S. parnassum,* this species has been assigned to the *S. tuberosum* species group (Crosskey and Howard 1997), ostensibly because of perceived similarities in the male genitalia, notably the presence of a medially directed lobe on the gonostylus. The homology of this lobe, however, is questionable based on differences in location, size, and vestiture. Accordingly, a reevaluation of the phylogenetic placement of *S. parnassum* is warranted.

Simulium parnassum was described from females taken in northern New Hampshire (Malloch 1914). Dyar and Shannon (1927) described a male from eastern Virginia as *S. hydationis,* which was synonymized with *S. parnassum* by Stone and Jamnback (1955). All life stages of *S. parnassum* have been described and figured (Stone and Jamnback 1955, Davies and Peterson 1956, Davies et al. 1962, Wood et al. 1963, Stone

Table 1. Numbers of two pupal forms (rugose, smooth) of *Simulium parnassum* collected at Smith and Abner Creeks in Pickens County, South Carolina, 1998–1999.

	Smith Creek			Abner Creek		
Year	Date	Rugose	Smooth	Date	Rugose	Smooth
1998	25 April	2	0	2 June	0	12
1998	5 May	0	4	9 June	0	20
1998	14 May	2	0	11 June	1	23
1998	23 May[1]	1	4	23 June[1]	0	1
1999	10 May	1	0	1 June	1	12
1999	15 May	1	1	5 June	0	11
1999	18 May	1	5	10 June[1]	0	26
1999	21 May	3	36			
1999	24 May	1	30			
1999	28 May	0	17			
1999	1 June[1]	0	4			

[1] No pupae found beyond these dates.

and Snoddy 1969, Adler and Kim 1986). The polytene chromosomes have not been studied, although Rothfels (1979) commented briefly on the banding sequence of two of the six chromosomal arms. Consequently, *S. parnassum* has not been investigated for sibling species, which are common among black flies (Adler 1988).

This species occurs in eastern North America from Canada to the southern end of the Appalachian Mountains (Stone and Snoddy 1969), with an isolated record from Missouri (Doisey et al. 1986). The immature stages occupy cool, rocky, forest streams (Davies et al. 1962, Adler and Kim 1986). Females are mammalophilic (Fuller 1940, Downe and Morrison 1957, Addison 1980) and can be pests of humans (Adler and Kim 1986, Gibbs et al. 1986).

Our objectives were to screen *S. parnassum* for sibling species, using morphology and polytene chromosomes, and to resolve its chromosomal banding sequence relative to the subgeneric standard of Rothfels et al. (1978), with the intent of gaining phylogenetic insight.

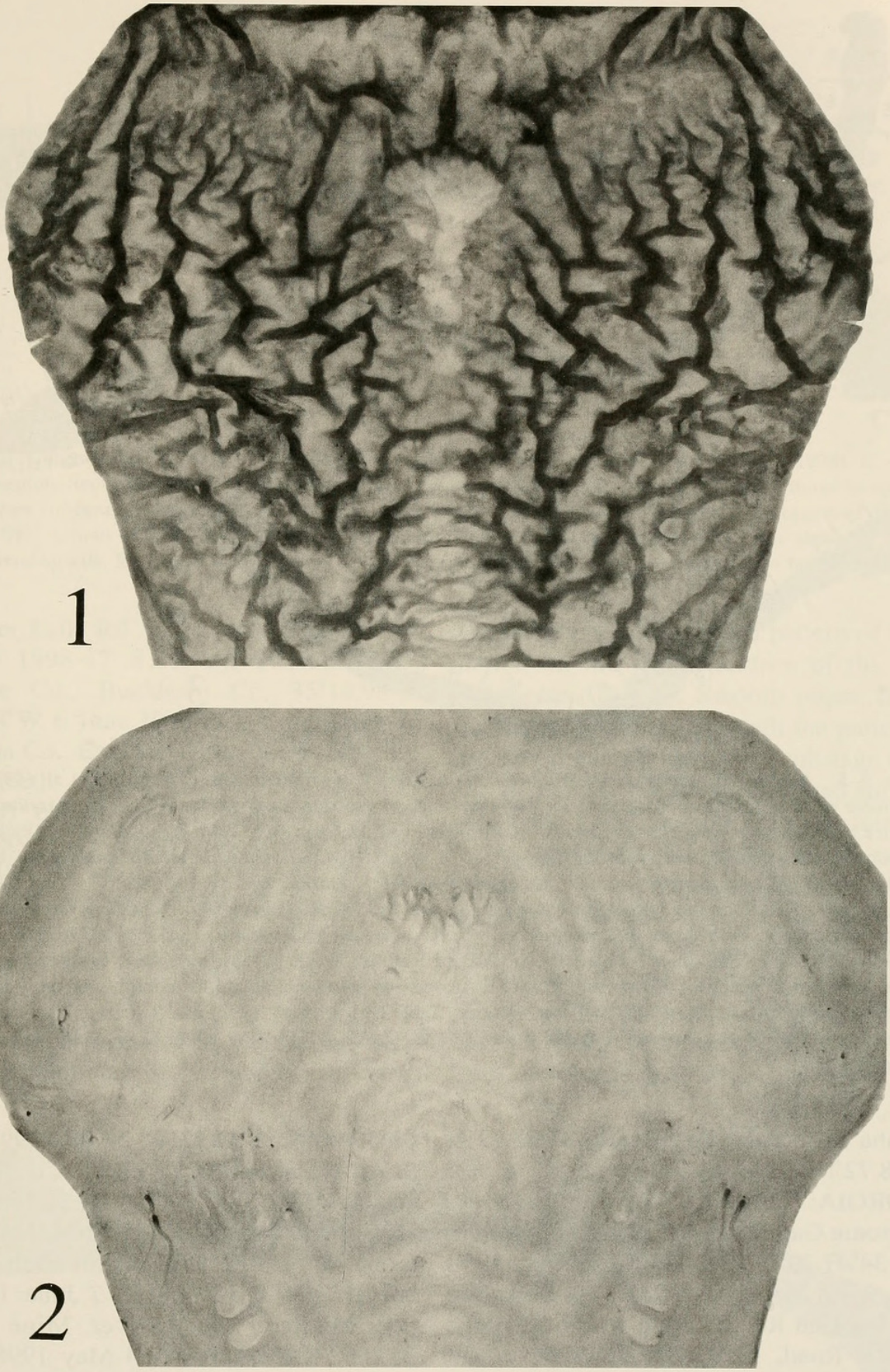
Materials and Methods

Larvae and pupae of *S. parnassum* were collected from 1998 to 1999 at 44 sites from the Gaspé Peninsula of Quebec to northern Alabama, and were fixed in Carnoy's solution (1 part glacial acetic acid: 3 parts 95% ethanol). Additional pupae were reared individually to adults on moist filter paper in petri dishes.

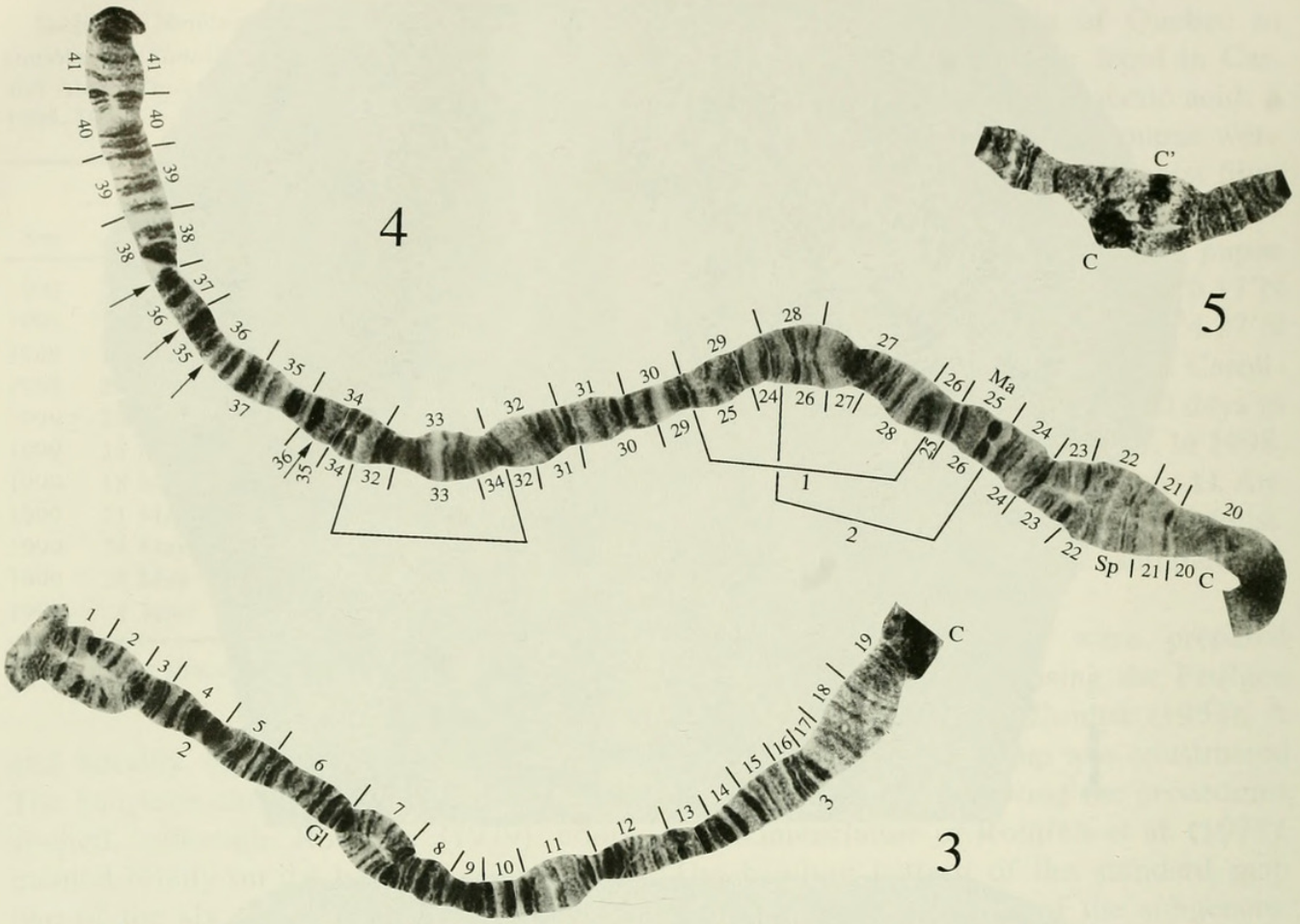
Seasonal sampling of larvae and pupae was conducted at Smith Creek (35°0.17′N 82°49.01′W) and Abner Creek (35°4.27′N 82°47.11′W) in Pickens Co., South Carolina. Streams were sampled every 10 days in 1998 and every three days in 1999. In 1998, Smith Creek was sampled 3 March–11 August and Abner Creek 13 May–11 August. In 1999, both streams were sampled 17 March–26 June.

Polytene chromosomes were prepared from larval silk glands, using the Feulgen method of Rothfels and Dunbar (1953). A standard chromosome map was constructed for *S. parnassum,* following the procedures and nomenclature of Rothfels et al. (1978). The banding pattern of the standard map was compared with that of the subgeneric standard of Rothfels et al. (1978). For chromosomal arms of *S. parnassum* that had banding patterns identical to those of the subgeneric standard, the subgeneric numbering was preserved. Chromosome arms of *S. parnassum* that were rearranged relative to the subgeneric standard were given new section numbers to provide a continuous sequence. Slide-mounted chromosomes of larvae from 15 of the 44 collection sites (see Chromosomal Material Examined) were compared band for band against the standard map of *S. parnassum* in a search for rearrangements within and between populations of *S. parnassum.* Specimens and chromosomal photographic negatives and working maps were deposited in the Clemson University Arthropod Collection, Clemson, South Carolina.

Chromosomal material examined (numbers of each sex refer to larvae for which all chromosomal bands were read).—ALABAMA: Talladega Co., Cheaha State Park, Cheaha Creek, 33°28.27′N 85°49.07′W, 27 March 1998 (1 ♂); 19 May 1998 (9 ♀);



Figs. 1–2. Cephalic plates of pupal exuviae of *Simulium parnassum*. 1, Rugose form (Smith Creek, Pickens Co., South Carolina, 18 May 1999). 2, Smooth form (Abner Creek, Pickens Co., South Carolina, 11 June 1998).



Figs. 3–5. Silk-gland chromosomes of *Similium parnassum,* with landmarks of Rothfels et al. (1978); C = centromere region. 3, Chromosome arm IS. Numbers indicate section numbers for both *S. parnassum* standard sequence and *Simulium* subgeneric standard of Rothfels et al. (1978). Male from South Carolina, Pickens Co., Oil Camp Creek, 8 May 1998 (sections 1 to center of section 13) plus female from Massachusetts, Berkshire Co., 29 June 1998 (section 20 to center of section 13); Gl = glazed, 2 = two blocks, 3 = three heavy. 4, Chromosome arm IL. Numbers on top of chromosome indicate *S. parnassum* standard banding sequence; bottom numbers correspond with those of *Simulium* subgeneric standard of Rothfels et al. (1978). Brackets indicate inversions relative to *Simulium* subgeneric standard of Rothfels et al. (1978); numbered brackets indicate sequence in which overlapping inversions occurred. Arrows indicate breakpoints for complex of three inversions relative to subgeneric standard. Female from Georgia, Dade Co., 19 May 1998; Ma = marker, Sp = spongy. 5, Centromere region of chromosome I. Male from Georgia, Dade Co., 19 May 1998; C′ = condensed, Y-linked centromere band.

Cheaha State Park, Dry Creek, 33°28.23′N 85°48.72′N, 19 May 1998 (1 ♂, 6 ♀); GEORGIA: Dade Co, Johnson's Crook, Newsome Gap Road, 0.8 km west of Moore Rd., 34°47.23′N 85°28.25′W, 19 May 1998 (2 ♂, 7 ♀); MASSACHUSETTS: Berkshire Co., Jug End Rd., 0.24 km west of Guilded Hollow Road, 42°8.99′N 73°26.98′W, 29 June 1998 (2 ♂, 16 ♀); NEW HAMPSHIRE: Carroll Co., Bear Notch Rd., 3.2 km north of SR 112, 44°1.50′N 71°19.13′W, 24 July 1998 (1 ♂); Grafton Co., SR118, 10.5 km southwest of SR 112, 43°59.00′N 71°47.93′W, 24 July 1998 (2 ♂, 7 ♀); NEW JERSEY: Sussex Co., Tillman Brook, 41°0.15′N 74°0.86′W, 22 June 1998 (1 ♂, 4 ♀); NORTH CAROLINA: Haywood Co., US Rt. 276, 1.6 km north of Cruso, 35°26.4′N 82°48.8′W, 7 June 1998 (2 ♂); Madison Co., Silver Mine Creek 35°0.53′N 82°0.48′W, 14 May 1998 (3 ♂, 2 ♀); SOUTH CAROLINA: Pickens Co., Abner Creek, SR 1105, 4.8 km east of Hwy 178, 35°4.27′N 82°47.11W′, 23 May 1998 (14 ♂, 5 ♀); 2 June 1998 (10 ♀); Oil Camp Creek, Oil Camp Creek Rd., 0.8 km west

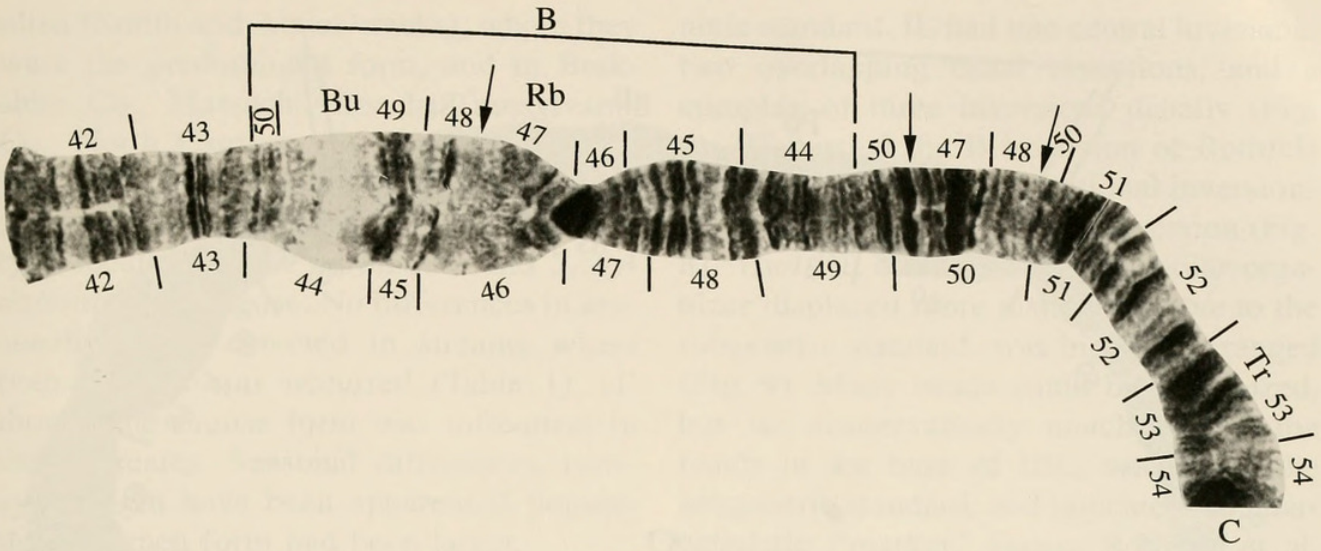


Fig. 6. Chromosome arm IIS of *Simulium parnassum,* with landmarks of Rothfels et al. (1978). C = centromere region. Section numbers on bottom indicate *S. parnassum* standard banding sequence; those on top refer to *Simulium* subgeneric standard sequence of Rothfels et al. (1978). Bracket indicates B inversion of Rothfels et al. (1978). Arrows indicate breakpoints of two overlapping inversions relative to subgeneric standard. Female from Massachusetts, Berkshire Co., 29 June 1998. Bu = bulge, Rb = ring of Balbiani, Tr = trapezoidal.

of River Falls Rd., 35°6.65′N 82°34.08′W, 8 May 1998 (7 ♂, 1 ♀); TENNESSEE: Monroe Co., Buckhorn Cr., 35°19.98′N 84°9.83′W, 6 June 1998 (2 ♂); VIRGINIA: Augusta Co., East Dry Branch, SR 688, 3.2 km north of SR 42, 38°12.70′N 79°16.31′W, 25 June 1998 (8 ♀); NEW BRUNSWICK: Restigouch Co., Collector Hwy. 180, 37 km west of Bathurst, 27 July 1998 (3 ♂, 12 ♀); QUEBEC, Gaspé Peninsula, Hwy. 132, 5.6 km south of Routhierville, 29 July 1998 (5 ♂, 19 ♀).

Results

Two pupal forms of *S. parnassum* were discovered among the 472 pupae examined. Rugose pupae had a raised pattern of reticulation on the dorsal surface of the head and thorax (Fig. 1). Smooth pupae lacked surface sculpturing, although the pattern of rugosity could be seen, with substage lighting, as a vague outline on the head and thorax of the pupal exuviae (Fig. 2). Intermediates between the smooth and rugose forms were not found. No additional morphological characters in larvae, pupae, or adults were found that correlated with the smooth and rugose pupal forms.

Smooth pupae were collected from four streams. They were found with the rugose form in streams in Pickens Co., South Car-

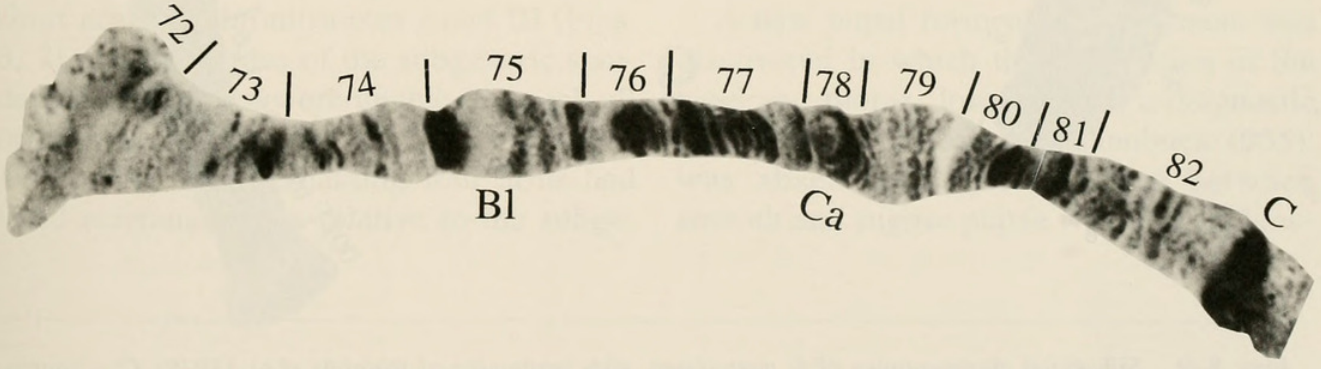
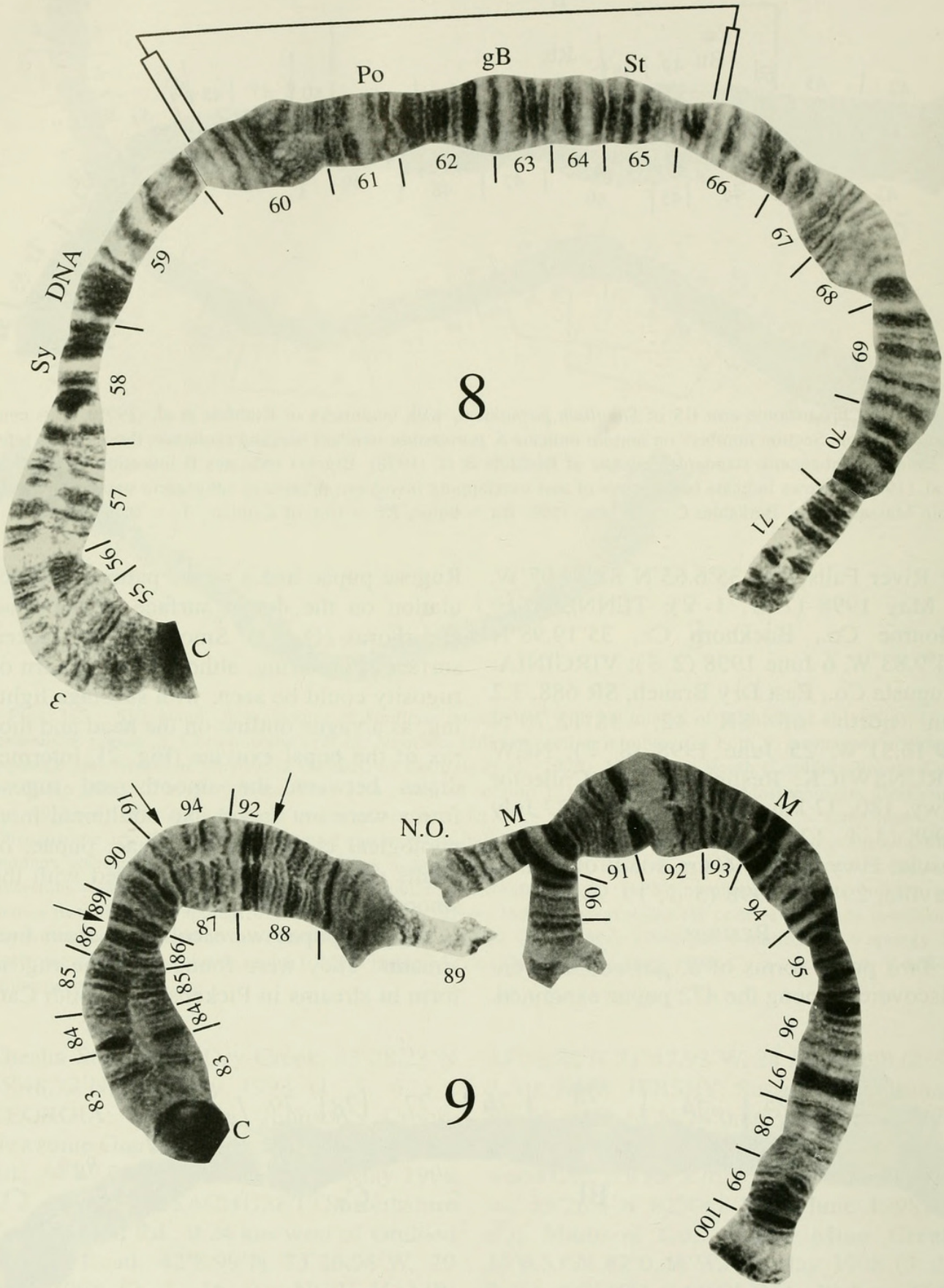


Fig. 7. Chromosome arm IIIS of *Simulium parnassum,* with landmarks of Rothfels et al. (1978). C = centromere region. Section numbers are those of both *S. parnassum* standard banding sequence and *Simulium* subgeneric standard of Rothfels et al. (1978). Male from South Carolina, Pickens Co., Oil Camp Creek, 8 May 1998. Bl = blister, Ca = capsule.



Figs. 8–9. Silk-gland chromosomes of *S. parnassum,* with landmarks of Rothfels et al. (1978). C = centromere region. 8, Chromosome arm IIL. Section numbers refer to *Simulium parnassum* standard banding sequence. Bracket indicates simple inversion relative to *Simulium* subgeneric standard of Rothfels et al. (1978). Female from Tennessee, Monroe Co., 6 June 1998 (sections 55 through 70) plus female from North Carolina, Haywood Co., 7 June 1998 (section 71). DNA = DNA puff, gB = gray band, Po = polar, St = saw tooth, Sy =

olina (Smith and Abner creeks), where they were the predominant form, and in Berkshire Co., Massachusetts. In Transylvania Co., North Carolina (South Prong Gladdy Fork), one smooth pupa and no rugose pupae were collected. Pupae from near the type localities of *S. parnassum* and *S. hydationis* were rugose. No differences in seasonality were detected in streams where both pupal forms occurred (Table 1), although the rugose form was infrequent in these streams. Seasonal differences, however, might have been apparent if populations of each form had been larger.

In Smith Creek, where a minimum-maximum thermometer had been placed, larvae first appeared on 13 March 1998; water temperature was 3.0–9.0°C for the three days prior to collection. Pupae were first found in Smith Creek on 25 April 1998; water temperature was 11.0–12.0°C during the 11 days prior to collection. In 1998, the last larvae were found at Smith Creek on 2 June (water temperature for 9 days prior to collection = 17.5–18.5°C) and in 1999 on 1 June (water temperature for 3 days prior to collection = 17.5–18.0°C). Pupae were first collected from Abner Creek on 2 June 1998 (temperature at time of collection = 18.0°C) and 1 June 1999 (16.0°C). The last larvae and pupae were collected from Abner Creek on 23 June 1998 (18.0°C) and 10 June 1999 (15.0°C).

Of 211 larvae prepared for chromosomal analysis, 72% were read, band for band, in their entirety. The banding sequences of the short arms of chromosomes I and III (Figs. 3, 7) matched those of the subgeneric standard, although we were unable to match all of the fine bands in the base of IS (sections 19 and 20). The remaining four arms had fixed rearrangements relative to the subgeneric standard. IL had one central inversion, two overlapping basal inversions, and a complex of three inversions distally (Fig. 4). IIS carried the B inversion of Rothfels et al. (1978), plus two additional inversions (Fig. 6). IIL had one central inversion (Fig. 8). The IIIL arm, with the nucleolar organizer displaced more distally relative to the subgeneric standard, was highly rearranged (Fig. 9). Many bands could be recognized, but we conservatively matched only the bands in the base of IIIL, relative to the subgeneric standard, and indicated the characteristic "marker" (*sensu* Rothfels et al. 1978), which was partitioned by rearrangements into two pieces.

We found no fixed or floating inversions in our material relative to the *S. parnassum* standard sequence. Chromosome I is implicated as the sex chromosome. Two males from Georgia (Newsome Gap Road) had differentiated centromere regions in chromosome I; the centromere band of one homologue was expanded (standard) more than the other (Fig. 5). About 52% of males (n = 44) from other sites showed a failure to pair on either side of the centromere of chromosome I. This failure to pair was found at sites with and without the two pupal forms. One male from Abner Creek (13 May 1998) was heterozygous for expression of the nucleolar organizer. *Simulium parnassum* was otherwise chromosomally monomorphic.

Discussion

A new pupal form of *S. parnassum* was discovered in which the reticulation of the head and thorax, long used as a diagnostic character (e.g., Stone and Jamnback 1955), was absent. No intermediates between smooth and rugose pupae were found; how-

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symmetrical, 3 = three sharp. 9, Chromosome arm IIIL. Section numbers on bottom indicate *S. parnassum* standard banding sequence; those on top refer to *Simulium* subgeneric standard sequence of Rothfels et al. (1978). Arrows indicate breakpoints of inversions. N.O. = nucleolar organizer, M = marker (divided in two pieces). Female from South Carolina, Pickens Co., Oil Camp Creek, 14 May 1998.

ever, we discovered no additional information, either morphological, chromosomal, distributional, or seasonal, to suggest that two species are present. The synonymy of *S. hydationis* with *S. parnassum* (Stone and Jamnback 1955) is justified, both morphologically and cytologically, because material from near the two type localities was morphologically and chromosomally homogeneous. However, the possibility that the two pupal forms of *S. parnassum* represent homosequential species or that homosequential sibling species exist, as they do in other black flies (e.g., Henderson 1986), cannot be excluded.

Relative to the *Simulium* subgeneric standard of Rothfels et al. (1978), we found only two inversions that are shared with other taxa. The IIS-B inversion of Rothfels et al. (1978) is shared with most species in subgenus *Simulium* (Adler et al. 1999), as is an inversion in IIIL that has one of its breakpoints at the 91/94 junction (Fig. 9) (Adler, unpublished). Because IIIL is not fully resolved, shared inversions with other taxa could be present. The remaining inversions in IL, IIS, and IIL are apparently autapotypic for *S. parnassum.* Chromosomal evidence does not suggest a relation with the *S. tuberosum* species group, which is defined by at least eight unique rearrangements, including four in IIL (Adler and Kusela 1994), nor does it provide resolution of relationship with other species groups. Tentative placement of *S. parnassum* in a separate species group, the *S. parnassum* species group, is therefore more appropriate.

Simulium parnassum was univoltine in South Carolina. In Pennsylvania, it has been considered univoltine but with overlapping cohorts (Tessler 1991). Other authors (e.g., Stone and Snoddy 1969, Cupp and Gordon 1983) suggest that *S. parnassum* completes more than one generation per year, although conclusive evidence supporting these claims is lacking.

Based on the results of our study, we consider *S. parnassum* a single species, albeit polymorphic in pupal surface texture. We suggest, however, that additional (e.g., molecular) evidence be brought to bear to corroborate or falsify this hypothesis.

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