THE DISTRIBUTION AND LIFE HISTORY OF AXYMIA FURCATA MCATEE (DIPTERA: AXYMYYIIDAE), A WOOD INHABITING, SEMI-AQUATIC FLY

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Abstract.—Axymia furcata McAtee (Diptera: Axymyiidae) is a xylophilic, semi-aquatic fly from eastern North America. As part of a comprehensive study of the fly’s distribution, life history, and phylogeography, we surveyed populations of A. furcata in the eastern United States and Canada. Collecting and rearing methods are described, and use of the niche modeling software, DIVA GIS, to locate regions with potentially suitable habitat is presented. Based on historical records and our recent survey, A. furcata is confirmed to occur from southern Ontario and Quebec, across the northern tier of states from Minnesota to Maine, and south along the Appalachian Mountains to Georgia and South Carolina. Our survey resulted in several new state records and demonstrated that A. furcata, a fly once considered quite rare, can be abundant (> 200 individuals in a single log) in suitable habitats. Larvae and pupae are most abundant in wet and partially submerged logs in small streams and seeps. Axymia furcata has four larval instars, overwinters as larvae, and has adults that emerge primarily in March and April in the southern Appalachians and somewhat later (e.g., to late May) in the northern Appalachians, upper Midwest, and southern Canada.

Key Words: habitat, xylophagy, rearing, larval instars, phenology, ecological niche modelling

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The Axymyiidae are an enigmatic group of semi-aquatic nematocerous flies, considered to be the most ancestral family in the infraorder Bibionomorpha in some classifications (Oosterbroek and Courtney 1995) but whose placement is uncertain in others (e.g., Wood and Borkent 1989, Bertone et al. 2008). The family includes three extant genera, Axymia McAtee, Mesaxyymia Mamayev, and Protaxymia Mamayev and Krivosheina (Mamayev 1968), containing seven described species. Of the described species, all but one occur in the Palearctic Region, where they are found in Hungary and Russia (Mamayev 1968), China (Yang 1993), Taiwan (Papp 2007), and Japan (Hiroshi 1953). Nearctic axymyiids include one
described species in the east, *Axymyia furcata* McAtee (McAtee 1921), and a single undescribed species in Oregon (Wood 1981). Prior to 2005, *A. furcata* had been reported from two Canadian provinces and 10 U.S. states but was considered rare throughout its range. In addition, three extinct genera, *Sinaxymyia* Zhang, *Juraxymyia* Zhang, and *Psocites* Hong, are known from Kazakhstan and China (Zhang 2010).

Published information on the ecology of axymyiid flies has been limited. Early records (e.g., Krogstad 1959) indicated that larvae reside inside self-dug chambers within saturated, decomposing woody debris. After two years, the larvae pupate inside the same logs and emerge as adults in early to mid-spring (Krogstad 1959, Wood 1981). Adults possess vestigial mouthparts and are thought to be short-lived (Wood 1981). Published information on most aspects of *A. furcata* life history (e.g., oviposition, overwintering stage, phenology) and general biology (e.g., adult behavior, “host” log preference) are lacking.

The objectives of our research included a detailed study of axymyiid life history and an intensive survey of these flies in eastern North America, partly to test if axymyiids are indeed rare or merely under-collected.

**Materials and Methods**

Comparative material.—Specimens collected during this study were compared with material from several research collections: Canadian National Collection of Insects, Arachnids, and Nematodes, Ottawa, ON (CNC); Cornell University Insect Collection, Ithaca, NY (CUIC); Museum of Comparative Zoology, Cambridge, MA (MCZ); United States National Museum of Natural History, Washington, DC (USNM), including holotype; University of Guelph Insect Collection, Guelph, ON (UGIC); University of Minnesota Insect Collection, St. Paul, MN (UMIC); and University of Wisconsin Insect Research Collection, Madison, WI (UWIC). We also consulted original descriptions of adults (McAtee 1921) and larvae (Krogstad 1959) of *A. furcata* as well as more recent accounts of the family (e.g., Wood 1981, Krivosheina 2000) and available keys to the North American fauna (e.g., Courtney and Merritt 2008).

Ecological niche modeling.—The ecological niche modeling program DIVA-GIS 5.2 (Hijmans et al. 2005) was used as a secondary tool to identify potential *A. furcata* populations. DIVA-GIS works by running GPS coordinates against 19 bioclimatic variables based on temperature and precipitation in the BIOCLIM algorithm. The program uses the results to construct a map that estimates the distribution of *A. furcata* and applies a basic color gradient to project the level of probability of *A. furcata* being present within that distribution. The GPS coordinates used to create the map originated from prior collecting by our lab, as well as points based on literature and specimen labels from various university and museum research collections. Existing records included adult females collected in Pennsylvania and Virginia (McAtee 1921) and various life stages from Massachusetts (Alexander 1942), Minnesota (Krogstad 1959), Ohio (Peterson 1960), New York (McAtee 1921), North Carolina (Wood 1981), and Wisconsin (Young and Lisberg 2001) in the United States, and Ontario and Quebec in Canada (Wood 1981). Unpublished records from Maryland and New Jersey were obtained from pinned material in the USNM. On completion of the DIVA-GIS analysis, we then used state atlases, park descriptions, and past publications to find possible collecting
sites within areas DIVA-GIS mapped as having reasonable probability of finding axymyiids.

Collection methods.—Once a seemingly suitable habitat was located, any saturated and somewhat decomposing woody debris was checked for axymyiid larvae or pupae. An effective initial check is to balance the log between two rocks or logs and apply pressure to the log with the heel. When A. furcata is present, the log typically breaks where larval galleries have weakened the wood, thereby exposing resident larvae or pupae. For careful and thorough removal of all larvae and pupae, each inhabited log can then be dissected with a knife or screwdriver. In our study, we often also scanned the resulting woody detritus under a dissecting microscope, which was especially helpful in finding early instars. When pupae were present, they were either preserved in 95% EtOH or placed in a vial or dish containing damp paper towel, moss, or small pieces of damp wood, where specimens could be held for adult rearing (see below). Emergence and Malaise traps were also used to collect adult A. furcata. Traps usually were placed in the flyway over a seep or small stream, usually near woody debris suspected of harboring axymyiids. All field-sampled, laboratory harvested, and reared specimens were collected into 70% or 95% ethanol for further examination. Various sites were searched throughout the year for the presence of A. furcata. The assumed two-year larval stage was apparent at most sites, as evidenced by the presence of either of two size classes of larvae or mid-stage larvae and pupae. Assuming a two-year life cycle is typical of most populations, we considered any “negative” collections as evidence that A. furcata was absent from that location. Specimens collected during this study have been deposited in the Iowa State Insect Collection, Ames, IA (ISIC), USNM, and CNC.

Rearing methods.—Because of the short adult life and the paucity of pinned adult material in university and museum research collections, the rearing of larvae and pupae was critical to evaluating characteristics of adult A. furcata. An effective rearing method involved the placement of field-collected pupae in individual wells of a 24-well cell-culture cluster plate (or standard Petri dish), with each specimen placed either between layers of damp paper towel or in moss or soft, finely-crumbled wood. Alternatively, especially when enumeration of all individuals or rearing of all adults from a log were objectives, we collected intact pieces of colonized logs for transport back to the laboratory. All such logs were gathered just prior to adult emergence (determined by samples from adjacent logs), maintained in either loosely sealed plastic tubs or plastic bags, and checked daily for emerged adults and misting with water (to ensure the log remained moist). In most cases, the log was kept cool during transport back to the lab and then placed in an incubator set to mimic the approximate high and low temperatures of the original habitat at the time of collection. However, we also maintained several logs on a laboratory bench at ambient room temperature.

Larval-instar determination.—Two measurements were recorded from the dorsal surface of the head capsule: (1) across the widest point and (2) at the anterior margin of the head capsule, just posterior to the base of the mandibles. Both measurements were taken from the more heavily sclerotized regions of the head because they show less variation within each instar and provide better separation between instars. This is unlike overall body length, where substantial overlap occurs between instars.
RESULTS AND DISCUSSION

Distribution.—A DIVA-GIS analysis identified several potentially suitable areas for *A. furcata* and formed the partial basis for subsequent searches in eastern North America. After extensive collection throughout the eastern United States, the first records of the family can be confirmed in 10 states: Connecticut, Georgia, Iowa, Kentucky, Maine, New Hampshire, Tennessee, South Carolina, Vermont, and West Virginia. A summary of recent collecting records in the eastern U.S. is presented in Appendix 1. Although our records fill significant gaps in the known distribution of these flies, we predict that additional collecting in the eastern United States and Canada will expand the known range of *A. furcata*. A DIVA-GIS analysis that incorporated all records from the current study provided the same prediction.

Habitat and larval behavior.—*Axymyia furcata* occurs in a variety of aquatic environments. Although first identified from damp logs in a “swampy woodland” (Krogstad 1959), our study indicates that *A. furcata* occurs primarily in small, lotic habitats, including seeps, springs, and streams (Figs. 1a–e). The lentic habitat (swampy woodland) originally described by Krogstad (1959) appears to be unique to that population in northern Minnesota. Furthermore, we found that *A. furcata* does not generally occur in intermittent or torrential habitats or in areas prone to frequent and rapid flooding. Because the larval stage must survive throughout the year (see below) and requires a continuously moist habitat (Wood 1981, M. Wihlm and G. Courtney unpbl.), intermittent water bodies do not appear to support these flies. Likewise, larval habitats must remain in the stream or seep for extended periods, so large, torrential, and/or flood-prone systems do not generally support populations of this species. That said we occasionally found colonized logs above the water level of larger streams, but usually in a floodplain where evidence of recent scouring of tributary streams was apparent.

The characteristics of the riparian zone appear to be important in the distribution of *A. furcata*. Our records indicate that areas with this species are well forested, consisting of a hardwood or a mixed canopy, with trees close enough to the aquatic habitat to provide limbs or other coarse woody debris for potential larval colonization. Most hardwood forests at known *A. furcata* sites consist primarily of maple, beech, birch, aspen, and oak. In some of the fly’s range, habitats also had smaller amounts of hemlock, white pine, or rhododendron incorporated into the hardwood stand. Our data confirm that *A. furcata* larvae occur primarily in elm (Peterson 1960), maple (Alexander 1920), aspen (Krogstad 1959), and hickory (genus *Carya*), and rarely in rhododendron. *Axymyia furcata* varies significantly from the axymyiid (*Protaxymyia* sp.) reported from Oregon, which resides primarily in softwoods (i.e., conifers). Although the western species was first recorded from alder (Dudley and Anderson 1982), the larvae appear to be most common in cedar (S. Fitzgerald pers. comm.) or Douglas fir (G. Courtney unpbl.).

Krogstad (1959) was the first to describe the wood in which *A. furcata* larvae reside. He noted that the wood must be in the beginning stages of decomposition, free of moss and bark, wet enough to yield water when a knife blade is inserted, and soft enough to push a pencil into it. Wood (1981) also noted that the wood, though rotting, should be light in color and firm enough that prying it apart is difficult. During our investigations we found these criteria to
be mostly true; however, we often found larvae in logs covered by moss or with bark remnants (see Fig. 1f). Level of decomposition varied from that similar to the description by Krogstad (1959) to wood that was so fresh it was difficult to insert a knife or flat head screwdriver. Our larval samples have come from wood ranging from small branches (3–5 cm diameter) to larger tree trunks (> 20 cm diameter).

Although we have not tested the relationship between microhabitat suitability and log position, we suspect a correlation. We have sampled logs from a variety of locations, and the position of the log seems to influence the pattern of colonization by *A. furcata* larvae. Wood lying horizontally on a cool, damp substratum or in a few centimeters of water was often colonized only in the wettest strip along the ventrolateral section of the log. Wood lying in deeper pools (i.e., mostly submerged) was usually inhabited only across the top of the log down to the water surface but rarely below the surface. When logs were leaning from the bank into the water, larvae were concentrated in a saturated band just above the water surface where water had wicked up the wood. In some cases we found colonized logs just above the water, supported by rocks or other woody debris, and where water splashed onto the underside of the log. In such logs *A. furcata* larvae inhabited the saturated bottom and lower sides kept wet from the splashing.

Early descriptions of the larval chambers of *Axymyiidae* suggested that the chambers are usually short (3–6 cm) and primarily straight (Krogstad 1959, Mamayev and Krivosheyna 1966). Wood (1981) reported further that each larva occurs in an individual gallery, with each gallery an oval tunnel that gradually increases in diameter as the larva grows and burrows deeper toward the center of the wood. Although some variation is apparent across geographical areas and population densities, we frequently observed more complex galleries resulting from multiple larvae residing in the same cross section of a log (Fig. 1f). Regardless, the original opening of the gallery is quite small and formed when the first instar burrows into the log after hatching from the egg.

Once the gallery is created, the larva often resides in a distinctive position, with the distal tip of the anal respiratory siphon at the outside opening of the log (Fig. 1i). Krogstad (1959) suggested that the larva uses its siphon to remain suspended in the chamber, a behavior thought possible with the aid of the spinelike structures at the apex of the siphon (Krivosheina 2000; see figs. 4.177–178 in Courtney et al. 2000). However, we found little evidence that larvae remain in such a position for long periods. The larval siphon is quite elastic, stretching and contracting as the larva moves within the chamber (Krivosheina 2000, W. Wihlm and G. Courtney pers. obs.). *Axymyia furcata* was reported to spend...
most of its larval stage with the head capsule pointed toward the distal end of the chamber (i.e., away from wood surface) and turning back toward the opening just prior to pupation (see fig. 2 in Krogstad 1959). However, we have observed larvae of nearly all instars (II–IV) in this position (Fig. 3b), as well as a variety of other positions, and larvae appear to move easily throughout their chambers. Prior to pupation larvae will reposition themselves as aforementioned; they then round out and enlarge the opening to the chamber (Krogstad 1959). At pupation, the two thoracic respiratory organs project from the gallery opening and become the primary respiratory organs (Fig. 1g), while the anal siphon shrinks and presumably loses all or most of its respiratory function.

Palearctic axymyiids are known to push debris out of the gallery and onto the outside surface of the log. This forms small piles of wood particles that are easily recognizable as evidence of axymyiid activity (Krivosheina 2000). This behavior was occasionally observed in *A. furcata*, but more often we noted a moist, pasty mixture of minute wood particles and frass within the gallery. As the larva burrows farther into the log, this pasty mix was often packed behind the larva. A similar phenomenon was reported in Palearctic species and was assumed to indicate that larvae were feeding on microorganisms growing on the paste (Mamayev and Krivosheyna 1966, Wood 1981, Krivosheina 2000); however, the exact composition of the larval diet remains unknown. Axymyiid

![Fig. 2. Scattergram of cranial measurements for larval Axymyia furcata. iI, 1st instar (n = 18); iII, 2nd instar (n = 9); iIII, 3rd instar (n = 94); iIV, 4th instar (n = 96).](image)
larvae may also feed on the wood itself, as evidenced by the gut often being filled with particulate wood (Mamayev and Krivosheyna 1966, Pereira et al. 1982).

Krogstad (1959) reported on the co-occurrence of *A. furcata* and *Temnostoma* Lepelletier & Serville, a syrphid whose larvae frequent rotting logs. We also found this association at many locations, especially in the southern Appalachians. In most situations, *Temnostoma* were in sections of the log that were less saturated and contained wood that was less decayed. Other frequent inhabitants of “A. furcata” logs were larvae of the tipuloids *Lipsothrix* Loew and *Epiphragma* Osten Sacken. When present, *Lipsothrix* was usually restricted to outer sections of the most saturated part of the log, whereas *Epiphragma* seemed to occur in essentially the same microhabitat as *A. furcata*.

Collecting and rearing.—As is true for many groups, the key to collecting axymyriids is to locate suitable habitat and microhabitat and to sample appropriately. Once the appropriate habitats are found, these flies can be quite abundant. Population densities varied greatly within and between collecting sites, with the number of larvae colonizing a single piece of wood ranging from a few individuals to several hundred; e.g., one small log (122 cm length and 12.75 cm average circumference) from Coweeta Hydrological Laboratory, North Carolina contained 289 specimens. Krivosheina (2000) noted comparable densities in Palearctic species, recording between 50 and 150 larvae in logs measuring one meter in length.

Various methods were successful for rearing pupae to adults, though some mortality was expected due to injuries sustained during extraction of the pupa from the wood. However, we found it more difficult to rear extracted pre-pupal fourth-instar larvae to the imago, presumably because provision of food is required. Food apparently was not a limiting factor in rearing larvae and pupae in situ from colonized woody debris, which was the most successful rearing method. Many if not all individuals reared in this manner were able to complete development and emerge. This included specimens reared in incubators as well as those left at ambient temperature in the laboratory. In fact, at least two logs from North Carolina that were reared at ambient temperature provided more than 100 emerged adults. For one of these logs, the first male emerged one day after the log was collected and the first female two days later. The last individuals (2 males and 3 females) emerged on day 14. Most males emerged on days 5–9 and most females on days 9–11; however, adults of both sexes emerged through most of this period.

Although both emergence and Malaise traps were used in this study, only the latter were successful in capturing adult *A. furcata*. Malaise traps were effective at collecting sites in Iowa, North Carolina, and Virginia. In 2007, an Iowa trap yielded several adult specimens between the sixteenth of April and the third of May. Traps set at Coweeta Hydrological Laboratory, North Carolina, during short-term March or April visits from 2006–2008, yielded a small number of adults. Malaise traps placed in a swampy riparian habitat in Virginia also trapped a
small number of individuals (D. Smith pers. comm.).

Larval instars.—Using measurements from the larval head capsule, we confirmed that *A. furcata* has four larval instars (Fig. 2), which is typical of most nematocerous flies. The mean widths at the anterior margin of the head capsule, just posterior to the base of the mandibles and at the widest portion of the head capsules of each instar follow respectively: instar I (0.20 mm, 0.24 mm [n = 18]), instar II (0.31 mm, 0.39 mm [n = 9]), instar III (0.49 mm, 0.63 mm [n = 94]), and instar IV (0.74 mm, 1.01 mm [n = 96]). Interestingly, *A. furcata* is unusual in that the first instar apparently lacks an egg-burster (Wihlm et al. In press), a structure present in most first-instar nematocerous Diptera.

Phenology and adult behavior.—Most of an axymyiid life cycle is spent in its larval stage. Based on the presence of two or three larval instars at any given time and on adults being present for only a short time in the spring, Krogstad (1959) suspected a two-year life cycle for *A. furcata*. Other studies have also suggested a semivoltine, two-year life cycle for this species (Wood 1981) and other axymiids (Krivosheina 2000). Although most of our data support this prediction, data from some sites suggest that sympatric univoltine and semivoltine populations may exist. This pattern can be seen in the highly variable size among pupae and adults, in which a portion of the individuals may be noticeably smaller than other individuals from the same log (Fig. 3g). For example, single logs from North Carolina (Fig. 4a) and New York (Fig. 4b) yielded some females that were strikingly larger (e.g., 6.9–8.0 mm wing length from NC, 7.6–7.7 mm wing length

![Fig. 4. Histogram of wing size and number of emerged Axymyia furcata from individual logs. a, Tributary of Rough Fork, Great Smoky Mountains National Park, North Carolina; b, Tributary of Michigan Creek, Danby State Forest, New York.](image)
from NY) than others (e.g., 5.0–6.5 mm wing length from NC, 5.1–6.8 mm wing length from NY). The same pattern was observed in adults of both sexes in populations scattered across the eastern U.S. The mechanism for this polymorphism remains unclear. Perhaps smaller individuals resided in a microhabitat that provided better nutrition, thereby allowing the larva to move more quickly through its development and to pupate in a single year. Alternatively, smaller individuals may have originated from a microhabitat with lower-quality resources, which stunted their growth, resulting in a much smaller size at emergence. Our data, which include both large and small adults emerging from the same region on many logs, seem to refute the latter alternative.

After overwintering as a larva and just prior to pupation in the early spring, the larva enlarges the opening of its gallery, which will soon be filled by the anterior end of the pupa (mostly the dorsal part of the thorax, with the respiratory organs). Prior to eclosion the pupa positions itself so that the head, thorax, and sometimes the anterior-most abdominal segments project out of the chamber (Figs. 3d). The adult then emerges directly onto the surface of the log and may remain near the cast skin for several minutes, presumably while the cuticle hardens (Fig. 3e). Adults are known to emerge in April to early May in the Midwest and northeastern North America (McAtee 1921, Krogstad 1959, Wood 1981, Young and Lisberg 2001, M. Wihlm and G. Courtney unpubl.); however, emergence in the southeastern United States can begin in early to mid-March. Adults of the Palearctic species have also been recorded to emerge in April and May (Krivosheina 2000). Specimen labels indicate that the undescribed species from Oregon emerges in November, differing from all other documented species.

Few reports of adult axymyiid behavior exist. Adults of Palearctic species have been observed swarming in slow flight on warm sunny days (Krivosheina 2000). This behavior has not been witnessed in A. furcata. In lab situations adults often seem apprehensive to fly and prefer to walk, which makes them easy to capture. Furthermore, if an adult takes flight, it is usually quite slow and clumsy. Although these behaviors are perhaps an artifact of laboratory rearing, we have several field-collected adults that were easily captured out of the air by hand. The adult life span is unknown, but the possession of vestigial mouthparts suggests these flies are short lived (Wood 1981). In our study, laboratory reared adults survived only a few (2–7) days. Although we never observed oviposition in the field, we recorded several females in our rearing chambers that placed eggs individually on logs containing larvae or pupae.

Conclusion.—Many discoveries about axymyiid biology remain for future dipterists and aquatic biologists. Historically, these flies have been considered quite rare, presumably because adults are seldom seen in the environment and rarely trapped or caught in sweep nets. This may reflect the short adult lifespan, their reluctance to fly, or some other aspect of their biology. Regardless, our study has demonstrated that the immature stages, especially the larvae, can be very abundant in the appropriate habitat. A better understanding of larval habitat has lead to the discovery of many additional populations of A. furcata. We have clearly shown that A. furcata is more widespread in eastern North America than was previously thought and that these flies are not rare—they are merely under-collected. The use of the niche modeling software, DIVA GIS, predicts that many other areas of eastern North America should harbor these unusual flies. However, as is the
case for many specialized insects, the key to future discoveries will be to effectively sample their unusual habitats. Axomyiid distributions have undoubtedly been shaped by diverse variables, including not only historical factors (e.g., distribution of their woody host species) but also subtle features of their microhabitat (e.g., age and orientation of woody debris). Data from future collections and controlled field and laboratory experiments may test the relative importance of these and other environmental variables and provide additional insights into the biology of these xylophilic flies.

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LITERATURE CITED


Appendix 1. Records of Axymyia furcata from recent collections in the United States. States listed in bold indicate new state records. CHL=Coweeta Hydrological Laboratory, GSMNP=Great Smoky Mountains National Park, and IF=Issaqueena Forest (Clemson University).

**Connecticut:** Litchfield Co: Macedonia Brook State Park, 41°45.66’N 73°29.71’W.

**Georgia:** Rabun Co: Warwoman Dell, 34°52.88’N 83°21.15’W; Towns Co: Charlie’s Creek above Tallulah River, 34°57.67’N 83°33.54’W.

**Iowa:** Dubuque Co: tributary of Catfish Creek, Swiss Valley Nature Center, 42°25.31’N 90°45.44’W; farm creek near town of Durango, 42°33’N 90°46’W.

**Kentucky:** Letcher Co: Bad Branch, 37°04.20’N 82°46.30’W; Rockcastle Co: small creek NE of Livingston, 37°17’N 84°12’W.

**Maine:** Oxford Co: White Mountain National Forest, 44°22.48’N 70°59.36’W; tributary of Bog Brook @ Bog Road, 44°22.91’N 70°54.29’W; Maryland: Frederick Co: Buzzard Branch @ Mink Farm Road, 39°35.22’N 77°29.60’W; Garret Co: Savage River State Forest, 39°35.19’N 79°10.16’W; Massachusetts: Franklin Co: Mount Toby State Forest,
42°28.84’N 72°34.94’W; Mount Sugarloaf State Reservation, 42°28.94’N 72°31.48’W; Minnesota: Clearwater Co: Itasca State Park, 47°13.72’N 95°11.02’W.

New Hampshire: Grafton Co: White Mountain National Forest, 44°04.22’N 71°41.68’W; New York: Tompkins Co: Danby State Forest, 42°18.92’N 76°29.69’W; Danby State Forest, 42°17.80’N 76°28.97’W.

North Carolina: Macon Co: CHL, Hugh White Creek (WS 14), 35°03.28’N 83°25.90’W; CHL, Grady Branch (WS 18), 35°03.07’N 83°26.17’W; CHL, seeps near Ball Creek gate, 35°03.53’N 83°25.82’W; CHL, upper Reynolds Branch, 35°02.30’N 83°27.10’W; CHL, Cold Springs Gap seeps, 35°01’N 83°27’W; CHL, Bates Branch, 35°03’N 83°27’W; CHL, small creek above weir 16, 35°03’N 83°26’W; CHL, 35°02’N 83°27’W; Haywood Co: GSMNP, seeps near Cataloochee Creek, 35°38.75’N 83°04.56’W; GSMNP, seeps near Rough Fork, 35°36.90’N 83°07.26’W; GSMNP, tributary of Palmer Creek, 35°37.70’N 83°06.90’W.

Ohio: Hocking Co: Hocking Hills State Park, 39°27.54’N 82°33.52’W.

Pennsylvania: Clinton Co: Hyner Run State Park, 41°21.97’N 77°37.86’W; Kettle Creek State Park, 41°20.54’N 77°54.54’W.

South Carolina: Oconee Co: small tributary of Brasstown Creek, 34°43.15’N 83°18.24’W; Pickens Co: IF, Indian Creek, 34°45.55’N 82°51.45’W; IF, Wildcat Creek, 34°45.35’N 82°51.38’W; IF, small tributary of Six-Mile Creek, 34°44.86’N 82°51.25’W.

Tennessee: Sevier Co: GSMNP, small creek near Husky Gap trail, 35°39’N 83°31’W.

Vermont: Bennington Co: Green Mountain National Forest, 43°02.56’N 73°05.48’W.

Virginia: Fairfax Co: Great Falls National Park, 38°59.10’N 77°14.80’W.

West Virginia: Nicholas Co: Monongahela National Forest, 38°18.01’N 80°30.61’W; Monongahela National Forest, 38°16.24’N 80°31.46’W.