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ANOPHELES PERPLEXENS FROM ARTIFICIAL CONTAINERS AND INTERMITTENTLY FLOODED SWAMPS IN NORTHERN FLORIDA

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ABSTRACT. *Anopheles perplexens* was collected from habitats previously unreported for this species in northern Florida. These habitats included intermittently flooded swamps, water-filled tires, and plastic oviposition cups. First-instar *An. perplexens* larvae were recovered from soil samples collected in an intermittently flooded swamp that were flooded in the laboratory, suggesting that *An. perplexens* eggs may survive in the soil during dry periods. *Anopheles perplexens* larvae were collected from water-filled tires and plastic oviposition cups at sites near Gainesville, FL. Of 30 dissected *An. perplexens* females collected in updraft CDC traps, all had ovarioles in Christophers's stage II and blood was absent in the midgut. The physiological state of these females indicates that updraft CDC traps collect predominately host-seeking females and that females take one blood meal per gonotrophic cycle.

Anopheles perplexens Ludlow, originally described from a single female collected in Pennsylvania (Ludlow 1907), was considered a melanistic form of *Anopheles punctipennis* (Say) by Howard et al. (1917). Bellamy (1956), however, demonstrated that *An. perplexens* was a species separate from *An. punctipennis*. *Anopheles perplexens* has been collected from scattered locations in Alabama, Florida, Georgia, North Carolina, Ohio, Pennsylvania, Tennessee, and Louisiana (Darsie and Ward 1981, Chapman and Johnson 1986). Because *An. perplexens* has been infrequently collected and has been recognized as a separate species for a limited period of time, little is known about its biology and ecology.

While studying the biology of anopheline mosquitoes in northern Florida, we collected *An. perplexens* adults and larvae, providing the opportunity to observe aspects of the biology of this little known species. The objectives of this paper are to describe habitats and mosquito species associated with *An. perplexens* larvae in northern Florida and describe the bloodfeeding status, parity status, and degree of egg maturation of *An. perplexens* collected in CO_2-baited, updraft CDC traps.

We distinguished *An. perplexens* from *An. punctipennis* females by *An. perplexens* being generally darker in appearance (King et al. 1960), with dark scales predominant on the scutum and reduced white scaling on the wings. We confirmed our ability to distinguish the 2 species by comparing, under the dissecting microscope, the extent to which the ventral egg surface is covered by the plastron in 20–50 eggs from each of 10 and 6 presumed *An. perplexens* and *An. punctipennis* females, respectively. *Anopheles perplexens* and *An. punctipennis* can be reliably distinguished by differences in ventral egg surface area covered by the plastron (Bellamy 1956, Linley and Kaiser 1994).

We sampled swamps, rivers, man-made and natural lakes, shallow ponds, roadside ditches, intertidal marshes, tree holes, artificial containers, springs, and spring outlet streams in northern Florida within the area shown in Fig. 1 for *Anopheles* mosquito larvae during 1991–93 using standard 500-ml larval dippers. All *Anopheles* larvae collected were reared and identified to species as 4th-instar larvae or newly emerged adults.

Because *An. perplexens* larvae were collected along with larvae of floodwater mosquito species from temporary pools in intermittently flooded swamps, we investigated whether the eggs of this species survived in the soil during dry periods. Samples (15 cm^2 × 3 cm) of decaying vegetative matter and soil were collected from dry depressions in swamps, held for 48 h, then immersed in distilled water and checked for the presence of mosquito larvae daily (Jensen et al. 1994). Larvae were reared and identified.

Collections of *An. perplexens* larvae from water-filled tires indicated that females oviposited in artificial containers. To confirm this behavior, we set out 15 black plastic oviposition cups filled with 350 ml of tap water in an auto wrecking yard southeast of Gainesville, Alachua County, on March 26, 1991. The site was selected because *An. perplexens* had been collected from tires in the yard. Other potential oviposition sites in the area included other water-filled containers, a stream, and a swamp located approximately 200 m away. Cups were examined 3 times weekly for 3 wk, and mosquito larvae were reared and identified as adults.

Three inverted CDC light traps baited with 2 kg of dry ice were used to collect *Anopheles* mosquitoes over a 24-h period on April 7–8, 1993, in an intermittently flooded swamp in Sumter County (Fig. 1). The site had been used in a study of the adult biology of *Anopheles quadrimacu-*

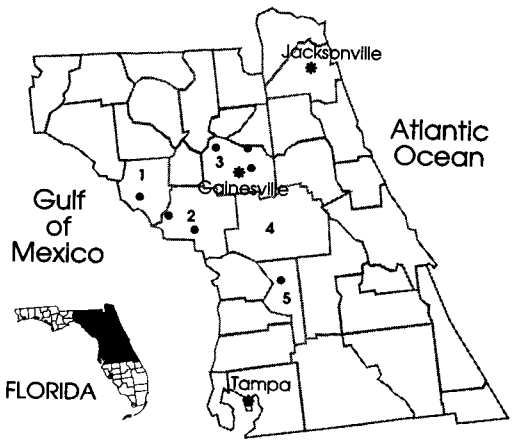

Fig. 1. Map of Florida indicating locations where *Anopheles perplexens* were collected. 1. Dixie Co., 2. Levy Co., 3. Alachua Co., 4. Marion Co., 5. Sumter Co.

latus Say species C1 (Jensen et al. 1993). Intact female mosquitoes were examined to determine bloodfeeding status and then dissected to determine the degree of egg maturation (Christophers 1911) and the parity of females with ovarioles in stage II based on examination of the ovarian tracheoles (Detinova 1962).

At the CDC trap site, collections of resting *Anopheles* mosquitoes were aspirated from 3 natural tree cavities, 3 1-ft^3 wooden red boxes, and 5 Arkansas red boxes (Weathersbee and Meisch 1988) at 3-day intervals, from August 15 to September 4, 1991, and screened for *An. perplexens.* In addition, 100 collections of *An. quadrimaculatus* mosquitoes from approximately 25 natural resting sites throughout northern Florida during 1991–93 were screened for resting *An. perplexens.*

From 1991 to 1993, *An. perplexens* larvae were collected from temporary pools in swamps (Fig. 2) at the following locations: Santa Fe River, Alachua Co. (May 10, 1991), Wacassassa River, Levy Co. (28 May 1991), Coleman Landing, Sumter Co. (June 5, 1991, and January 6–May 5, 1993), and Bear Bay, Dixie Co. (March 9–July 7, 1993). Bald cypress, *Taxodium distichum* (Linn.), and sweet gum, *Liquidambar styraciflua* Linn., were the dominant tree species in all these swamps indicating that these swamps were intermittently flooded. *Taxodium distichum* occurs in sites that are regularly inundated; however, *L. styraciflua* is limited to sites where surface water persists for short periods and there is little likelihood of prolonged immersion (Penfound 1952).

Anopheles perplexens was less abundant than other *Anopheles* mosquito larvae in pooled collections from Coleman Landing on March 12, 16, 22, and 30, 1993. Of 202 anopheline larvae reared from these collections, 13 (6.4%) were *An. perplexens,* 107 (53%) *Anopheles crucians* Wied. *s. l.,* and 82 (41%) *An. quadrimaculatus s. l.* Other mosquito species collected with *An. perplexens* larvae in the swamps were *Aedes infirmatus* Dyar and Knab, *Aedes vexans* (Meigen), *Psorophora ferox* (von Humbolt), *Psorophora howardii* Coquillett, *Culex territans* Walker, *Uranotaenia sapphirina* (Osten Sacken), *An. quadrimaculatus* species A and C1, and *s. l. Anopheles perplexens* larvae were absent from collections from other habitat types.

Five *An. perplexens,* 1 *An. crucians s. l.,* 2 *An. quadrimaculatus* species C1, and 12 *Ae. infirmatus* larvae were obtained from 10 soil samples collected from a depression in Coleman Landing Swamp, Sumter County, on May 4, 1993. No standing water was present on May 3, 24 h before the sample was collected and, standing water had been present on April 14, 20 days before the soil sample was collected, indicating that *An. perplexens* eggs survive 3–20 days in the soil. *Anopheles perplexens* larvae were absent from 20 samples collected from 2 other depressions at the same time and from soil samples collected from Coleman Landing swamp on May 10, 13, and 18, and June 1 and 10, and from soil samples obtained from other swamps in Dixie, Marion, and Sumter counties.

Four *Anopheles* larvae were collected from a water-filled tire in the auto wrecking yard. Three of these were reared and identified after emergence, as 2 male and 1 female *An. perplexens.* Eighteen km away, in Waldo (Alachua Co.), 2 *An. perplexens* larvae were also collected from water-filled tires.

Five 1st-instar *An. perplexens* larvae were collected from the oviposition cups in Gainesville. Two larvae were collected on April 2 and 8 were collected on April 5, 1991, 5 and 7 days, respectively, after the cups were set out. Another anopheline larva, collected on April 15, died as a 2nd instar and could not be identified to species.

Thirty of 54 (56%) *An. perplexens* females collected in updraft CDC traps were dissected. The remaining females were too desiccated or damaged to be dissected. None of the midguts of dissected females contained blood or traces of blood. The ovarian primary follicles of all dissected females were in stage II (Christophers 1911); 4 of 30 (13%) females were parous. Other mosquitoes collected in the CDC trap were *An. quadrimaculatus s. l., An. crucians s. l., Ae. infirmatus, Ae. vexans, Ps. ferox, Ps. howardii,* and *Culex* spp.

Fig. 2. Temporary pool in an intermittently flooded swamp near Coleman, Sumter County, FL, from which *Anopheles perplexens* larvae were collected.

One blood-engorged female and one male *An. perplexens* were collected from an Arkansas red box in Coleman Landing swamp on August 15, 1991. No *An. perplexens* were collected from wooden red boxes, Arkansas red boxes, and 3 natural tree cavities on August 20, 23, 27, 29, and September 4. A single *An. perplexens* was collected from a tree cavity in an intermittently flooded swamp on the Lower Suwannee National Wildlife Refuge, Levy County, FL, on April 5, 1994.

Prior to our study, *An. perplexens* larvae have been collected only from springs and outlet streams associated with limestone outcroppings in northern Florida (King et al. 1944, 1960) and southern Georgia (Bellamy 1956) and a millpond and associated quiet stream in Georgia (Kreutzer and Kitzmiller 1971). Because *Anopheles* mosquito larvae are typically found in heavily vegetated permanent and semipermanent aquatic habitats (Bates 1949), prior surveys may not have sampled artificial containers or intermittently flooded swamps for anopheline mosquitoes.

Floodwater *Aedes* and *Psorophora* mosquitoes have adapted to temporary pools by means of eggs that survive out of water for periods of up to many years but hatch shortly after the site is inundated (Bates 1949). The finding of viable *An. perplexens* eggs in the flooded soil samples suggests that this species may be similarly adapted. Eggs of several anopheline mosquitoes that develop in temporary pools are also capable of surviving in the soil for limited periods (Stone and Reynolds 1939, Rajapaksa 1971, Rosenberg 1982, Beier et al. 1990). Eggs of *An. quadrimaculatus* species C1 and *An. crucians s. l.,* which occur in the same pools from which we collected *An. perplexens* larvae, survive 34 days when out of water (Jensen et al. 1994; T. Jensen, unpublished data). The relative scarcity of *An. perplexens* in the larval collections from Coleman Landing in March 1993 suggests that the absence

of viable *An. perplexens* eggs in the soil samples collected after May 3 could have resulted from the absence of eggs in the collected soil samples.

The presence of larvae during January, February, and March indicates that *An. perplexens* females remain gonotrophically active and do not enter a reproductive diapause during winter in northern Florida. The presence of *An. perplexens* larvae in the oviposition cups indicates that females will oviposit in small artificial containers even though the creek and nearby swamp areas may provide available habitats. However, it is not known if *An. perplexens* can complete development in these containers. Limited food resources and competition from other larvae may prevent completion of development.

The physiologic state of *An. perplexens* females collected in the updraft CDC traps was similar to that of *An. quadrimaculatus* species C1 females collected at the same location (Jensen et al. 1993). Moreover, the degree of egg maturation of dissected females indicates that the traps collected primarily host-seeking females. The absence of blooded females or females with ovarioles in the intermediate stages of oogenesis suggests that a single blood meal is required for completion of each gonotrophic cycle.

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