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THE STRUCTURE AND FUNCTION OF THE LARVAL SIPHON AND SPIRACULAR APPARATUS OF *COQUILLETTIDIA PERTURBANS*

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ABSTRACT. The structure of the larval siphon and spiracular apparatus of *Coquillettidia perturbans* and the mechanism of attachment to roots of emergent aquatic macrophytes were examined by utilizing dissection and scanning electron microscopy. The roots of these plants contain large air-filled aerenchyma channels that larvae of *Cq. perturbans* pierce with their specialized siphon and spiracular apparatus to breathe. The siphon contains the spiracular apparatus, comprising the saw, postabdominal spiracles, inner spiracular teeth, and the spiracular apodeme. These are the primary structures that are utilized by larvae to pierce root tissue. Once entry is made into a root, the outer spiracular teeth open fully, anchoring the larva in place.

KEY WORDS *Coquillettidia perturbans,* cattail mosquito, aerenchyma, siphon, emergent aquatic macrophyte

INTRODUCTION

Some of the most remarkable larval respiratory adaptations in the family Culicidae occur in the genera *Mansonia* and *Coquillettidia.* The species of these genera have a specialized siphon to pierce the roots, stems, or submerged leaves of aquatic plants, enabling them to utilize oxygen from plant tissue. One species, the cattail mosquito (*Coquillettidia perturbans* (Walker)) is a common nuisance mosquito in North America that has been implicated as a bridge vector of eastern equine encephalomyelitis virus (Carter et al. 1981, Francy 1982, Sofield et al. 1983, Crans and Schulze 1986, Nasci et al. 1993). Walker described the adult stage of this mosquito in 1856, naming it *Culex perturbans,* but because of its unusual behavior the larva remained undescribed for more than 50 years. The mystery was solved in 1907 when J. Turner Brakeley, a volunteer field observer in New Jersey, washed a 3rd-stage larva from the roots of an aquatic grass growing in a marsh near Trenton. He reported the find to John B. Smith of Rutgers University, who in turn published a detailed account of the discovery (Smith 1908). Smith's publication incorporated John A. Grossbeck's description of the larva and several drawings of its morphology, including that of the siphon. After Brakeley's discovery, other mosquito species that utilized some type of aquatic plant as a means for larval respiration were discovered in other parts of the world (Dyar and Knab 1910, Ingram and Macfie 1917, Wesenberg-Lund 1918, Edwards 1919, Gillett 1946, Laurence 1960, Burton 1965). Over the years, researchers have verified many of Smith's initial observations regarding *Cq. perturbans*; however, apparently no close examination was made of the structure and function of the siphon and spiracular apparatus.

In this paper, the morphology of the siphon and spiracular apparatus of *Cq. perturbans* are reexamined and the mechanism for penetration and attachment to roots of aquatic macrophytes are described. Harbach and Knight (1980) are followed for morphological terminology.

MATERIALS AND METHODS

Larval *Cq. perturbans* were collected at Colliers Mills Wildlife Management Area, Colliers Mills, NJ, by using the modified bilge pump method of Walker and Crans (1986). In the laboratory, 4th-stage larvae were isolated and placed in a 250-ml beaker with 100 ml of bottled water. Fourth-stage larvae were selected because this size enabled the clearest view of the siphon and the attachment site. Living roots of known host plants were cut from masses in lengths of approximately 10–15 cm and placed in beakers with several larvae. During the warmer months, the setup was left at ambient temperature, usually overnight, until a number of the larvae attached to the roots. During the colder months, the setup was placed in refrigeration at 6°C overnight. Some larvae would not attach for various reasons, and those that were going to attach would usually do so overnight; allowing more time did not result in significantly more larval attachment and may have actually resulted in some detachment. Once the larvae attached, the setup was gradually frozen overnight. With this method, larvae slowly freeze while remaining attached to the roots. Larval *Cq. perturbans* are very cold tolerant and may even withstand freezing for short periods, so it is important to freeze the setup overnight. Once thawed, small sections of root with attached dead larvae were cut free with dissecting scissors, carefully removed with forceps, and placed in a petri dish containing water. Free-hand cross-sectioning of the root with attached larvae was performed under a Leica StereoZoom 6 Photo dissecting microscope (Leica Microsystems Inc., Buffalo, NY) at 40×. After sectioning, the siphon and the attached root cross-section were dissected from the larva. The prepared sections were placed in a 10% NaOH solution and incubated at 35°C overnight for

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clearing and subsequently stained with a weak solution of Safranine O.

For scanning electron microscopy, 4th-stage larvae were collected as above and living specimens were air-dried on filter paper. Photographs of structural details were taken with a Hitachi™ S510 scanning electron microscope (Hitachi Instruments, San Jose, CA) after coating specimens with gold-palladium.

RESULTS

The siphon and spiracular apparatus (SAp) of *Cq. perturbans* is a modified culicine type, and is dark brown and strongly sclerotized. The siphon is continuous, lacks sutures, and tapers gradually for approximately one half of its length. The apex of the siphon is abruptly constricted and bears the anteriorly curved spiracular apparatus that terminates sharply. As with other mosquito larvae, *Cq. perturbans* rely on valves to open and close the SAp as needed. When larvae are detached, either swimming or resting, the valves remain closed and are composed of several sclerotized movable plates that are relatively smooth (Fig. 1a). The posterior aspect is less complex than the anterior and consists of the posterior spiracular plate and posterior spiracular lobe (PSL), 2 structures involved in covering the spiracular apparatus (Fig. 1b). The anterior aspect consists of a number of structural elements, the most prominent of which is the saw. The sclerotized saw, situated within a furrow in the center of the anterior portion of the siphon, is bordered externally by the anterolateral spiracular lobes (LSL) (Fig. 1c). Internally the saw is fused basally to the postabdominal spiracles (PAS) and these in turn are joined to the spiracular apodeme (SAd). The PAS are 2 fused, rigid tubes that connect with the large flexible tracheal trunks in the 8th abdominal segment. The SAd is a laterally compressed tubular structure that has a strong muscular attachment at its proximal end and distally forms a daggerlike structure. Lateral to this structure, on each side, are triangular plates, and at their apices are the inner spiracular teeth (IST). The IST, saw, PAS, and the SAd collectively represent the SAp (Fig. 2).

When the SAp is open, the IST and outer spiracular teeth (OST) are fully everted. The IST are distal to the OST and are situated 1 set on either side of the spiracular opening (Fig. 3a). These structures are greatly reduced in comparison to the OST, are dark in color, and are highly sclerotized.

The OST are lightly sclerotized and occur in 2 rows below and lateral to each set of IST. Each of the rows consists of 3 hooklike teeth that are stacked one upon the other and when everted curve backward and appear to be connected basally to the PSL (Fig. 3a).

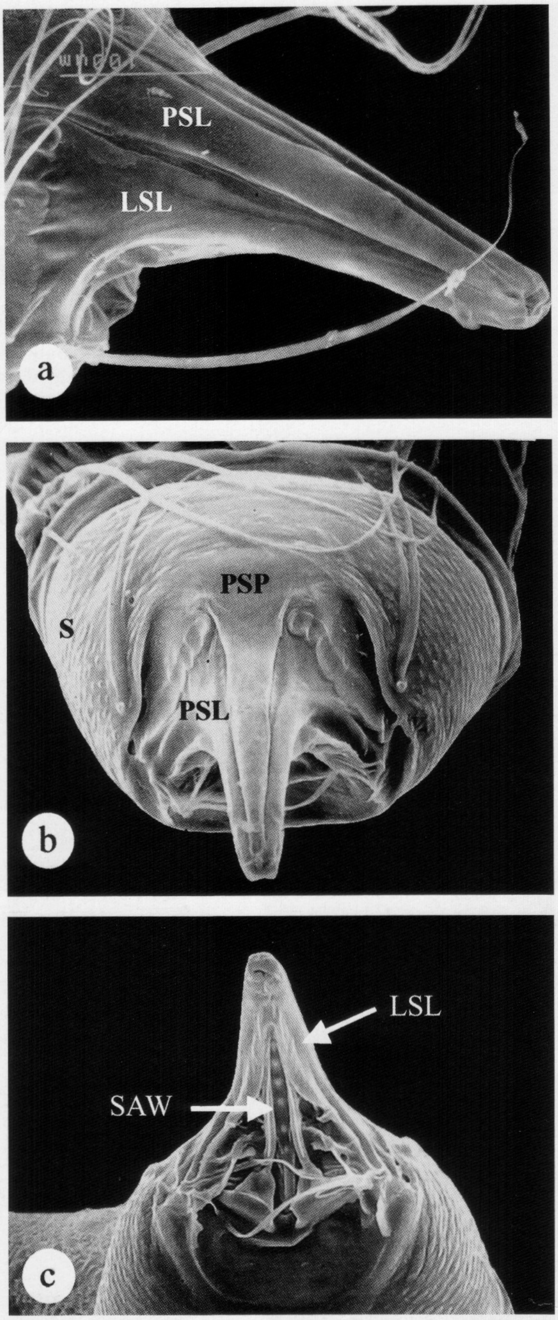

Fig. 1. Siphon and spiracular apparatus (closed) of *Coquillettidia perturbans.* (a) Lateral aspect. LSL, anterolateral spiracular lobe; PSL, posterolateral spiracular lobe. (b) Posterior aspect. S, siphon; PSP, posterior spiracular plate; PSL, posterolateral spiracular lobe. (c) Anterior aspect. SAW, saw; LSL, anterolateral spiracular lobe.

DISCUSSION

Most culicine larvae hang headfirst from the water's surface, venturing below either to feed or escape danger. Regardless of the time spent sub-

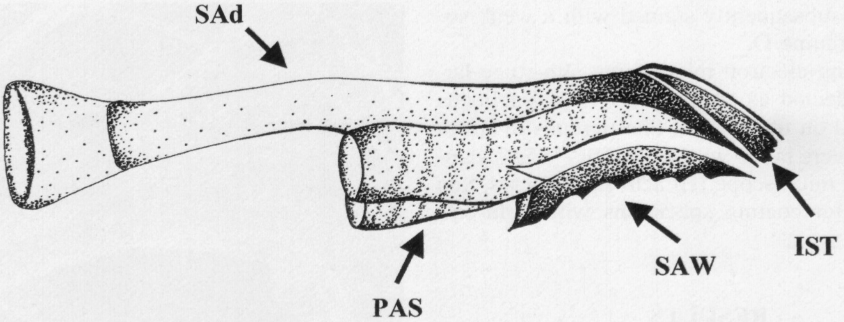

Fig. 2. Internal structure of the spiracular apparatus of *Coquillettidia perturbans.* SAd, spiracular apodeme; PAS, postabdominal spiracles; SAW, saw; IST, inner spiracular teeth.

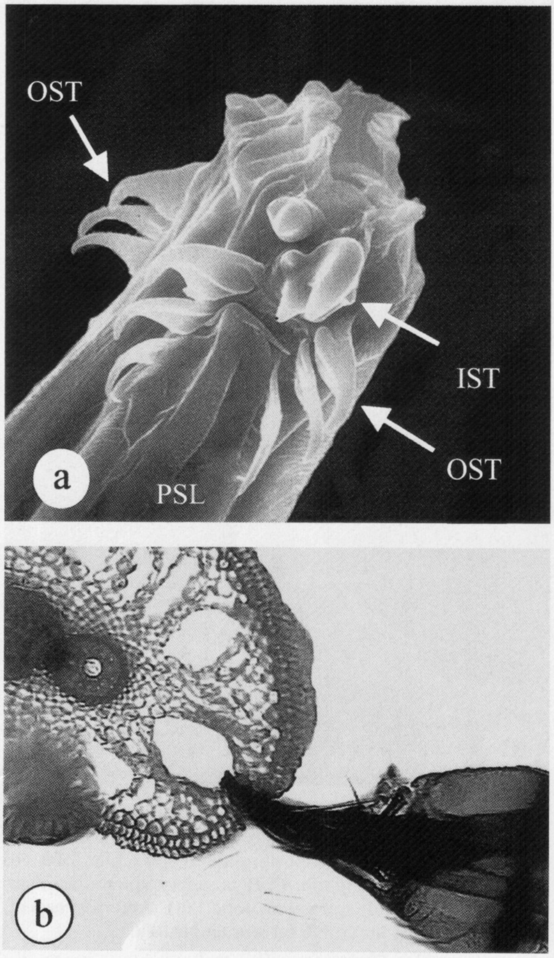

Fig. 3. (a) Spiracular apparatus (open) of *Coquillettidia perturbans.* OST, outer spiracular teeth; IST, inner spiracular teeth; PSL, posterolateral spiracular lobe. (b) Cattail (*Typha latifolia*) root cross-section exposing air-filled aerenchyma channel pierced by spiracular apparatus of *Cq. perturbans.*

merged, they must return to the surface to respire through a siphon. However, exceptions exist and a number of species found in permanent freshwater habitats have evolved unique ways to circumvent their need to surface for air, and perhaps reduce their exposure to predation (McNeel 1932, Van den Assem 1958, Armstrong 1980). Some larvae rely on trapped air bubbles in and among vegetation whereas others remain submerged by utilizing gill or cuticular respiration or both. *Coquillettidia perturbans* and its near relatives respire by piercing the roots of emergent aquatic macrophytes with their highly specialized siphons. Emergent aquatic macrophytes are plants that are at least partly rooted in sediment and whose leaves extend into the atmosphere. The nutrient-rich medium in which these plants grow is nearly anoxic, and because roots need oxygen to function, some have evolved an elaborate gas transport system. Dacey (1981) demonstrated that aquatic plants such as the yellow water lily (*Nuphar luteum*) maintain a pressurized flow-through ventilation system in which atmospheric air enters newly unfurled leaves against a gradient in pressure and travels down the petioles to the rhizomes and roots via a continuous network of large open-channeled aerenchyma tissue. As a result of the pressure generated by the younger leaves, the by-products of root metabolism (carbon dioxide and methane) are forced to the atmosphere through the plants' older leaves. Compared with the roots of terrestrial plants, those of emergent aquatic plants contain a greater proportion of aerenchyma (Armstrong 1978), and it is these larger air-filled channels that larval *Cq. perturbans* utilize for underwater respiration.

In New Jersey, 3 plant species in particular are host to larval *Cq. perturbans*: cattail (*Typha* spp.), rush (*Juncus* spp.), and swamp loosestrife (*Decodon verticillatus*) (Crans et al. 1986). Larvae searching for an attachment site move along the length of the root tapping the epidermis with the apex of their siphon, occasionally everting the OST. Ingram and Macfie (1917) proposed that fringed bristles on the siphon of *Mansonia africana* (Theobald) might have a sensory function that aids larvae in the location of suitable attachment sites. Our examination of the siphon and SAp of *Cq. perturbans* did not reveal any fringed bristles and in our opinion larvae simply test the respiratory suitability of the substrate by probing. The depth to which the SAp is embedded in root tissue is dependent upon its contact with suitable aerenchyma, and very often these channels can be found just inside the root epidermis (Fig. 3b). Robust muscles attached to the SAd provide the strength needed to pierce roots (Ingram and Macfie 1917). Penetration of the epidermis is accomplished by rapid, repeated thrusts of the terminal segments in concert with the exertion of the daggerlike SAd and saw. Once a suitable portion of the aerenchyma is penetrated, the IST and OST open fully. In this position, the OST curve backward and anchor the larva in place. This attachment is a strong one, but larvae can quickly release and swim away if disturbed. The IST seem to play a lesser role in anchoring the larvae and may represent a vestigial or ancestral character.

Larval *Cq. perturbans* have been assumed and widely advocated to possess a siphon equipped with an external saw that is used to cut into the roots of aquatic plants. Our examination of the siphon and SAp reveals a much more complex structure composed of several internal structures that operate in unison to 1st pierce and then anchor larvae into the large air-filled aerenchyma channels found in the roots of emergent aquatic macrophytes.

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