

## OPERATIONAL AND SCIENTIFIC NOTES

### DISCOVERY OF AN OVERWINTERING ADULT FEMALE OF *CULISETA ANNULATA* IN BALTIMORE

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During an ongoing study on the overwintering biology of *Culex* (*Culex*) *picipiens* Linnaeus 1758 and St. Louis encephalitis virus, a team from the Walter Reed Army Institute of Research collected an adult female of *Culiseta* (*Culiseta*) *annulata* (Schrank 1776). The single female was found on 8 March 1978, resting on a wall inside "Outer Battery Bomb Proof No. 2" at Fort McHenry National Monument and Historic Shrine, Baltimore, Maryland. Fort McHenry, built to protect Baltimore against water approaches by enemy vessels, is located on Whetstone Point on the Patapsco River, 4.8 km from the center of Baltimore. Outer Battery Bomb Proof No. 2, constructed during the 1860's or 70's, is a red brick, munitions bunker partially buried under a 2 m earthen mound. The floor of this bunker is about 4 m below ground level and is reached by stairs entering at the northwest corner. The female of *annulata* was resting upon the north wall of the bomb proof approximately 1 m above the floor and 2 m east of the stairs. The wall was moist and coated to varying degrees with a layer of mineral deposits. The floor of the bunker was flooded with water to a depth of about 0.3 m. In addition to the single female of *annulata* captured, 41 adult females of *Cx. picipiens* were collected.

The specimen of *annulata* was taken to the laboratory and was held for 20 days at 27°C ± 0.5, 60–90% relative humidity and a long day photoperiod of 16:8 h light: dark cycle. The female fed on a 1-day old chick on 29 March and was then isolated in a 9-dram vial partially filled with water in the hope of obtaining eggs from which progeny could be individually reared. Unfortunately, the female was found dead on 3 April prior to oviposition. The female was then taken to the Medical Entomology Project, Smithsonian Institution, for detailed morphological study.

On first examination we believed that this female was a specimen of the western North American species, *Culiseta* (*Culiseta*) *particeps*

(Adams 1903), as it had subapical light-scaled bands on the femora which, in the Nearctic, are used to distinguish *particeps* from the Holarctic species, *Cs. (Cus.) alaskaensis* (Ludlow 1906). However, we found that this specimen of *annulata* could easily be distinguished from *particeps* in the adult female by the following characters; (1) a light-scaled medial band on tarsomere 1 of each pair of legs, (2) larger light-scaled basal bands on fore-, mid- and hind tarsomeres 2–4, (3) absence of a light-scaled basal band on hind tarsomere 5 and, possibly (4) cross-veins r-m and m-cu with fewer scales and scales usually restricted to anterior portion of crossvein.

Presently, *annulata* is listed by Knight and Stone (1977) as including 2 subspecies, *annulata* and *subochrea* (Edwards 1921). We concur with Mohrig (1969) and treat these latter taxa as separate species. We observed that the adults of *annulata* differ from those of *subochrea* in several different characters as do, reportedly, the larvae and male genitalia (Marshall 1938; Natvig 1948). The specimen from Ft. McHenry is clearly *annulata* (Fig. 1) and not *subochrea*.

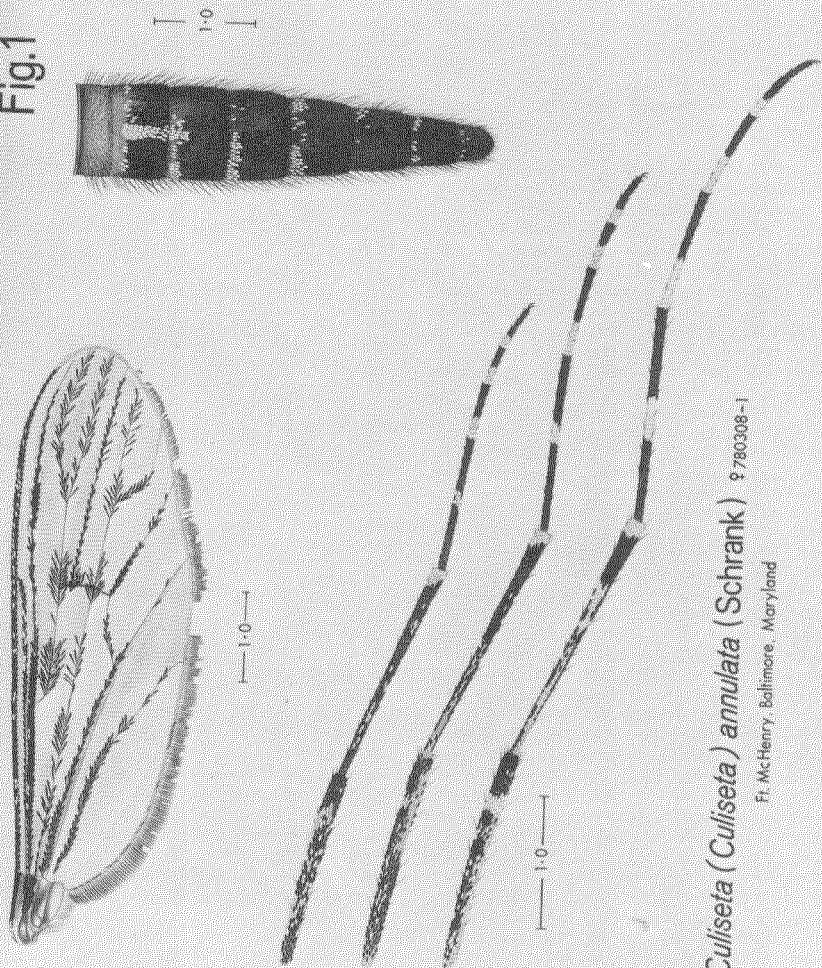
*Culiseta annulata* is distributed throughout western and central Europe, Scandinavia, USSR (east to Leningrad region, north to Estonia, and the Caucasus and Transcaucasian mountains), Middle Asia, the Mediterranean and North Africa.

The immatures have been collected in the Palearctic region in both artificial and natural aquatic habitats such as ditches, stagnant pools, puddles, marshes, barrels and cement reservoirs, in open as well as shaded areas, and in fresh and brackish water (Wesenberg-Lund 1921; Marshall 1938; Natvig 1948; Gutsevich, Monchadskii and Shtakel'berg 1971). The immatures seem to tolerate, and possibly prefer, habitats with moderate amounts of organic pollution. They have been collected in large numbers in "manure-water employed for gardening purposes" (Marshall 1938) and in ditches with *Cx. picipiens* "mainly consisting of [cattle] urine" (Wesenberg-Lund 1921).

There is evidence to suggest that Tahyna virus, a member of the California group of arboviruses, is transovarially transmitted in *annulata*. Bardos et al. (1978) isolated the virus from a pool of first generation, field collected, *annulata* larvae from Moravia, Czechoslovakia.

In regions having relatively mild winters, reportedly *annulata* is capable of overwintering

Fig.1



*Richard Mackintosh*

*Culiseta (Culiseta) annulata (Schrank)* ♀ 780308-1

Fr. McHenry, Baltimore, Maryland

as an adult female or as an immature. Marshall (1938) stated that adult males, pupae and larvae of all 4 stages have been collected every month of the year on Hayling Island, Great Britain. Likewise, Gutsevich et al. (1971) stated that along the southern coast of Crimea this species hibernates in the larval stage. Marshall wrote that in Great Britain the adult females undergo what he termed partial hibernation. Whenever conditions are favorable during the winter females become active and seek a blood meal, and then, develop eggs and oviposit. In areas where the winters are more severe such as in northern Europe and northern USSR, *annulata* overwinters as an adult female. Throughout most of its range (except in the most southern regions) the females spend the winters in places such as stables, attics, cellars, hollow trees, stacks of wood, etc. (Marshall 1938; Wesenberg-Lund 1921). Pertinent to the female collected at Ft. McHenry, Wesenberg-Lund wrote, "If in the winter we examine the deep frostless cellars of our houses, we find among the numerous *C. pipiens* a few larger ones, highly characteristic because of their spotted wing and ringed tarsi. This is *T. [Theobaldia] annulata* [= *Cs. annulata*] the largest of our mosquitoes."

Previously, Hughes (1961) reported that a specimen of *annulata* was collected dead on an aircraft. We assume this report refers to a specimen in the Smithsonian collection with the following labels, "NY Int. Airport/J. Hughes 890/IX-59-25993/Aircraft/Culiseta subochrea/Stone (Edw.)." Unfortunately, as the label indicated, this was a female of *subochrea* and not *annulata*. We did, however, examine an adult female of *annulata* that had been collected on an airplane with the following labels, "17. Plane SAS.OY-AAP/Arr. N.Y. 10-2-50/From Stockholm//Culicidae Culiseta annulata (Schrank)." Since the closest international airport to Ft. McHenry is 20 km removed, the possibility that this female of *annulata* was introduced by an aircraft is remote.

Because of this species' ability to inhabit diverse aquatic environments and to overwinter as an adult female (or immature, climate permitting), it is possible that a specimen or specimens of *annulata* could easily survive a transatlantic voyage protected in any number of places aboard a ship. Since many large freighters do moor at docks adjacent to, or very near, Ft. McHenry, it is most likely that this specimen or one of its ancestors was introduced into the Ft. McHenry-Baltimore area by a ship traveling from Europe. As the specimen was in good condition, it is possible that a

breeding population of *annulata* has been established in the vicinity of Ft. McHenry, and the single specimen did not simply ride aboard a ship as an adult, then find its way into the bunker. Although fresh water habitats are scarce near Bomb Proof No. 2, there was, until October 1978, a brackish marsh about 500 m southwest of the bomb proof. It is conceivable that the immatures may have occurred in that marsh, or, perhaps *annulata* carries out its entire life cycle inside Bomb Proof No. 2 or one of the other bunkers, as water was present on the floor of the former on every occasion that we visited the fort. A search for the immatures of *annulata* on 27 April 1979 was unsuccessful.

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### RECONSTITUTED COLLAGEN SAUSAGE CASINGS FOR THE BLOOD FEEDING OF MOSQUITOES<sup>1</sup>

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The increasing cost and manpower requirements associated with the mass rearing of blood-feeding arthropods for research and biological control programs have made blood-feeding through membranes a viable alternative or adjunct to the use of living animals (Bailey et al. 1978). Numerous researchers (Tarshis 1958) have developed and evaluated techniques for the feeding of arthropods through natural and synthetic membranes. These have ranged from bat wing skin to latex prophylactic condoms, each with its advantages and disadvantages.

We have tested collagen sausage casings for the blood-feeding of 6 species of mosquitoes (*Aedes aegypti*, *Ae. taeniorhynchus*, *Anopheles albimanus*, *An. quadrimaculatus*, *An. stephensi* and *Culex pipiens*) and 2 species of blood-sucking bugs (*Rhodnius prolixus* and *Triatoma barberi*). The casings used are formed from processed beef hide corium collagen which has been chemically rearranged to form the pure collagen casings (Devro, 1976). Two sizes of sausage casings, #300-812-0 and #360-911-0 (Devro, Inc., Somerville, N.J.), were tested (Table 1). No difference between the 2 casings was noted in respect to mosquito probing activity or feeding to repletion after 14 trials using 28 sections of each size casing. Both sizes became

Table 1. Collagen sausage casings used for insect blood feeding.

Casing Number	Stuffed Diameter (mm)	Thickness (microns)*	Price \$/8 in Section
300-812-0	30	2.86 ± 0.20	\$0.0213
360-911-0	36	3.92 ± 0.16	\$0.0310

\* Mean ± s.d. of measurements taken on 9 casings 1 hr after immersion in distilled water.

supple after a few seconds of exposure to blood or water, remained pliable through numerous feedings and were resistant to tearing. In tests using over 200 sections of casing only one hole, attributable to a defect in the casing, was detected.

We used blood-feeding methods similar to those of Bailey et al. (1978). Outdated human blood, to which 2.5 mg adenosine triphosphate per ml was added as a feeding stimulant, was used in all tests. Eight-inch sections of casing were cut and tied shut at one end with heavy string. The open end of the casing was slipped over the stem (20 mm diameter) of a powder funnel and secured with a second piece of string that could be pulled tight after slipping the filled casing off the funnel stem. The funnel was placed on a ring stand with the closed end of the casing in contact with the base of the stand (Fig. 1). Approximately 50 ml of blood was poured into the casing, which was then removed from the funnel. The air was expelled to prevent distention during warming, and the casing was tied shut. The blood filled casings were heated in a 44°C water bath for 10-15 minutes and transferred to the screened top of the insect rearing cage for 10-15 minutes to permit feeding. Placing a warm damp cloth over the heated casing extended the feeding time. The filled casings were reheated and reused until the colonies had been fed.

Mosquito colonies were allowed to blood feed once per week using the casing (#300-812-0, Table 1) and twice per week on anesthetized rabbits for 12 weeks. This regimen was followed by biweekly casing feedings and weekly rabbit feedings for 4 weeks. No noticeable changes in egg production or fecundity occurred. Observations indicated that, with the exception of *Cx. pipiens*, all strains would feed as well on the casings as on rabbits in respect to the number of mosquitoes feeding to repletion. *Cx. pipiens* were observed to feed less readily; however, this species showed no drop in egg production. After 2 weeks of feeding by the casing method exclusively, egg production

<sup>1</sup> The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.