WORLD CERATOPOGONIDAE GROUP

Proceedings of Meetings, August 23—September 1, 1976

This series of abstracts summarizes the combined proceedings of 2 meetings held in connection with the XV International Congress of Entomology in Washington, D. C. from August 19 to 27, 1976.

A symposium, "Systematics and Biology of Ceratopogonidae," was sponsored by Section 9 of the Congress (Medical and Veterinary Entomology) jointly with Section 1 (Systematics), with a program held during the afternoon and evening sessions on August 23. The Honorary Chairman was Botha de Meillon, Tzaneen, South Africa; the Chairman was Michel Kremer, Strasbourg, France; and the Moderator was J. A. Downes, Ottawa, Canada. The program was organized and the meeting convened by W. W. Wirth. Thirteen contributions were given under the 10 topics listed below.

A Post-Congress International Workshop, "The Ceratopogonidae," was held at the Mountain Lake Biological Station near Blacksburg, Virginia, August 28 to September 1, 1976. The workshop was organized by J. R. Linley, and E. C. Turner, Jr. The contributors from the Congress symposium served as introductory speakers for each topic, followed by informal discussions and demonstrations. At the Workshop a Certificate of Commendation was awarded to John P. T. Boorman of Pirbright, England, in appreciation of his initiative and management of the "Ceratopogonid Information Exchange," which has circulated CIE Newsletters semi-annually since 1968.

Contributions to these proceedings are arranged numerically according to the major topics discussed. Reprints should be requested from W. W. Wirth.

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1. REMARKS OF THE CHAIRMAN OF THE SYMPOSIUM.

The Study of Ceratopogonidae—a history. M. Kremer. Institute of Parasitology, Faculty of Medicine, University of Strasbourg, Strasbourg, France.

A brief history of the study of Ceratopogonidae was presented. The development of this subject can be divided in 3 periods. Prior to the present century, the important works were those of Derham, Linnaeus, Latreille, and Meigen, followed by the studies of Zetterstedt, Staeger, and Winnertz. The 2nd period commenced with this century, and included the work of Malloch, Kieffer, and Goetghebuer. Some portraits of Kieffer were shown. The 3rd, modern era, is characterized by an abundance of both systematic and biological research. The author concluded with the hope that informative newsletters like the Ceratopogonid Information Exchange and the organization of meetings specifically on Ceratopogonidae will continue. He thanked all those contributing to the symposium and especially the organizer, Dr. W. W. Wirth.

2. SYSTEMATICS.

Systematic study of the *Leptoconops kerteszi* complex. J. Clastrier. National Museum of Natural History, Laboratory of Entomology, Paris France.

Originally described from Egypt by Kieffer in 1908, *Leptoconops (Holoconops) kerteszi* has since been reported from most of the northern hemisphere as well as from South Africa. The use of known and new morphological characters in both female and male sexes has shown that several distinct species were confused under this name. In the Mediterranean subregion 2 names have been restored (*L. laurae* (Weiss, 1912) and *L. peneti* (Langeron, 1913)), and 8 other species have been described as new. In North America, 11 species have been differentiated, as discussed more fully in the abstract written with W. W. Wirth.

A systematic study of the *Leptoconops kerteszi* complex in North America. J. Clastrier and W. W. Wirth. National Museum of Natural History, Laboratory of Entomology, Paris, France, and Systematic Entomology Laboratory, ARS, USDA, Washington, D. C., U.S.A.

Females of the *Leptoconops kerteszi* complex are frequently extremely annoying bloodsucking pests in coastal or desert regions, and the immature stages have been found usually in wet sandy soil in depressions near lakes, ponds, streams, springs, or coastal lagoons, usually in conditions of considerable salinity or alkalinity. Using characters that have proved useful in differentiating 3 species of this complex in southern Europe and 7 others in North Africa, a critical study was made of the species that had previously gone under the name of *L. kerteszi* in North America. It was concluded that the species *L. kerteszi* does not occur in North America, but 11 species were recognized, one of which, *L. americanus* Carter, had been described from Great Salt Lake, Utah, while 10 species were described as new. These species fall into 3 taxonomic groups with one or more common and widespread species in each group, along with several species of quite restricted distribution. The most useful characters for species recognition were found in the number of subapical black setae on the last segment of the female antenna, the number and position of the setae on the female clypeus, the number of stout ventral spines on the midportion of the mid basitarsus, the shape of the basal seta on the ventral side of the male 5th tarsomere, the distribution of the ventral setae and the shape of the tip of the male dististyle, and the presence of a dorsal process on the male 9th tergum.

***Leptoconops*—Leptoconopidae? J. A. Downes, Biosystematics Research Institute, Ottawa, Canada.**

Krivosheina (1962, 1969) has argued, chiefly on larval characters, that *Leptoconops* should be excluded from the Ceratopogonidae and given family rank. The larvae differ widely in many important respects (see also Clastrier, 1971; Laurence and Mathias, 1972), although the complex pharyngeal apparatus may be homologous.

The adult, therefore, should be reexamined. The thickened anterior vein is usually interpreted as representing the compact radial system of Ceratopogonidae s.s. Loss of the cross-vein and development of a remarkably strong 'intercalary vein' must, therefore, be posited. But perhaps the anterior vein represents less than the whole radial system, and the 'intercalary vein' is really R_{4+5}, or even R_5. The problem of the cross-vein would be modified, and a widely different picture of the wing would emerge. In the antenna, there is no indication of the differentiation of 3 (♂) or 5 (♀) apical segments seen in Ceratopogonidae s.s. The terminal denticle of the dististyle and the large flattened cerci (♀) are not found in Ceratopogonidae s.s., although both occur in Psychodidae. The rather similar mouthparts of *Leptoconops* and Ceratopogonidae s.s. are a plesiomorphic feature shared also with *Phlebotomus*, and do not tell against the differences noted. Krivosheina's conclusion is probably well founded.

The Nearctic species of *Parabezzia* Malloch. W. L. Grogan, Jr., and W. W. Wirth. Department of Entomology, University of Maryland, College Park, Maryland, and Systematic Entomology Laboratory, ARS, USDA, Washington, D. C., U.S.A.

The separation of the 14 Nearctic species of *Parabezzia* into 3 species groups was based on (1) basal costal swelling, (2) reduction of the costal fringe, and (3) normal costal fringe of the female wing. The evolution of the 3 species groups and the phylogenetic relationships with closely related genera were discussed.

Systematics of the *obsoletus* group of *Culicoides* (subgenus *Avaritia*) in the Palaearctic Region, with remarks on some types. **M. Kremer and C. Rebholtz (technical assistance—J. C. Delecolle). Institute of Parasitology, Faculty of Medicine, University of Strasbourg, Strasbourg, France.**

	Int. Oc. space (triangle formed by the 3 hairs)	Eyes	Sperma-thecae	Space between terminal sternite thickening	Palpus
scoticus	equilateral	bare	subequal	tight	clumpy
obsoletus	equilateral	bare	subequal	large	clumpy
montanus	equilateral	bare	subequal	tight	special form special setae on antenna
abchazicus	equilateral	bare	unequal	?	clumpy
dewulfi	isoceles, large	bare	unequal	tight	cylindrical
chiopterus	equilateral	hairy	unequal (±)	tight	cylindrical
obsoletiformis	equilateral	bare	?	?	cylindrical

The characters used for the *obsoletus* group and the key of the species were tabulated as below. The males were easily recognized by their hypopygium. The females were classified according to the following characters: eyes and interocular space, palpus, last sternite with chitinized thickening, and size of the spermathacae. A problem arose in the character of hairy eyes which were never described in *C. chiopterus*. Thus, we described *C. dobyi* (Callot and Kremer) in 1969 as possessing hairy eyes. But, later we found that the eyes of all specimens of *C. chiopterus* were more or less hairy, and *C. dobyi* was recognized as synonymous with *chiopterus*. The existing types of *C. obsoletus* and of *C. chiopterus* did not permit a clear resolution of this problem, because the two hypopygia were missing and the eyes were bare. Another difficulty was discovered in some specimens of *C. dewulfi*, which also had hairy eyes. The character "hairy eyes" could be a variable one and of limited use.

Wing pattern analysis in *Culicoides*—preliminary results. **R. P. Lane. Department of Entomology, British Museum (Natural History), London, England.**

As part of a study of the *Culicoides pulicaris* group, wing patterns were investigated using 2 different strategies. A representative sample of 15 operational taxonomic units (OTU's) was used. The 1st, a purely descriptive approach, involved superimposition of a grid onto a wing and subsequent scanning for pigmentation. This produced a series of matrices which constituted numerical images of the specimens. Significant among the theoretical objections to this method was that the components of the matrices describing the wing pattern, derived purely by operational homology, were not true 'biological' characters in the general sense but pseudocharacters. This would inevitably have resulted in a restrictive phenetic classification. The 2nd approach relied on defining wing pattern elements and determining their homologies by use of a sequentially arranged morphological series. A system was developed by which the variation of homologous pattern elements could be described. The 2 approaches were also evaluated by examining their influence on the relationships of the 15 OTU's using principal components analysis. By this means the classification based on the scanning data was more diffuse, being composed of fewer groups of OTU's than that derived from the data of the 2nd approach.

Distribution and differentiation in the subgenus *Monoculicoides*. J. A. Downes. Biosystematics Research Institute, Ottawa, Canada.

Monoculicoides is a small and specialized subgenus of *Culicoides* characteristic of the Holarctic region. Two groups, based on the Old World species *stigma* Meigen and *nubeculosus* Meigen are easily recognized, and Gluchova (1971) distinguishes further a *nubeculosus* group s.s. and a *riethi/puncticolis* group.

In North America *Monoculicoides* is western only, except for the transcontinental *variipennis* complex. *C. variipennis* is probably an outlying member of the *riethi* group. Widespread in western Canada there is also *C. gigas* Root and Hoffman, hard to separate from Old World *riethi*. The *stigma*-group has undescribed species in Washington and Alberta, and another Alberta species may belong to the *nubeculosus* group. Thus *Monoculicoides* has made several independent crossings of Beringia.

The basal species are bloodsucking, and often associated with large mamals, but reduced forms have arisen independently in each group. *Culicoides rithi* in Britain and *C. gigas* in Canada are autogenous in the first cycle but remain able to bite. The Alberta species near *stigma* has weakened mandibular teeth (autogenous and nonbiting). The Alberta species supposedly allied to *nubeculosus* has a strongly reduced proboscis and other peculiarities.

Evolution of feeding habits in Ceratopogonidae. J. A. Downes. Biosystematics Research Institute, Ottawa, Canada.

The feeding habits of the females (blood-meal or analogue) conform well with the probable phylogeny (see diagram).

Food sources of Forcipomyiinae are very varied; possibly this is plesiotypic for Ceratopogonidae. Sources include blood of large insects; blood of amphibia, birds and mammals; juices of dead insects; pollen-sap. The females hover around the host, attracted and controlled by substances given off, as in typical biting flies, but the host substances must often be unusually specific (species of *Chrysopa*, Odonata, pollen of *Nuphar*, etc.). *Dasyhelea* has lost both protein-meal and functional mandibles; the habits of its stem-group are unknown.

Culicoidini, the more plesiomorphic sister group of other Ceratopogoninae, retain the typical hunting process and feed on vertebrate, blood (reptiles, birds, mammals). The more evolved Ceratopogoninae (stem A) have a radically new food source and method of hunting, evidently very successful. They feed on small insects, Chironomidae, small Ephemeroptera, etc., usually males, captured in flight by the raptorial legs from the mating swarm. The female responds first to the swarm-

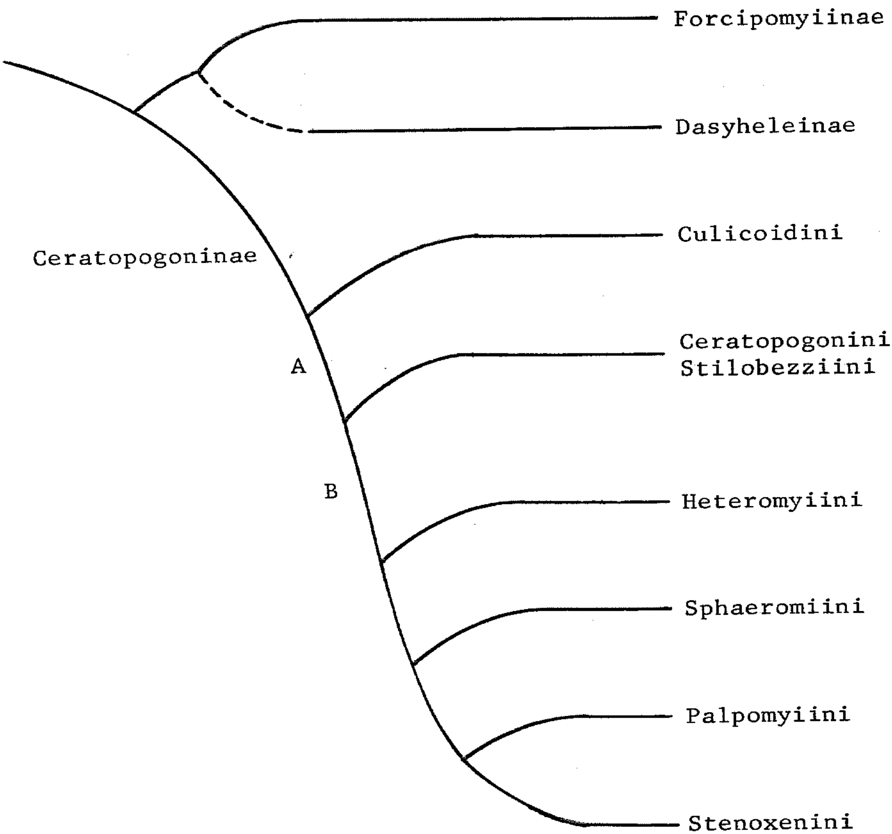

Fig. 1. Downes' diagram.

marker, hovers until able to strike, and sucks prey dry. In stem B, the female remains in hunting phase on entering her own (conspecific) mating swarm, and eats the male during mating. The behaviour of Stenoxenini is unknown, and perhaps abberrant.

3, LARVAL BIOLOGY AND SYSTEMATICS.

Biology and immature stages of the *Culicoides* of Laos. F. G. Howarth. Bernice P. Bishop Museum, Honolulu, Hawaii, U.S.A.

During 1967–1968, 25 species of *Culicoides* were reared, 18 for the first time, from 55 breeding sites in Laos. The pupa and adult of 17 of these species were associated, 14 for the first time. The 55 sites were grouped into 11 habitats and included a variety of water margins, rotting plant material, and animal manure. For breeding sites at water margins the following factors were important in delimiting species of *Culicoides*: sunny vs shaded conditions; content of moisture, organic detritus and sand; and substrate texture. Although often obscured, the recent past history of perturbations at a site, such as wallowing by animals, the addition of organic matter, and flooding or drying, had a profound effect on breeding.

Taxonomically, the pupae fitted reasonably well into the same species groups as the adults. Characters useful for identification included morphology of the respiratory trumpet, chaetotaxy, and integumental ornamentation. Three laboratory demonstrations were also presented at the meeting: (1) dark field phase microscopy for accurate interpretation of wing patterns in *Culicoides*; (2) a dish with silicone sealant barriers forming troughs as wide as a chosen optical field, to facilitate sorting of fluid collections; (3) a bent, flattened wire microspatula mounted on a hypodermic needle and syringe for transferring individual living immatures safely and rapidly.

Distribution of *Culicoides* within salt marsh habitats in North Carolina. D. L. Kline. Department of Biology, The Citadel, Charleston, South Carolina, U.S.A.

The relative distribution of several *Culicoides* species was studied within several types of North Carolina salt marsh habitats. Irregularly flooded marshes, inundated only by very high tides, contained 2 major plant communities—*Distichlis spicata* (L.) Greene, and *Juncus roemerianus* Scheele. Regularly flooded marsh areas with silt substrate, inundated twice daily, supported cord grass, *Spartina alterniflora* Loiseleur, which occurred in 3 forms (tall, medium, short) presumably due to duration of inundation. A regularly flooded sandy tidal creek beach with vegetated (*S. alterniflora*) and non-vegetated areas was also studied. The distribution of the *Culicoides* spp. was determined by sticky cylinder traps, emergence traps, and substrate sampling.

Essentially the same distributional pattern was shown by all 3 methods. *C. bermudensis* Williams was found in irregularly flooded areas only. *C. hollensis* (Melander and Brues) was found in all areas of the silt substrate marsh, but had a definite preference for tall *S. alterniflora* (> 1.2 m in height). *C. furens* (Poey) was found in all habitats studied, but was most abundant in short form *S. alterniflora* (< 0.3m). *C. melleus* (Coquillett) was recovered only from the sandy habitats and mostly from non-vegetated areas.

Approaches to collecting and extracting immature Ceratopogonidae (Diptera). W. L. Knausenberger and E. C. Turner, Jr. Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, U.S.A.

In a biosystematic study on immature Ceratopogonidae, an intensive sampling program has been developed in and around Virginia to obtain living immatures for individual rearing.

An array of tools is used, including a variety of hand shovels, scoops, corers, limnological grabs, and drift nets. Artificial substrates were developed. Two identical subsamples from each site are brought to the laboratory. One of these is held at least 90 days for adult emergence in specially-designed rearing cartons. The other subsample is processed according to a flow chart of sequential procedures, adjusted according to the nature of the substrate. The basic techniques employed, in order of frequency, are: (1) sieve-washing (0.175 mm mesh size) and sucrose flotation, (2) heat extraction (by Berlese-type funnels), (3) live organisms sorting themselves (through screening or sand), (4) elutriation, (5) hand-picking unwashed samples, and (6) centrifugation-flotation.

A standardized *ad hoc* collecting data form provided entries for (1) pertinent weather data, (2) detailed locality information (including geographic coordinates and elevation), (3) habitat descriptions, (4) vegetative characteristics, such as biotic province, dominant species, and shading, and (5) detailed chemical/physical characteristics.

Success in obtaining viable larvae and pupae increased from ca. 60% to 80% of the sites as the procedures were developed. These procedures have proven to be least effective for the semi-terrestrial representatives of the Forcipomyiinae and Dasyheleinae.

***Culicoides* breeding sites in Panama. Gary Vitale. Yale University School of Medicine, New Haven, Connecticut.**

The purpose of this project was to search for species of *Culicoides* breeding in bromeliads at the Bayano River encampment of the Gorgas Memorial Laboratory in Panama. Other likely breeding sites were also examined. The duration of the field study was from May to August of 1976.

In total, 202 collections were made and processed. Of these, 82 were from bromeliads, 53 from tree holes, 21 from water-containing bamboo internodes, 19 from moist soil and leaf debris, 11 from cut palm trunks, 7 from streams, 3 from *Heliconia* (Musaceae) inflorescences, 3 from palm crowns, 1 from the inflorescence of *Costus* (Costaceae), 1 from *Xanthosoma* (Araceae) and 1 from a water-holding palm frond. The bromeliads and tree holes were distributed from ground to canopy level.

In all, 363 larvae of *Culicoides* were isolated from the collections, 41 live pupae and 256 adults (reared) while 44 larval skins and 68 pupal skins were preserved. The larvae were found in tree holes, bromeliads, streams, leaf humus, cut palm trunks, bamboo internodes and *Heliconia* inflorescences. *Culicoides* adults were successfully reared from tree holes (206), bromeliads (15), cut palm trunks (22) and trapped leaf humus (13). A total of 43 *Culicoides* species was identified (Dr. Willis W. Wirth, personal communication). Those from tree holes included *Culicoides debilipalpis* Lutz, *C. paraensis* (Goeldi), *C. quasiparaensis* Clastrier, and 1 possibly new species. The bromeliads *Viriesia heliconioides* (HBK) and *Aechmea pubescens* Baker each yielded adults of *C. debilipalpis*.

Besides the *Culicoides*, other insects were collected as follows: 359 adults (reared), 29 larvae, 16 pupae, 27 larval skins and 135 pupal cases. All collections were associated with complete data on habitat. Of special interest were 45 adult crane flies (Tipulidae) with 44 pupal cases, which were reared mainly from bromeliads. Preliminary identifications disclosed several genera of mosquitoes as well as predaceous ceratopogonid genera such as *Bezzia* and *Palpomyia*.

Within the study period, rainfall during June and July was below normal. For this reason, the breeding sites of *Culicoides* were restricted and the numbers of *Culicoides* were reduced. It is possible that species found breeding in tree holes make use of new breeding sites which become available as rainfall increases. One potentially excellent breeding site is the palm crown. In the Bayano region species in several genera of palms are among the most abundant understory trees and form a significant component of the canopy. I consistently was able to find moist dirt in the leaf bases of palms and it is likely that with the daily rains of the wet season these would be converted into good breeding sites for *Culicoides* and other insects.

4. ADULT BEHAVIOR.

A latex agglutination test for the identification of blood meals of *Culicoides*. J. P.

T. Boorman. Animal Virus Research Institute, Pirbright, Surrey, England.

A latex agglutination test has been developed which is suitable for identifying the origin of blood meals of *Culicoides* midges. Due to the small amount of blood ingested, the precipitin test is sometimes difficult to apply; micro-haemagglutination inhibition or micro-gel diffusion tests are also used for midge blood meals. The latex agglutination test has several advantages—it requires the minimum of reagents, is very rapid and can be performed in the field.

Anti-species antisera of high titre and specificity are prepared in rabbits and treated with caprylic acid to remove most of the serum proteins except 1 gG; this is then conjugated with polystyrene latex. A platinum loopful of conjugate is mixed with a blood meal extract. In a positive test agglutination occurs within 30 seconds. The sensitivity and specificity of the test depend upon the quality of the antisera used, but the method can be as specific as a standard precipitin test. The technique will be described in full in the *Bulletin of Entomological Research*.

The role of the female in the sexual behavior of *Culicoides melleus*. J. R. Linley. Florida Medical Entomology Laboratory, Vero Beach, Florida, U.S.A.

In a series of detailed studies of sexual behavior in *Culicoides melleus*, remarkably complex behavioral and physiological interactions have been found to take place between male and female. Many of these interactions depend upon the behavior of the female and particularly upon her defensive kicking involving the hind legs—movements which are very similar to those executed in rear abdominal grooming. The legs are alternately moved closely along the sides of the abdomen and beyond the posterior abdominal tip. If the legs engage with some obstacle, for example a sexually united male, strenuous kicking and pushing against the male terminalia is seen.

The functions and effects found to be associated with this behavior are—(i) a stimulus to the male to release the female at the end of copulation and the main force effecting subsequent separation, (ii) the means whereby the spermatophore is removed from the female genitalia, (iii) the female contribution to a mechanism which prevents copulation between recently mated insects, (iv) the behavioral basis for female selection of a potent mate, (v) an effect in prolonging copulation if the female has mated previously and, associated with this, (vi) an effect that causes reduction of the quantity of male ejaculate in such prolonged copulations.

Ejaculation and spermatophore formation in *Culicoides melleus*. J. R. Linley and M. J. Hinds. Florida Medical Entomology Laboratory, Vero Beach, Florida, U.S.A.

A series of borax-carmine stained whole mounts, prepared by cold-fixation at progressive times early in copulation at 25–27°C, was shown to illustrate the stages of ejaculation and spermatophore formation. Ejaculation starts almost immediately after genital union is established and is complete to the stage of the completely formed and filled spermatophore within 2 minutes. Within 30 seconds of sexual union, sperm are moved into the paired seminal vesicles, whence they quickly pass as two broadly ovoid bundles into the anterior portions of the glutinous glands. Even before copulation is initiated, these glands contain the clear viscous mucoprotein that will form the spermatophore. Contraction of circular muscles investing the entire accessory gland forces the sperm bundles, preceded by the mucoprotein, towards the ejaculatory duct. Within 1 minute of genital union, the mucoprotein emerges from the duct as a pear-shaped mass, while the closely following sperm bundles become elongated and are extruded, without becoming fused, through the dilated ejaculatory duct and centrally into the mass of mucoprotein. Complete investiture of the 2 still distinct sperm bundles takes place posteriorly as the spermatophore assumes its final shape, confined predominantly by the male genitalia.

Concerning some parasites found in *Culicoides*. C. Rebholtz, F. Zenner, and M. Kremer (technical assistance—J. C. Delecolle). Institute of Parasitology, Faculty of Medicine, University of Strasbourg, Strasbourg, France.

Several parasites were found in different species of *Culicoides* captured in light traps: mermithid worms, flagellates, ciliates and gregarines. 1) Adult male and female mermithid worms (Tetradonematidae) were found in *C. obsoletus* from 2 parts of France (Strasbourg and Biarritz), but also in other species from other localities. Passages of these parasites in our colonies of *C. nubeculosus* and *C. riethi* have never been successful. 2) Promastigote forms of *Herpetomonas* were observed in the gut and Malpighian tubules of laboratory-reared *C. nubeculosus*. Other flagellate parasites were found in *C. punctatus, C. pulicaris,* and *C. salinarius*. 3) Ciliates were rarely found in *Culicoides*. However, many (perhaps genus *Tetrahymena*) were seen in the ovaries of 1 female *C. punctatus*. Cysts (?) were also discovered in the gut of wild-caught *C. punctatus* and *C. obsoletus*. 4) Finally, the mid-guts of wild-caught *C. obsoletus* were observed to contain from 1 to 20 or more cysts of gregarines (of more than 70 micrometers diameter). After dissection in physiological saline, secondary cysts were seen in the primary cysts. After crushing, the secondary cysts showed a maximum of 8 banana-shaped corpuscles which were liberated after a short time.

In the tissues of *C. nubeculosus* larvae and *C. obsoletus* adults, sporozoite-like cells were seen, but it is unknown whether a correlation exists between these 2 series of parasites, and if the latter are indeed parasites at all.

Acknowledgment: The authors are indebted to Professor Chaubaud, Madame Landau, and Dr. Hommel for their help in this preliminary report.

5. COLONIZATION.

Colonization of biting midges: progress and prospects. J. P. T. Boorman. Animal Virus Research Institute, Pirbright, Surrey, England.

The Ceratopogonidae have always been regarded as a "difficult" group to breed; but over the last few years a number of laboratory colonies of *Culicoides* and *Forcipomyia* have been successfully set up. The diverse methods used reflect the ecological diversity of the species; simulation of the natural habitat in the laboratory is much easier to achieve in some groups than in others. Many problems remain: some important species are reluctant to feed and mate under laboratory conditions and the means must be sought to overcome these problems. Methods are now available for rearing smaller numbers of midges and this opens whole new fields—for example, genetic studies of midges. Above all, we need to know more about the biology of these insects, both in the wild and in those species already successfully adapted to colony conditions.

***Culicoides variipennis*, a model for vector competence studies. R. H. Jones. Arthropod-borne Animal Disease Research Laboratory USDA, Denver Federal Center, Denver, Colorado, U.S.A.**

The biting midge *Culicoides variipennis* (Coquillett) is the most important biting fly attacking livestock in the United States when its role as a pest and disease vector is defined by the large number of flies attacking throughout its wide range in North America. It is the vector of several arboviruses (Orbiviruses, Bunyaviruses) and probably of *Onchocerca* filarial worms.

Oral susceptibility tests with bluetongue virus (BTV) showed that different field populations were differently susceptible to the same BTV strain (0–69%), and that each population was differently susceptible to different BTV serotypes. A field population collected at an epizootic was most susceptible to the BTV serotype from the epizootic. Colonies based on small numbers of field females were non-representative: a field population and the colony derived from it were differently susceptible to BTV, and each colony appeared homogeneous to infection with different serotypes. Different colonies were differently susceptible to BTV, and, although homogeneous to infection with different BTV serotypes, a colony was still highly polymorphic for susceptibility characteristics in genetic selection studies.

A homogeneous colony was an effective control in vector competence studies with field populations. Both colony and field flies are readily used with laboratory procedures for vector competence studies.

Agar rearing of *Culicoides*. H. G. Koch and R. C. Axtell. Department of Entomology, North Carolina State University, Raleigh, North Carolina, U.S.A.

Culicoides furens (Poey), *C. hollensis* (Melander and Brues), and *C. melleus* (Coquillett) were reared from eggs to adults in 1% laboratory grade agar. The agar medium was supplied with nematodes, *Panagrellus redivivus* (L.), every 3–4 days from a culture grown on oatmeal, yeast, and water. Previous work in 1974 and 1975 had shown that field-collected 3rd and 4th instar larvae would continue development, pupate, and emerge from the agar medium.

A variety of containers of different dimensions were used to rear the insects. Individual *Culicoides* were successfully reared in petri dishes (35 x 10 mm) about three-fourths filled with agar. Individual progeny groups (40–100) were reared in 40 dram vials (55 x 90 mm) about one-half filled with agar. The agar was poured into the containers placed on a slight slant to prevent excess water from overlaying the medium surface. *C. furens* adults were obtained in greatest numbers by this technique. It was obvious from these studies that the larval diets and/or substrate requirements of the species differ considerably. Work is continuing in an attempt to obtain further laboratory-reared generations.

6. ARBOVIRUS TRANSMISSION.

Recent advances in arbovirus studies involving *Culicoides*. M. D. Murray. McMaster Animal Health Laboratory, CSIRO, Glebe, N.S.W., Australia.

The two principal areas of endeavor have been (1) colonization of *Culicoides*, which has led to basic investigations on the transmission of Bluetongue and Button Willow viruses by *C. variipennis*, and (2) field studies which have been developed extensively in Australia, Kenya, Nigeria, and California, U.S.A.

The 20 recognized arboviruses which have been isolated from *Culicoides* spp. are, in the main, Bunyaviruses, particularly in the Simbu group, or Orbiviruses. Many are apparently closely associated with ungulates, particularly cattle, goats, and sheep, and in Africa and Australia the *Culicoides* spp. from which they have been isolated are mostly members of the subgenus *Avaritia*, species of which attack livestock in great numbers. It is therefore somewhat urgent that the taxonomic problems of this important vector group of Africa, Asia and Australia be resolved.

Isolation of viruses has made possible serological surveys to determine correlations between presence of antibodies and clinical disease, as in the recent studies of Akabane disease in Japan and Australia. This virus infects the foetus in cattle, goats and sheep, causing abnormalities such as hydranencephalus, micranencephalus and arthrogryposis. Passage of virus across the placenta occurs in early pregnancy and no clincial disease is produced in the dam.

There is increasing evidence that *Culicoides* spp. are important vectors of arboviruses of livestock, and transmit a greater variety than realized. It is likely that several of these viruses will be shown to cause disease.

***Culicoides* as potential vectors of viruses to livestock in Kenya. A. R. Walker. Veterinary Research Laboratory, Kabete, Kenya, Africa.**

The ecology of *Culicoides* in relation to the epidemiology of bluetongue and ephemeral fever was studied over 7 years by monitoring seasonal population fluctuations, blood feeding, longevity, virus infection, and by distribution surveys.

The most important species were considered to be *C. pallidipennis* C. I. & M. (= *C. imicola* Kieffer) and *C. schultzei* (End.); both were very widespread and occasionally occurred in large numbers in hot dry areas, although their larval habitats seemed to be mainly swamps and watercourses. Seasonal population fluctuations were variable and did not relate closely to rainfall.

Both species fed mainly on bovids. *C. schultzei* had a strong preference for cattle and *C. pallidipennis* a fairly strong preference for cattle, but both fed on sheep and goats even when cattle were immediately available.

Natural survival rates of both species were about 0.80, at which both could take up to 4 blood meals within 17 days efore the percentage of survivors dropped below1%. This longevity was sufficient to permit incubation of bluetongue virus, which was isolated from *C. pallidipennis* on 3 occasions but not from *C. schultzei*. Ephemeral fever virus was isolated from a mixed pool of *Culicoides* in which *C. schultzei* was predominant and Palyam group viruses were isolated from pools in which *C. pallidipennis and C. schultzei* were predominant.

7. NONVIRAL DISEASE TRANSMISSION.

***Culicoides* as vectors of Haemosporida. E. C. Greiner and G. F. Bennett. International Reference Centre for Avian Haematozoa and Department of Biology, Memorial University, St. John's, Newfoundland, Canada.**

The status of *Culicoides* in relation to haemosporidan transmission was reviewed. *Culicoides* spp. have been incriminated as vectors of species of *Haemoproteus* (= *Parahaemoproteus*), *Hepatocystis*, and *Akiba* are suspected of being vectors of *Simondia* and *Saurocytozoon*. There is evidence that each haemosporidan species may be transmitted by more than one species of *Culicoides*, and that one species of *Culicoides* may transmit more than one species of haemosporidan. Incriminated vectors include: *C. adersi* and *C. fulvithorax* (development to oocysts)—*Hepatocystis kochi*; *C. downesi*—*Haemoproteus nettionis; C. sphagnumensis*—*Haemoproteus canachites, Haemoproteus velans*, and *Haemoproteus danilewskii*; *C. crepuscularis*—*Haemoproteus danilewskii* and *Haemoproteus fringillae* (development to oocysts); *C. stilobezzioides*—*Haemoproteus velans* and *Haemoproteus fringillae* (development to oocysts); *C. arakawae, C. circumscriptus, C. odibilis*, and *C. schultzei*—*Akiba caulleryi*. Although the sequence of the cyclopropagative development was the same, differences in the rate and site of development occurred among the parasite genera. The difficulty of working with *Culicoides* and the scarcity of colonized ornithophilic species of *Culicoides* have inhibited the progress of knowledge relating to *Culicoides*—haemosporidan interactions. It is hoped that the co-ordinated efforts of entomologists and parasitologists will continue to expand our minimal understanding of these relationships.

Neotropical filariasis: vector studies involving *Culicoides*. R. C. Lowrie, Jr. and T. C. Orihel, Delta Regional Primate Research Center, Covington, Louisiana and J. R. Linley, Florida Medical Entomology Laboratory, Vero Beach, Florida, U.S.A.

Each year the Delta Regional Primate Research Center receives monkeys harboring naturally-acquired filarial infections. Most belong to the *Dipetalonema* group. Presently, there is very little information regarding the arthropod species that serve as natural vectors of these parasites. To maintain these filariae in the laboratory, local bloodsucking arthropod species must be found that can serve as intermediate hosts. A filariid of special interest in our laboratory is *Dipetalonema marmosetae*, a common parasite of squirrel monkeys (*Saimiri sciureus*). *Culicoides furens* gnats were collected in Florida and blood-fed on a squirrel monkey infected with this parasite. A sample of gnats was dissected daily, and the larvae examined microscopically. The morphological changes seen in these larvae correlate well with the developmental pattern observed with other filarial species. Infective larvae were harvested on day 10 and inoculated subcutaneously into an uninfected laboratory-born squirrel monkey. Unfortunately, the animal died from unrelated causes before the life cycle could be completed. We hope that these limited observations will stimulate more interest in the dipetalonematid filariae and their vector requirements. Possibly such studies will aid us further in resolving the taxonomic problems of the *Dipetalonema* group.

8. ADULT POPULATION STUDIES.

Geographical distribution and ecology of *Culicoides* in Haiti—preliminary results. C. Raccurt, French Institute of Haiti, Port-au-Prince, Haiti, West Indies and C. Rebholtz and M. Kremer, Institute of Parasitology, Faculty of Medicine, University of Strasbourg, Strasbourg, France.

Ten species of *Culicoides* are presently known in Haiti. *C. furens, C. barbosai* and *C. trinidadensis* have been collected while biting man and *C. furens* and 7 other species have been reared to the adult stage from immatures collected in the field.

C. furens is entirely a coastal species. *C. insignis, C. pusillus* and *C. borinqueni* are ubiquitous. The larvae of *C. furens* breed in coastal sand and in inland brackish water. Sometimes they are found in fresh-water mud, and once they were collected from a tree-hole. Unlike *C. furens, C. insignis* is found predominantly in fresh water and becomes less abundant as the water becomes more saline.

The following species from Haiti are reported here for the first time with notes on their habitats: *C. pusillus* most commonly breeds in animal excreta, but is also found in mud containing either fresh or brackish water. *C. foxi* is a fresh-water species and *C. phlebotomus* is a marine species. *C. borinqueni* and *C. eadsi* breed in tree holes. We found *C. jamaicensis* breeding in a habitat not reported before, namely, a rotting cactus plant (*Opuntia sp.*).

The most important pest species in Haiti is *C. furens* for 2 reasons: firstly, it attacks man readily and it is found along the entire coastline where it adversely affects tourism and economic development; secondly, it is important because it is found in areas where *Mansonella ozzardi* is present.

***Culicoides* of Northern Thailand. N. C. Ratanaworabhan. Applied Scientific Research Corporaton of Thailand, Bangkok, Thailand.**

A total of 35 species of bloodsucking midges was collected from 4 provinces of Northern Thailand: Chiang Mai, Chiang Rai, Phrae and Lampang. The collection included 33 species of *Culicoides*, a species of daytime-biting midge, *Forcipomyia anabaenae*, and *Leptoconops xuthosceles*. Species of *Culicoides* were: *arakawai, circumscriptus, guttifer, hegneri, homotomus, actoni, amamiensis, boophagus, brevipalpis, brevitarsis, gemellus, geminus, huffi, insignipennis, jacobsoni, malayae, mcdowelli, orientalis, peregrinus, recurvus, schultzei, shortti, sigaensis, similis, anophelis, barnetti, flaviscutatus, humeralis, macfiei, palpifer* and *paraflavescens*. Among these, *actoni, amamiensis, peregrinus, shortti, anophelis, circumscriptus, schultzei, flaviscutatus* and *humeralis* are man-biting species. *C. arakawai, actoni, schultzei, brevitarsis, peregrinus, shortti* and *similis* feed on cattle. *C. homotomus* feeds on horse and *C. arakawai* feeds on fowl.

The highest population density of bloodsucking midges occurred during the rainy season, June through October. The average catch from each light trap was 11, 169 midges per day. During the cold season, the population density was much lower with an average catch of only 6 per day.

The dominant species of *Culicoides* in the northern region was *C. peregrinus*. Its population comprised about 40% of the total catch of *Culicoides* species. *C. barnetti* is a new record for Thailand.

The species of *Culicoides* of Northern habitat which have been reported as vectors or suspected vectors of diseases of man and animals are shown below with the associated pathogens.

arakawai–Leucocytozoon caulleryi; homotomus–Onchocera cervicalis; amamiensis–Maccacanema formosana; brevitarsis–potential vector of blue-tongue disease; *peregrinus–microfilaria; schultzei*–African horse-sickness virus and *Onchocera gibsoni; anophelis*–possibility of transmitting malaria parasite; *Forcipomyia (Dacno.)—anabaenae* related to F. (L.) *taiwana*, which has been implicated in the transmission of Japanese B. encephalitis virus to man in China.

Preliminary note on the *Culicoides* of the Ried of Alsace, with special reference to halophilic species. J. P. Rieb and M. Kremer. Institute of Parasitology, Faculty of Medicine, University of Strasbourg, Strasbourg, France.

The Ried is a humid landscape in the plain of Alsace. It is characterized by swampy depressions and meadows alternating with thickets and forests. In the south of the plain are potash mines, from which salt (NaCl) disperses into the River Ill. Several species of *Culicoides* were found in 130 samples of mud from this region. Fresh-water biotopes yielded *C. odibilis, C. clastrieri, C. musilator, C. cubitalis, C. pictipennis, C. punctatus, C. pumilus,* and *C. poperinghensis*. The first four species were very common, and occurred in very pure as well as in polluted water. *C. clastrieri* and *C. odibilis* were often found together in great numbers.

Brackish water species, which were rare and associated together, included *C. riethi, C. circumscriptus,* and *C. salinarius*. The habitats of these species were along the margins of the Ill. Here saline conditions have not long existed, but in the neighboring region of Lorraine, salt has always been present and so were all Euopean species of salt marsh *Culicoides*. It is suspected that the colonization of the Rhine Plain perhaps originated from Lorraine.

The *circumscriptus* group (subgenus *Beltranmyia*) was problematical, for transitional forms were found between *C. circumscriptus* and *C. salinarius*. Further, the status of *C. kirovabadicus* as a valid species appeared to be in some doubt. Two apparent specimens were found in Lorraine. It is possible that *kirovabadicus* represents a variation in the markings of *C. circumscriptus*.

9. CONTROL.

Concepts and problems in biting midge control. R. C. Axtell, Department of Entomology, North Carolina State University, Raleigh, North Carolina, U.S.A.

The economic importance of Ceratopogonidae was discussed and the difficulty in achieving control was analyzed with emphasis on the problem of coastal *Culicoides*. The various methods which have been proposed for the control of Ceratopogonidae were reviewed and evaluated in terms of efficacy and the effects on non-target organisms and on the environment. Methods included were chemical (repellents, larviciding, and adulticiding by fogs, ULV aerosols, and residual deposits), cultural (impoundment, dredge and fill), and biological (pathogens, parasites, predators, and behavioral modification). Problems of sampling and population monitoring in relation to control were discussed. Integration of monitoring and control alternatives into an area-wide biting fly and mosquito pest management program was postulated.

10. POLLINATION.

Life cycles of cacao pollinating midges (*Forcipomyia* spp.) and some notes on the larval behavior in the laboratory. S. de J. Soria, Centro de Pesquisas do Cacau,

Itabuna, Bahia, Brazil, and W. W. Wirth, Systematic Entomology Laboratory, USDA, Washington, D. C., U.S.A.

Midges of the genus *Forcipomyia,* subgenus *Euprojoannisia,* were recently determined as the most important pollinators of cacao or cocoa (*Theobroma cacao* L.). In Brazil the embryonic and postembryonic development of the pollinating midges *F. (E.) spatulifera* Saunders and *F. (E.) blantoni* Soria and Bystrak, and the non-pollinating midges *F. (F.) genualis* (Loew), *F. (F.) poulaineae* Ingram and Macfie, and *F.* sp. 1, subgenus near *Lepidohelea,* have recently been studied in detail. The periods for development of eggs, larvae, pupae, and the life span of adults were recorded. Knowing the complete life cycle of each species, the potential number of generations per year was estimated. The midge *F.* (*Microhelea*) *fuliginosa* (Meigen) could not be successfully cultured in the laboratory.

The life-table technique was used for evaluating survival of *Forcipomyia* in the laboratory. Mortalities above 95% were observed in the adult stage. Eggs with the exception of *F.* (*M.*) *fuliginosa* suffered mortalities which varied between 50–86%. The egg mortality for *fuliginosa* was 100%. It was concluded that the rearing method must be improved for egg and adult stages, to achieve efficient mass-rearing in the laboratory.

Preliminary results describing the activity and behavior of the larvae were briefly discussed in the text of the paper.

Biology, activity, and behavior of cocoa pollinators. **J. A. Winder. Biological Control Station CSIRO, Curitiba, Parana, Brazil.**

Pollination of the cocoa or cacao tree (*Theobroma cacao* L.) depends on the activities of female ceratopogonid flies of the genus *Forcipomyia* s. lat. Since 1941, when the first ceratopogonid pollinator was observed in Trinidad, more than 75 species of Ceratopogonidae in 10 genera have been found frequenting cocoa flowers throughout the world, and about 50% of these have actually been observed pollinating. The most important pollinators belong in the subgenera *Euprojoannisia, Thyridomyia* and *Forcipomyia,* and their breeding sites include cocoa leaf litter, rotting cocoa pod husks, epiphytic bromeliads, and rotting banana trunks. The life-cycle of *Forcipomyia* from egg to adult lasts approximately 23 days (egg 3–4 days; larva 12–15 days; pupa 3–4 days) and the immature stages are important in taxonomy. Pollinators are most active during the early morning under sunny conditions and show distinct preferences for certain cocoa trees over others. High ceratopogonid populations in cocoa plantations are positively correlated with increased rainfall and excess soil water. Although insecticides seem to have no effect on pollination rates in cocoa, most experiments have suffered from inadequate experimental design and further research is needed. Natural pollination limits cocoa yield in some areas but increasing pollinator populations may not greatly increase yields since available evidence suggests that visiting cocoa flowers is only a small part of a pollinator's activities.