The Earliest Fossil Mosquito (Diptera: Culicidae), in Mid-Cretaceous Burmese Amber

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ABSTRACT *Burmaculex antiquus* new genus, new species, is described from a single partially preserved adult female in Burmese amber. The fossil has several plesiomorphic features, indicating that it is the sister group of all other fossil and extant Culicidae: a relatively short proboscis, the palpi extending beyond the apex of the proboscis, a clypeus with several setae, and the palpus without scales. Antennal and mouthpart structure suggest the female of this fossil species was a vertebrate blood feeder. The age of Burmese amber has been estimated as between Upper Albian to Turonian, 100–90 million years ago but the origins of the Culicidae are likely significantly older. The sister group of the Culicidae are the Chaoboridae, known as Jurassic fossils, and the Culicidae therefore must be equally as old. Although fossil adults of the two families may not be distinct at this early stage of evolution, the immatures would likely provide distinguishing features.

KEY WORDS amber, mosquito, Culicidae, Burmese, Cretaceous

MOSQUITOES ARE ONE OF the most familiar of all Diptera, largely because of their great impact on the health and comfort of humans and domestic animals. As vectors of malaria, yellow fever, and various encephalitides alone (not to mention other diseases), mosquitoes have affected more humans than any other insects. Indeed, mosquitoes are one of very few eukaryotes known to have affected human evolution, in the form of sickle cell anemia and the resistance it confers to malaria.

Primarily through the concerted efforts of medical entomologists, most extant species have been described, with ≈3400 validly named, and the majority of these are known in each life stage. Only very recently, however, has major progress been made in our understanding of the phylogenetic relationships among mosquitoes, providing a superior framework for interpreting the bionomic features of both extant and fossil taxa (Harbach and Kitching 1998; Judd 1996; Sallum et al. 2000, 2002). Cladistic studies based on morphology and molecular sequences have uncovered previously unsuspected relationships and major changes in the classification of the Culicidae are likely to take place. Despite the attention paid to mosquitoes, the fossil record of the Culicidae is very sparse, with only 12 substantiated species known from the Tertiary and one confirmed species from Upper Cretaceous Canadian amber, dated at 78 mya (Evenhuis

1994; Poinar et al. 2000; Szadziewski 1998). In this paper we describe the oldest known fossil species of Culicidae and interpret its phylogenetic position within the family.

Materials and Methods

The specimen was unique among nearly 3,500 inclusions of arthropods recently assembled in a large collection of Burmese amber at the American Museum of Natural History (AMNH), New York (Grimaldi et al. 2002). No mosquito is known in the collection of Burmese amber fossils at the Natural History Museum, London, which contains ≈1,200 arthropods (Rasnitsyn and Ross 2000). The AMNH Burmese amber was collected by Douglas Cruikshank of Leeward Capital Corp. in Kachin Province in northern Myanmar, near the village of Tanai, ≈105 km northwest of Myitkyina. Historically, the ages of strata from this area have been very confused, but the stratigraphic ranges of certain insect taxa in the AMNH collection indicate that this material is Cenomanian to Turonian, 95–90 mya (Grimaldi et al. 2002). Recently, limited evidence from fossil spores and an ammonite indicates an even slightly older age of upper Albian, ≈100 mya. The specimen was prepared according to the protocol described in Grimaldi et al. (2002). The fossil was examined, measured, and drawn using a Wild M3 dissecting microscope and a Jenaval compound microscope (Carl Zeiss, Thornwood, NY). Terms for structures follow those in the Manual of Nearctic Diptera (McAlpine 1981) and specialized terms for Culicidae are those in Harbach and Knight (1980).

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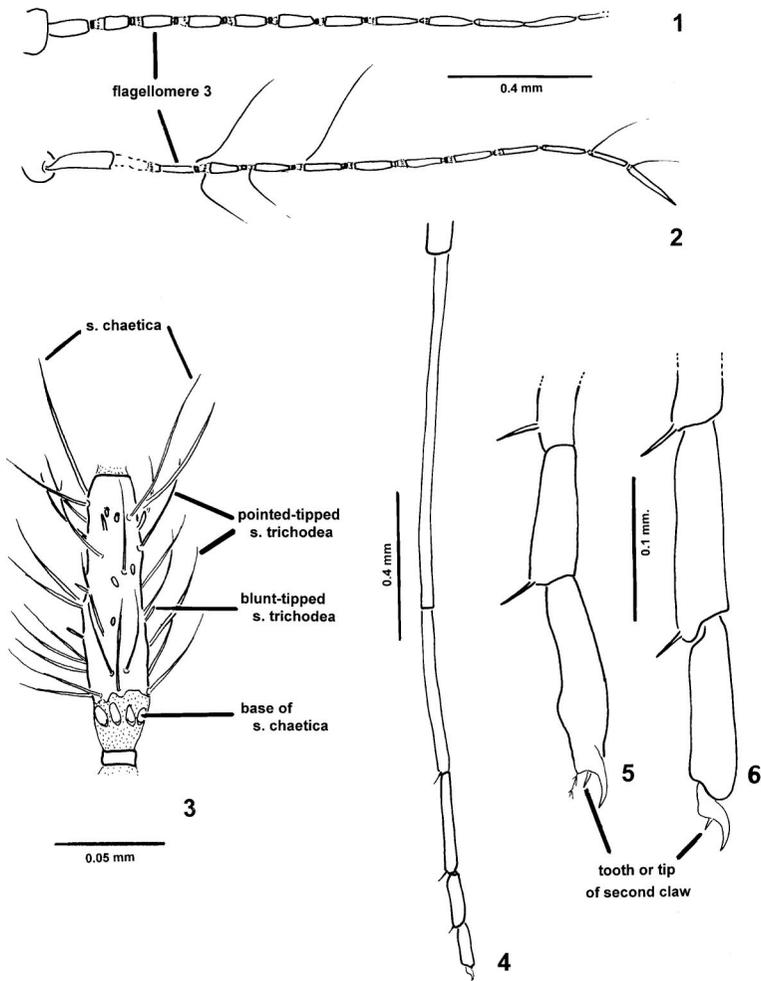
Figs. 1–6. Structures of *B. antiquus*. 1. Left antenna. 2. Right antenna, flagellomeres 1–3 distorted. 3. Flagellomere 7 of left antenna; s, sensillum. 4. Hindleg tarsomeres. 5. Midleg tarsomeres 4 and 5. 6. Hindleg tarsomeres 4 and 5.

Results

Burmaculex antiquus new genus, new species

Culicidae - Grimaldi et al. 2002: 53 (description, illustrations, photo).

Diagnosis. The only known Culicidae with moderately elongate mouthparts (≈1/3 the length of antenna). Also the only known Culicidae lacking body and wing vein scales (other than those on the posterior margin of the wing).

Description. Body lacking scales, except on posterior margin of wing. Ommatidia abutting or nearly abutting dorsomedially (details not clearly visible), extending ventrally to base of proboscis. Antenna with 13 flagellomeres (Figs. 1 and 2); flagellomeres 2–11 (possibly also 12 and 13) each with basal whorl of sensilla chaetica arising from ring of pale cuticle (Fig. 3); flagellomeres 2–13 with four types of sensilla (Fig. 3): subbasal whorl of very elongate sensilla chaetica, somewhat scattered subapical circle of shorter sensilla chaetica, numerous pointed-tipped sensilla trichodea scattered on surface, a few blunt-tipped sensilla trichodea (or perhaps grooved pegs); apex of flagellomere 13 bifid. Clypeus with a few setae (Fig. 8). Mouthparts (Figs. 7 and 8) moderately elongate (length of labium/length of flagellum 0.3). Labrum slender, elongate, curved in cross section, abruptly tapered apically. Mandible not visible. Lacinia with three to four small, barely discernible, apical teeth; with striae along length. Palpus longer than proboscis, with five segments (segment one not visible but must be very short), with segment three elongate, bearing numerous pegs (total number not visible). Labella well-developed, division not visible. Thorax with numerous setae on anterior portion of scutum, at least six katepisternal setae; further details not visible. Wing as shown in Fig. 10, length 1.9 mm, with slender setae on many veins (all shown, or, if missing, their bases), scales restricted to alula, posterior margin of wing, microtrichia, on membrane. Mid, hindleg with numerous setae, midleg with apex of tarsomeres 1–4
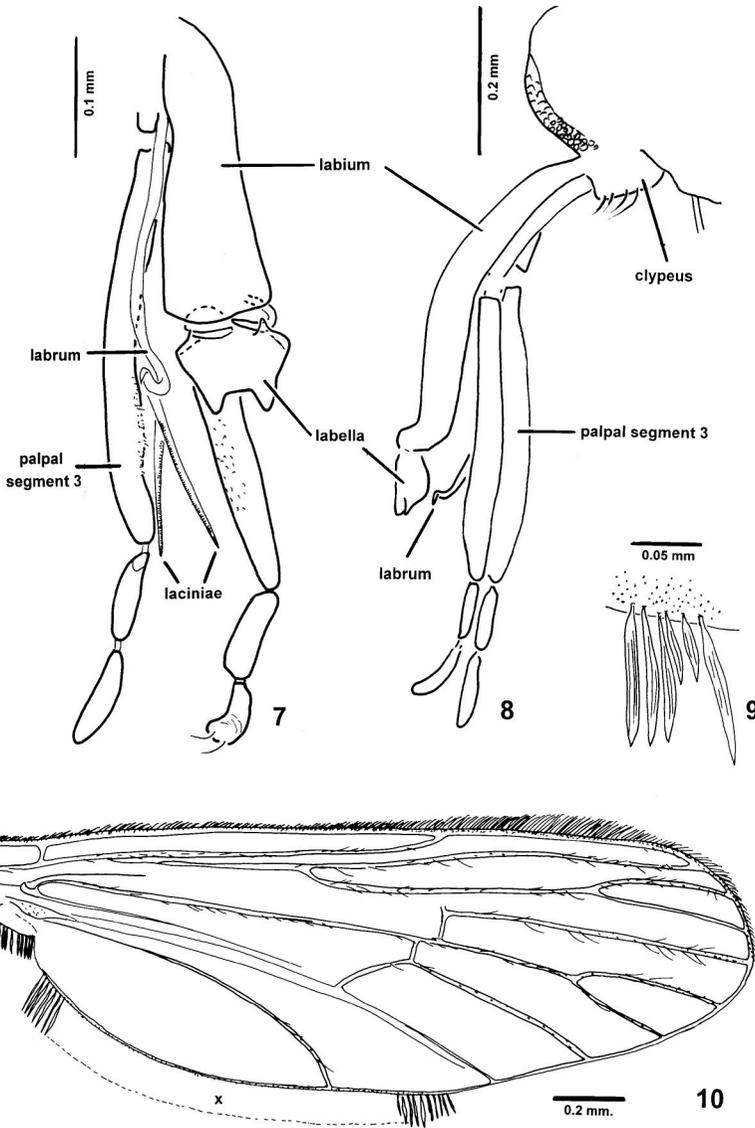
Figs. 7–10. Structures of *B. antiquus*. 7. Mouthparts in ventrolateral view. 8. Mouthparts in lateral view (laciniae not visible from this angle). 9. Wing scales from posterior margin of wing, marked with an "x" in Fig. 10. 10. Right wing.

each with pair of short spines, hindleg with apex of tarsomeres 2–4 each with pair of short spines (Figs. 4 and 6); mid, hindleg claws not clearly visible, either with basal tooth or "tooth" actually tip of second claw (Figs. 5 and 6). Midleg with empodium (Fig. 5).

Bionomics. Extant female biting Culicomorpha, including mosquitoes, have capitate or peg sensilla on palpal segment 3, which function to detect CO_2 (McIver 1972), and both pointed-tipped and blunt-tipped sensilla trichodea on their antennae (McIver 1978) to detect odors emanating from their vertebrate hosts (Bock and Cardew 1996). Similar sensilla on palpal segment 3 and the antennae of *B. antiquus* (Figs. 7 and 8) strongly suggest that these functioned in the same manner. The presence of stylate-like labrum and

laciniae in the fossil (Figs. 7 and 8) is shared with other Culicidae and is also likely indicative of a vertebrate biting habit (a small percentage of extant species have these features but are autogenous). Potential hosts for *B. antiquus* include reptiles and birds, because unidentified remains of these occur in Burmese amber (Ross and York 2000, Grimaldi et al. 2002). Other vertebrates are known from the mid-Cretaceous and are also potential hosts.

Sensilla coeloconica and ampullacea on the female antennae of extant Culicidae have been identified as thermoreceptors that are important in detecting their warm-blooded hosts (Boo and McIver 1975, Davies and Sokolove 1975). The antenna of *B. antiquus* does not have any sensilla coeloconica (all flagellomeres

examined) (Fig. 3), but we could not determine whether the small sunken pits characteristic of sensilla ampullacea were present. It is therefore not possible to use this character to determine whether the hosts of *B. antiquus* were ectotherms or endotherms.

There are several trichomes and an unidentified, elongate, segmented arthropod with very thin cuticle in the amber with *B. antiquus.*

Taxonomic Discussion. The holotype female is in relatively poor condition. The specimen seems partially decayed and some parts are distorted, which is typical for many inclusions in Burmese amber. The following parts are missing: apical one-half of left flagellomere 12 and all of 13; most of dorsum of the thorax, including the halters; and all of the dorsum of the abdomen (also most of its very apex), apex of the left wing, both forelegs, apex of the left midleg, and all of the left hindleg. Although the apex of the abdomen is missing, the specimen is clearly a female because the pedicel is small, typical of females of all Corethrellidae, Chaoboridae, and Culicidae, and the laciniae are elongate (short in males of all other Culicidae; Wahid et al. 2003).

There is a gap between palpal segments 2 and 3 (Figs. 7 and 8), which may be real (connecting membrane not seen) or an artifact of preservation. Similarly, there is a gap at the very base of R_{4+5} (Fig. 10), which may or may not be an artifact. If real, both these features would be unique within the Culicomorpha. The labrum was identified on the basis of its similarity to extant Culicidae: curved in cross section and tapering abruptly at its apex. The laciniae were identified on the basis of their small apical teeth and striae, both of which are similar to those of, for example, *Anopheles earlei* Vargas (personal observation).

The specimen is small, with a wing length of 1.9 mm. There are, however, some extant Culicidae which are equally as small (e.g., some *Wyeomyia* Theobald, some *Uranotaenia* Lynch Arríbálzaga some *Culex* (*Melanoconion* Theobald)).

A few features of this fossil were originally misinterpreted (Grimaldi et al. 2002). The labrum was labeled as a maxillary stylet and although true that there are no scales on the body, the hind margin of the wing actually bears a row of flattened scales (Figs. 9 and 10). The right antenna has 13 flagellomeres, not 11 as shown in the original drawing. The material in the abdomen includes two trichomes and therefore is unlikely to be gut contents (probably foreign material deposited after partial rotting of the mosquito's body). Furthermore, it is unlikely that a blood meal would be preserved when all the tissues of the thorax and abdomen have disintegrated.

Type. Holotype: female in a piece of amber embedded in epoxy, labeled "HOLOTYPE *Burmaculex antiquus* Borkent & Grimaldi," "AMBER: MYANMAR (BURMA) Cretaceous, Kachin: Tanai Village (on Ledo Rd. 105 km NW Myitkyna), coll. Leeward Capitol Corp., 1999, AMNH B-032" (AMNH).

Derivation of Generic and Specific Epithet. The generic name refers to the origin of this fossil Culicidae and the specific name to its old age.
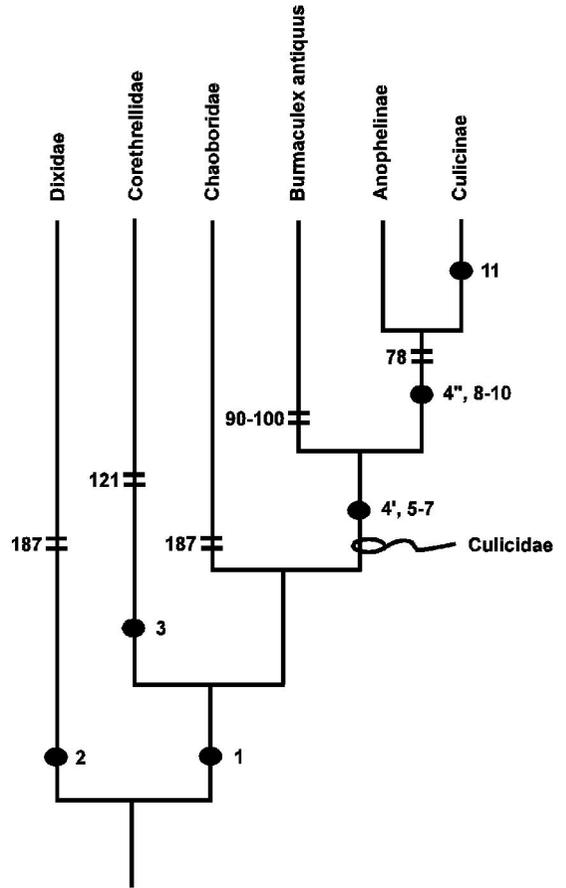

Fig. 11. Cladogram indicating the relationship of *B. antiquus* to other Culicoidea. Circles refer to synapomorphies discussed in the text, including only those which could be scored for the fossil. Double bars give the age of the oldest known fossil for each taxon.

Phylogenetic Position of *B. antiquus*

The following characters provide evidence for the relationships among major lineages of Culicoidea and *B. antiquus* (Fig. 11). Only character states that were visible in the fossil are included here. We paid particular attention to the characters used by Harbach and Kitching (1998) and Sallum et al. (2000) in their cladistic analyses of Culicidae, but very few of these could be seen in the fossil. Wood and Borkent (1989) discussed other characters, especially those of the immatures, important in interpreting the phylogenetic position of the families of Culicoidea.

1. Posterior margin of wing with setae (plesiomorphic); with scales (apomorphic).
   This feature is unique within the Culicomorpha and is restricted to Corethrellidae, Chaoboridae, and Culicidae (Figs. 9 and 10).
2. R_{2+3} straight or slightly curved (plesiomorphic); R_{2+3} strongly arched (apomorphic).
   The apomorphic condition is unique to Dixidae within the nematocerous Diptera.

3. R_1 extending to near apex of wing (plesiomorphic); R_1 short, ending in C near level of fork of R_2 and R_3 (apomorphic).

   The apomorphic condition is unique to Corethrellidae within the Culicoidea. A short R_1 is also present in most Chironomoidea, which is here considered to be convergent.

4. Short proboscis (plesiomorphic); moderately long proboscis ("apomorphic"); very elongate proboscis ("apomorphic").

   The elongate proboscis of Culicidae is unique within the nematocerous Diptera and is superficially approximated only by a few Tipulidae, Tanyderidae, and Mycetophilidae s. lat. Some Ceratopogonidae have somewhat elongated mouthparts, but this is clearly convergent. In extant Culicidae and those fossils for which the character has been described, the proboscis is either about as long as the antennae or is longer. The proboscis of *B. antiquus* is of intermediate length (Figs. 7 and 8), with the proboscis ≈1/3 the length of the antennae. We therefore consider this feature to be a morphocline, evolving from a short, to a moderately long, to a very elongate proboscis.

5. Female labrum relatively short, broad, and dorsal to remaining mouthparts (plesiomorphic); labrum stylate, partially enclosed by the labium (apomorphic).

   The derived condition is unique to the Culicidae within the Diptera. Although the labrum of *B. antiquus* was long and stylate (Figs. 7 and 8), the somewhat splayed arrangement of the mouthparts meant that we could not determine whether the labrum was originally enclosed by the labium. In other Diptera, the labrum may be very broad or somewhat narrowed, especially in other biting taxa, but these are never as narrow and slender as the labra of Culicidae.

6. Female lacinia either reduced or a flattened blade with retrorse hooks (plesiomorphic); lacinia a long, slender stylet bearing fine flattened ridges (apomorphic).

   The long, slender stylate lacinia is a characteristic feature of the Culicidae, including *B. antiquus* (Figs. 7 and 8) and is unique within the Diptera.

7. Palpal segment three short or of moderate length (plesiomorphic); palpal segment three disproportionately elongate (apomorphic).

   The derived condition is present in most Culicidae, including the fossil *B. antiquus* (Figs. 7 and 8). Other Culicomorpha have a comparatively shorter palpal segment 3, more or less equal in length to or shorter than the combined lengths of palpal segments 4 and 5. In many Culicidae, the terminal palpal segments are reduced (see character 11 below) and in some in the subfamily Culicinae the remaining palpal segments are short, which we considered to be secondarily derived.

8. Female palpus longer than other mouthpart elements (plesiomorphic); palpus equal in length or shorter than other mouthpart elements (apomorphic).

The palpus extends well beyond the apex of the proboscis in the fossil (Figs. 7 and 8) and most nonculicid Culicomorpha. Only some Ceratopogonidae have the palpus about equal to the length of the other mouthparts but this is likely to be independently derived, considering their phylogenetic position within the Chironomoidea. Among extant female Culicidae, most Anophelinae have the palpus more or less equal to the length of the mouthparts. Other Culicidae have the palpus significantly shorter than the mouthparts.

9. Clypeus with setae (plesiomorphic); clypeus without setae (apomorphic).

   The females of *B. antiquus* and all other Culicomorpha have at least one seta on the clypeus (most have at least several) (Fig. 8). Nearly all remaining Culicidae have a bare clypeus, the only exception being some species of *Trichoprosopon* Theobald, which bear setae on the margins of the clypeus [e.g., *Trichoprosopon digitatum* (Rondani), *Trichoprosopon compressum* Lutz] (Lane 1953). We consider this to have evolved independently to approximate the plesiomorphic condition.

10. Palpus without scales (plesiomorphic); with scales (apomorphic).

    The palpus of all Culicidae other than *B. antiquus* have scales. These are lacking in the fossil and all other Culicomorpha and therefore the scaled condition is considered derived. Otherwise, some Nematocera also have scales on their palpi (some Cecidomyiidae, some Psychodidae), but this is certainly independently derived.

11. Female palpus with five segments (plesiomorphic); with four or fewer segments, some with vestigial fifth (apomorphic).

    The palpus of *B. antiquus* has only four visible palpal segments (Figs. 7 and 8). However, it is almost certain that the first segment, always small in Culicomorpha, was merely not visible among the distorted base of the mouthparts. The third segment was identified on the basis of the presence of sensory rods, which are typical of Culicidae. In Culicidae that have three or four palpal segments, it is the terminal segments that are reduced, with the very elongate third segment remaining. Independent reductions occur in some derived lineages of Chironomidae and Ceratopogonidae.

Comments. Characters 4′ and 5–7 are all features that involve the elongation of various components of the mouthparts, and they may actually represent a single complex character state. Whether taken singly or not, this suite of derived states shows that *B. antiquus* is indeed a member of the Culicidae.

The wing veins of *B. antiquus* have only setae present (Fig. 10). Due to significant homoplasy in the outgroup, this character state cannot presently be interpreted phylogenetically. Wing vein scales are present in all Culicidae other than *B. antiquus* but they

are present or absent in both Corethrellidae and Chaoboridae. They are absent in all Dixidae. Within Chironomoidea, some Ceratopogonidae have wing vein scales.

Discussion

Our cladistic analysis shows that *B. antiquus* is the sister group to all other fossil and extant species of Culicidae, and it represents the oldest confirmed member of the family (Fig. 11). The 90–100-million-year-old Burmese fossil described here shows that the family must have yet earlier roots. What might the expected age of the Culicidae be? Dipterists consider the Chaoboridae to be the sister group of the Culicidae, and the Chaoboridae, with a relatively rich fossil record, is known from Lower Jurassic deposits ≈187 million years old (Fig. 11) (Borkent 1993). Therefore, we would expect the Culicidae to be equally old and suggest that fossil mosquitoes can be expected from much older deposits than the oldest fossil described here. However, older lineages of Culicidae may be increasingly difficult to recognize if only fossils of adults are available. The Chaoboridae have no recognized synapomorphies in the adult stage that define the family, and Mesozoic fossils known only as adults cannot be presently interpreted phylogenetically on the basis of their features. It is therefore possible that some Jurassic and/or Cretaceous taxa presently placed in the Chaoboridae, and which are known only as adults, are actually early lineages of Culicidae with short mouthparts. Jurassic fossils of Chaoboridae that are confidently identified as members of this family are those with synapomorphies in the immature stages: larval antenna elongate and bearing strong apical spines (modified to capture prey), larval anal fan in a single plane, pupal respiratory organ with a small aperture, and pupae with unique brush-like arrangements of pharate adult abdominal setae (Kalugina and Kovalev 1985). These synapomorphies indicate that the Chaoboridae are a valid, monophyletic taxon, including at least some Lower Jurassic taxa. We suggest that culicid-like larvae were present in the Jurassic but the odds of finding these as fossils is unlikely. Culicidae larvae are restricted to small, fishless bodies of water and are therefore unlikely to be preserved in the lake sediments that characterize the Jurassic freshwater fossil record.

Tertiary fossils of Culicidae are known only from the Eocene and Oligocene and their features have been summarized by Szadziewski (1998) and Poinar et al. (2000). All are members of extant genera or subgenera or are so poorly known as to be of uncertain status. Poinar et al. (2000) described the only other known Cretaceous Culicidae, *Paleoculicis minutus* Poinar, Zavortink, Pike & Johnston based on a single male from Canadian amber, 76.5–79.5 million years old. This monotypic genus has a unique combination of character states. Although not assigned a phylogenetic position within the Culicidae these authors suggested that overall the taxon was "closer" to the Culicinae. The presence of synapomorphy 7 described above indicates that *P. minutus* does indeed belong within the family and the presence of synapomorphy 10 shows that it belongs within the clade recognized as the sister group of *B. antiquus.* The proboscis of *P. minutus* was missing from the fossil and therefore character 4 cannot be appraised. However, an elongate palpus strongly suggests that it also possessed an elongate proboscis. In summary, the fossil record indicates that much of the generic differentiation of the Culicidae occurred during the Tertiary and that Cretaceous taxa are either generically distinct or represent earlier lineages.

Acknowledgments

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