

ARTICLE



Priscoculex burmanicus n. gen. et sp. (Diptera: Culicidae: Anophelinae) from mid-Cretaceous Myanmar amber

George Poinar^a, Thomas J. Zavortink^b and Alex Brown^c

^aDepartment of Integrative Biology, Oregon State University, Corvallis, OR, USA; ^bBohart Museum, Department of Entomology and Nematology, University of California, Davis, CA, USA; ^cBerkeley, CA, USA

ABSTRACT

A new genus and species of mosquito, *Priscoculex burmanicus* (Diptera: Culicidae: Anophelinae), are described from mid-Cretaceous Myanmar amber. Diagnostic characters for the holotype female are the presence of scales and setae on the wing veins, a setaceous, straight, proboscis that is subequal in length to the maxillary palpi, rounded margin of the scutellum, the third palpomere with a sensillum chaeticum, long setae on the proboscis and palpi, antennae longer than proboscis, absence of scales on the abdomen, lack of pulvilli and spiracular setae, truncate abdomen and oval cerci. The above-mentioned features are the basis for assigning *Priscoculex* as an early lineage of the Anophelinae that existed ~100–110 Mya. Discussion is presented on how the Anophelinae could have originated in Gondwana and colonized the interconnected lands before the continents separated.

www.zoobank.org/urn:lsid:zoobank.org:pub:C776F051-F345-4E15-9837-DF2D1AF22DF3
www.zoobank.org/urn:lsid:zoobank.org:act:1601C12D-6CFC-49E9-8731-A011F98E6556
www.zoobank.org/urn:lsid:zoobank.org:act:C5E4DBC1-CCE1-48F4-9A08-DAA6993BAA9D

ARTICLE HISTORY

Received 15 December 2018
Accepted 11 January 2019

KEYWORDS

Mid-Cretaceous amber;
Culicidae; Myanmar;
anopheline fossil

Introduction

Due to their blood-sucking habits and various pathogens they transmit to vertebrates, mosquitoes are one of the most medically important families of hematophagous arthropods. Just when mosquitoes evolved and what vertebrate groups served as their first hosts is unknown. The Cretaceous fossil record of mosquitoes is quite sparse with *Paleoculis minutus* from Upper Cretaceous Canadian amber (Poinar et al. 2000) and *Burmaculex antiquus* from mid-Cretaceous Myanmar amber (Borkent and Grimaldi 2004, 2016). The Myanmar amber female specimen described in the present paper possesses basic morphological features that align it with the subfamily Anophelinae (Diptera: Culicidae), such as the straight proboscis, extended maxillary palpi that are subequal in length to the proboscis, scutellum with rounded margin, absence of pulvilli and spiracular setae, abdomen lacking scales, tip of abdomen truncate and oval cerci. This discovery provides evidence that the radiation of anophelines was occurring 100 Mya in Gondwana, based on the proposal that Myanmar amber fossils originated in Gondwana.

Materials and methods

The amber originated from the Aung Bar Mine near Tanai in the Hukawng Valley in Myanmar. Based on palaeontological evidence, amber from this region was dated to the Upper Albian of the Lower-Mid Cretaceous (Cruickshank and Ko 2003), placing the age at 97–110 Mya. A more recent study using U-Pb zircon dating determined the age to be 98.79 ± 0.62 Ma (Shi et al. 2012). Nuclear magnetic resonance (NMR) spectra and the presence of

araucaroid wood fibres in amber samples from the Noije Bum 2001 Summit Site indicate an araucarian tree source for the amber (Poinar et al. 2007).

Observations and photographs were made with a Nikon SMZ-10 R stereoscopic microscope and Nikon Optiphot compound microscope with magnification up to 1000X. Helicon Focus Pro X64 was used to stack photos for better overall clarity and depth of field.

Results

The fossil is complete however the right eye is damaged and the thorax is somewhat distorted.

Description

Order: Diptera Linnaeus, 1758
Family Culicidae Meigen, 1818
Subfamily: Anophelinae Grassi, 1900

Genus: *Priscoculex* Poinar, Zavortink and Brown, n. gen.

LSID

Etymology. The generic name is taken from the Latin ‘prisco’ = ancient, of former times and the Latin ‘Culex’ = mosquito.

Type species. *Priscoculex burmanicus* Poinar, Zavortink and Brown, n. sp.

Diagnosis. Flagellum with 13 flagellomeres, palpi with 5 palpomeres; ten veins reaching wing margin; costal vein continuing around wing, although weaker along hind margin; subcostal vein ending in costal beyond middle of wing; vertex of head bearing scales; proboscis setaceous, straight; palpi and antennae longer than proboscis; third palpomere with sensillum chaeticum; scales on wing veins and tarsi; scutellum with rounded margin; pulvilli and spiracular setae absent; abdomen without scales; tip of abdomen truncate, cerci oval.

Included species. Type.

Priscoculex burmanicus n. gen. et sp. (Figures 1–10)

LSID:

Etymology. The specific epithet refers to the origin of the fossil.

Type material. Holotype female (no. B-D-70) deposited in the Poinar amber collection maintained at Oregon State University.

Type locality. Myanmar: Kachin State, Myitkyina District, Tanai Township, Hukawng Valley, Aung Bar Mine (26°21' N, 96°43' E).

Description. Body and appendages brown. Body length from top of curved scutum to tip of abdomen, 2.9 mm; wing length, 2.6 mm (Figure 1).



Figure 1. Holotype of *Priscoculex burmanicus* in Myanmar amber. Scale bar = 674 μ m.

Head. Vertex of head with numerous small, erect, broad scales; eyes reniform with facets of equal size; antennae longer than palpi and proboscis (Figures 2, 3), with 13 flagellomeres, flagellum length, 1.5 mm; each flagellomere bears scales and dense short setae; flagellomeres 5–13 have a basal whorl of long setae reaching up to 339 μ m in length (Figure 4); clypeus triangular-shaped, length 103 μ m; labrum straight, completely exposed, with pointed tip, length 875 μ m; proboscis bearing numerous setae, especially on ventral surface near tip; labellum comprising two incompletely separated sclerites, 126 μ m long; scape short, partially hidden by large, cup-shaped pedicel, length pedicel, 131 μ m, width pedicel, 140 μ m; palpus

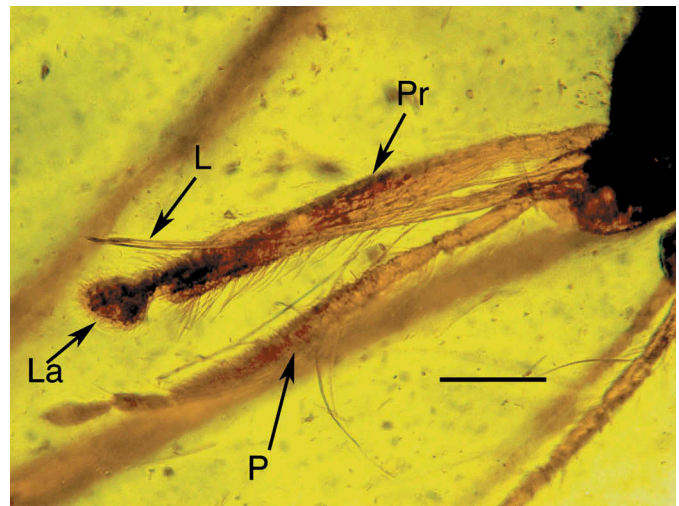


Figure 2. Head appendages of *Priscoculex burmanicus* in Myanmar amber. Pr = proboscis; P = palpus; L = labrum; La = labellum. Scale bar = 24 μ m.



Figure 3. Palpus of *Priscoculex burmanicus* in Myanmar amber with a sensillum chaeticum located in approximately the middle of the third palpomere. Scale bar = 30 μ m. Insert shows detail of sensillum chaeticum. Scale bar = 8 μ m.

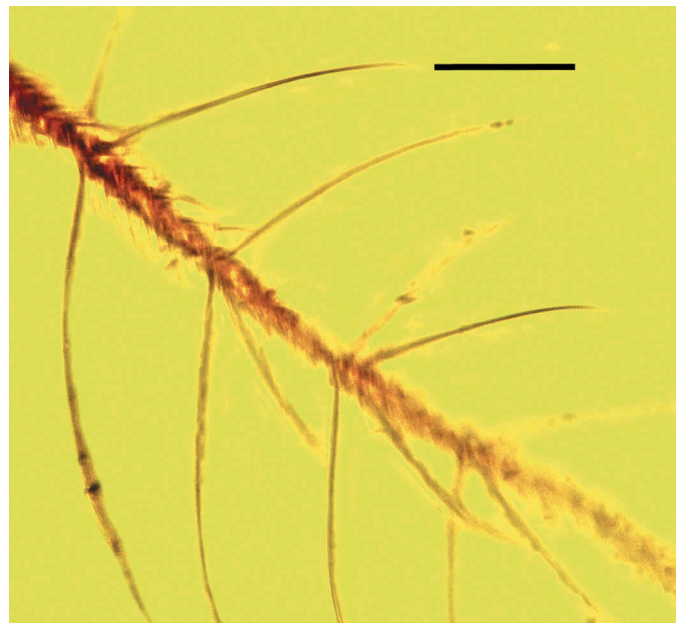


Figure 4. Setae on flagellomeres of *Priscoculex burmanicus* in Myanmar amber. Scale bar = 106 μ m.

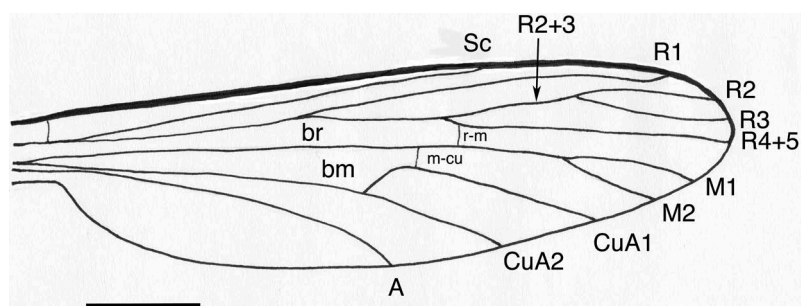


Figure 5. Wing of *Priscoculex burmanicus* in Myanmar amber showing veins. Scale bar = 386 μ m.

5-segmented, length 1.1 mm, slightly longer than proboscis, bearing numerous setae basically, third palpomere bearing a sensillum chaeticum on the middle of the inner surface; ratio proboscis/flagellum, 0.6.

Thorax. Scutellum narrow, evenly arched; wing elongate, length 2.6 mm (Figure 5); W/L ratio, 0.2; subcostal vein meets costal vein on wing margin beyond r-m crossvein; Sc and R1 reaching C in apical half of wing, distal to base of R2 and R3; M with 2 branches; R2 + 3 approximately equal to distance from fork of R2+ R3 to wing margin; cells br and bm more than half as long as wing; anal vein reaching wing margin distal to fork of CuA vein; wing membrane pebbled; basal portion of costal vein bearing row of wide, pale scales; basal posterior wing margin bearing long, slender, fringe scales and short secondary scales; alula bearing long, slender fringe scales (Figures 6, 7); wing veins with both long, slender scales and setae (Figure 8); legs 5-segmented, tarsomeres bearing scales; each leg bearing a pair of simple, curved claws; empodium absent (Figure 9).

Abdomen. With 8 distinct and 2 indistinct segments; scales absent; cerci oval, distinctly clubbed, setose, on 9th segment adjacent to postgenital lobe (Figure 10).

Male. Unknown

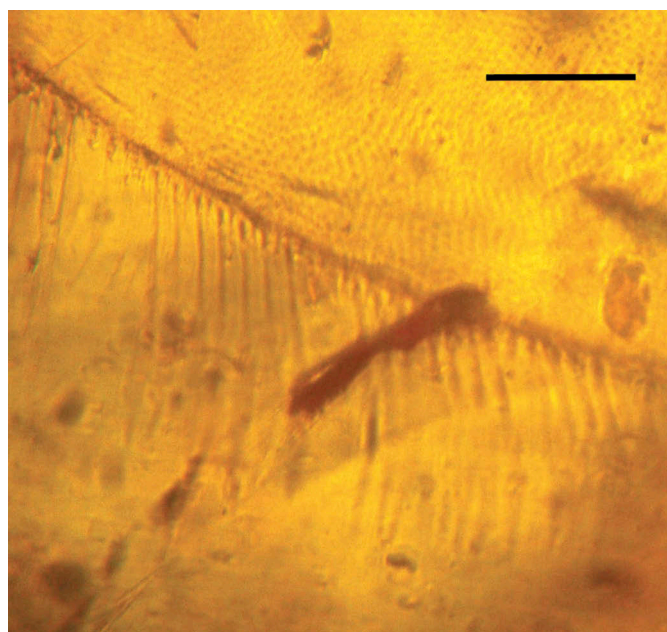


Figure 7. Long slender fringe scales and short secondary scales on the basal portion of the wing margin of *Priscoculex burmanicus* in Myanmar amber. Note pebbling (dotting) on wing membrane. Scale bar = 113 μ m.

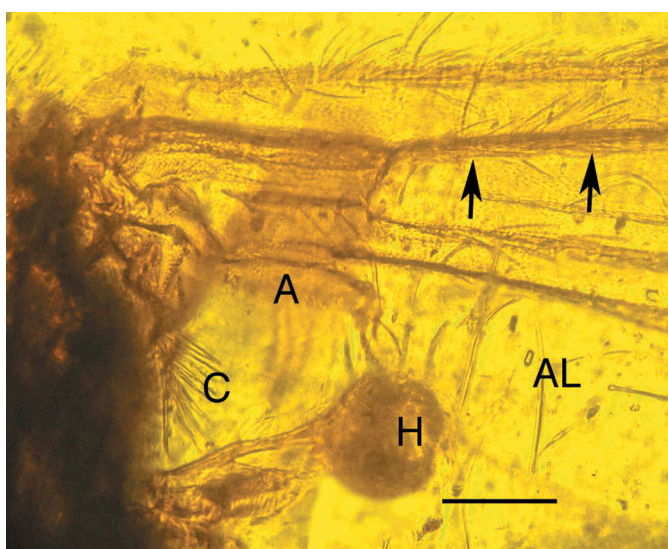


Figure 6. Basal posterior wing margin of *Priscoculex burmanicus* in Myanmar amber showing alula (A), upper calypter (C), anal lobe (AL), and halter (H). Arrows show elongate scales clasp the base of R vein. Scale bar = 38 μ m.

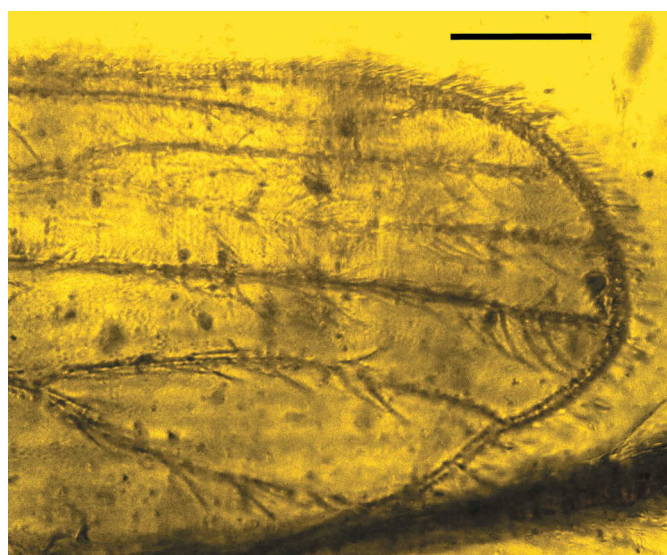


Figure 8. Long, slender scales and setae on wing veins of *Priscoculex burmanicus* in Myanmar amber. Note also shorter scales along margin of wing tip. Scale bar = 200 μ m.

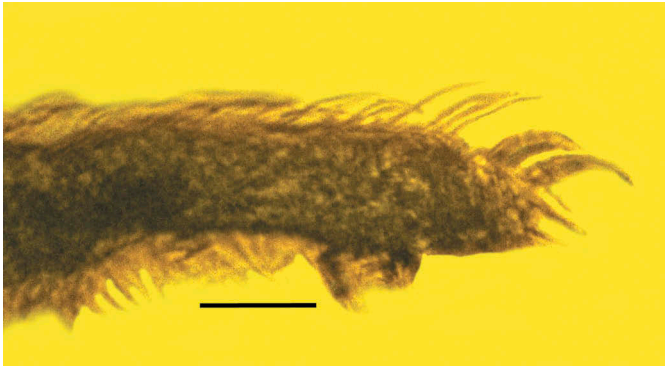


Figure 9. Claws on mesotarsus of *Priscoculex burmanicus* in Myanmar amber. Scale bar = 63 μ m.



Figure 10. Tip of abdomen with cercus of *Priscoculex burmanicus* in Myanmar amber. Scale bar = 56 μ m.

Comments

The predominance of anopheline features of *Priscoculex* (Belkin 1962; Stone 1981; Foster and Walker 2002) is the basis for assigning *Priscoculex* to the Anophelinae. However, some features of *Priscoculex*, such as the setaceous proboscis and palpi and the long setae on the wing veins are not typical of anophelines and are regarded as ancestral characters.

Aside from *Anopheles*, two additional genera have been placed within the subfamily Anophelinae. One of these is the Neotropical genus *Chagasia* Cruz, whose females have maxillary palpi slightly longer than the proboscis, similar to the condition in *Priscoculex* (Harbach and Howard 2009). While *Chagasia* and *Anopheles* have been considered sister groups (Sallum et al. 2002), *Chagasia* spp. are characterized by a strongly arched scutum, trilobed scutellum and antennae shorter than the proboscis, which separate members of this genus from *Priscoculex*.

The other anopheline genus, *Bironella* Theobald, is restricted to the Australasian region. Basic features of this genus are evenly rounded wings with vein M1 + 2 (stem of 2nd fork) and M3 + 4 distinctly wavy beyond the m-cu cross-vein, and short female palpi that are only about 0.2 the length of the proboscis (Tenorio 1975, 1977). These characters clearly separate *Priscoculex* from members of *Bironella*.

Priscoculex can be distinguished from the previously described Myanmar amber genus *Burmaculex* Borkent and Grimaldi (Borkent and Grimaldi 2004, 2016) using characters in the following key:

A. Proboscis with curved shaft that abruptly tapers at apex; ratio proboscis L to flagellum L = 0.3–0.4; palpus not bearing sensilla chaetica; wing fairly broad, length 1.6–1.9 mm, W/L ratio = 0.3; Sc meets C on wing margin at level of r-m cross-vein; R2 + 3 approximately twice the distance from fork of R2 + 3 to wing margin; cell R2 shorter than vein R2 + 3; anal vein meets wing margin proximal to origin of CuA1 – *Burmaculex*

AA. Proboscis straight; ratio proboscis L to flagellum L = 0.6; third palpomere bearing sensillum chaeticum; wing narrow, length 2.6 mm; W/L ratio = 0.2; Sc meets C on wing margin anterior to r-m crossvein; R2 + 3 approximately equal to the distance from fork of R2 + 3 to wing margin; cell R2 longer than vein R2 + 3; anal vein meets wing margin distal to origin of CuA1 – *Priscoculex*

The sensillum chaeticum on the inner surface of the 3rd palpomere of *Priscoculex* is thick-walled, externally scalloped, arises from a socket and is distally pointed (Figure 3, insert). Sensilla chaetica, which are probably sensitive to touch and/or air currents, have been noted previously on the palpi of both sexes of *Anopheles stephensi* Liston, *A. albimanus* Wiedemann, *A. quadrimaculatus* Say and on females of *A. gambiae* Giles (McIver and Siemicki 1975).

Regarding pebbling (minute ‘dots’) on the wing membrane of *Priscoculex*, this condition has been noted to occur in association with regular rows of dense microtrichia, with the interference patterns indicating reflection around each microtrichium (Shevtsova et al. 2011).

Discussion

The only previous fossil anopheline is *Anopheles (Nyssorhynchus) dominicanus* in Dominican amber (Zavortink and Poinar 2000) that establishes the genus in the Americas in the mid-Tertiary. Also in South America is the endemic basal anopheline genus *Chagasia* that differs from other anophelines by its combination of anopheline and culicine features.

A proposal that anophelines existed on Pangaea and diversified afterwards on the separated continents was suggested by Christophers (1933) and discussed by Harbach (2013). However, as pointed out by Harbach (2013), a Pangaea origin of anophelines would not explain the presence of the endemic genera *Chagasia* and subgenus *Stethomyia* in the Neotropics, the endemic subgenus *Baimaia* in the Orient or the presence of the genus *Bironella* in Australia and Papua – New Guinea. However a Gondwana origin of the anophelines would account for these distributions.

Suggesting that anophelines had a Gondwana radiation is not just based on their present distribution but on previous evidence showing that the Burmese amber mines, which are located on the West Burma Block, rafted from Australia to SE Asia in the Lower to mid-Cretaceous (Metcalf 1990, 1996; Mitchell 1993; Poinar 2018). This would explain the presence of the endemic African *Anopheles funestus* Giles, *A. gambiae* Giles, and *A. nili* species complexes in Africa (Sinka et al. 2010) and the origin of the endemic *A. culicifacies* Giles, *A. dirus* Peyton and Harrison, *A. sundaicus* (Rodenwaldt), *A. fluviatilis* James and *A. stephensi* Liston species complexes in India (Reisen 2003; Dev and Sharma 2013; Harbach 2013).

Several studies used molecular methods to date the origin of the Culicidae and Anophelinae. Bertone et al. (2008) used multiple nuclear genes to place the divergence time between Chaoboridae and Culicidae at 118 Mya. Using nuclear single-copy genes and mitochondrial genome annotation, Krzywinski et al. (2006) proposed that *Aedes* and *Anopheles* separated ~145–200 Mya, the split between *Anopheles* subgenera *Cellia* and *Anopheles* occurred at ~90–106 Mya and the split between lineages within the subgenus *Anopheles* occurred around ~70–85 Mya. A study by Moreno et al. (2010) used mtDNA genes to place the split between the Culicinae and Anophelinae at ~190 Mya, with the radiation of *Anopheles* occurring ~130 Mya. Reidenbach et al. (2009) used 6 nuclear genes and 80 morphological characters to show that the Culicinae and Anophelinae separated about 226 Mya. Based on aligned protein sequences of 1,085 single-copy orthologs, Neafsey et al. (2015) concluded that *Anopheles* began to diversify 100 Mya. The above molecular studies support a scenario of the Culicidae appearing in Jurassic Pangaea some 225–240 Mya with the anophelines beginning to diversify 100 Mya, which is the age of the present fossil.

It is proposed that *Priscoculex* represents a primitive anopheline lineage that was the result of an earlier diversification that occurred in the Lower Cretaceous some 130–140 Mya when the Gondwana continents were still interconnected, but Gondwana had already separated from Laurasia (Funnell 1990; Smith et al. 1994).

Acknowledgments

Thanks are extended to Ralph Harbach and Graham White for discussions and literature on anopheline distribution, speciation and divergence times, to two anonymous reviewers whose comments improved the paper and to Roberta Poinar for remarks on earlier drafts of this manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Belkin JN. 1962. The mosquitoes of the South Pacific (Diptera, Culicidae). Vol. 1 and 2, Berkeley: University of California Press; vol. 1, 620; p. vol. 2, p. 412.

Bertone MA, Courtney GW, Wiegmann BM. 2008. Phylogenetics and temporal diversification of the earliest true flies (Insecta: Diptera) based on multiple nuclear genes. *Syst Entomol.* 33:668–687.

Borkent A, Grimaldi DA. 2004. The earliest fossil mosquito (Diptera: Culicidae), in mid-cretaceous burmese amber. *Ann Entomol Soc Am.* 97:882–888.

Borkent A, Grimaldi DA. 2016. The cretaceous fossil *Burmaculex anti-quus* confirmed as the earliest known lineage of mosquitoes (Diptera: Culicidae). *Zootaxa.* 4079:457–466.

Christophers SR. 1933. The Fauna of British India, including Ceylon and Burma. Diptera. Vol. IV. Family Culicidae. Tribe Anophelini. London: Taylor and Francis.

Cruickshank RD, Ko K. 2003. Geology of an amber locality in the Hukawng Valley, northern Myanmar. *J Asian Earth Sci.* 21:441–455.

Dev V, Sharma VP. 2013. The dominant mosquito vectors of human malaria in India. Chapter 9, London: Intech; p. 239–271. doi:10.5772/55215

Foster WA, Walker ED. 2002. Mosquitoes (Culicidae). In: Mullen G, Durden L, editors. *Medical and veterinary entomology*. New York: Academic Press; p. 203–262.

Funnell BM. 1990. Global and European cretaceous shorelines, stage by stage. In: Ginsburg RN, Beaudoin B, editors. *Cretaceous resources, events and rhythms*. Dordrecht: Kluwer Academic; p. 221–235.

Harbach RE. 2013. The phylogeny and classification of *Anopheles*. In: Manguin S, editor. *Anopheles Mosquitoes – new insights into malaria vectors*. Rijeka (Croatia): InTech; p. 3–55. doi:10.5772/54695

Harbach RE, Howard TM. 2009. Review of the genus *Chagasia* (Diptera: Culicidae: Anophelinae). *Zootaxa.* 2210:1–25.

Krzywinski J, Grushko OG, Besansky NJ. 2006. Analysis of the complete mitochondrial DNA from *Anopheles funestus*: an improved dipteran mitochondrial genome annotation and a temporal dimension of mosquito evolution. *Mol Phylogenet Evol.* 39:417–423.

McIver SB, Siemicki R. 1975. Palpal sensilla of selected anopheline mosquitoes. *J Parasitol.* 61:535–538.

Metcalf I. 1990. Allochthonous terrane processes in Southeast Asia. *Philosoph Trans Royal Soc London.* A133:625–640.

Metcalf I. 1996. Pre-Cretaceous evolution of SE Asian terranes. In: Hall R, Blundell DJ, editors. *Tectonic evolution of Southeast Asia*. Vol. 106, London: Geol. Society, Spec. Publ; p. 97–122.

Mitchell AHG. 1993. Cretaceous-Cenozoic tectonic events in the western Myanmar (Burma)-Assam region. *J Geological Soc London.* 150:1089–1102.

Moreno M, Marinotti O, Krzywinski J, Tadei WP, James AA, Achee NL, Conn JE. 2010. Complete mtDNA genomes of *Anopheles darlingi* and an approach to anopheline divergence time. *Malaria J.* 9:127. <http://www.malariajournal.com/content/9/1/127>.

Neafsey DE. 2015. Highly evolvable malaria vectors: the genomes of 16 *Anopheles* mosquitoes. *Sci.* 347(issue 6217):43–50. doi:10.1126/science.1258522

Poinar G Jr. 2018. Burmese amber: evidence of Gondwanan origin and Cretaceous dispersion. *Hist Biol.* 1–6. doi:10.1080/08912963.2018.1446531

Poinar G Jr., Lambert JB, Wu Y. 2007. Araucarian source of fossiliferous Burmese amber: spectroscopic and anatomical evidence. *J Bot Res Inst Texas.* 1:449–455.

Poinar GO Jr., Zavortink TJ, Pike T, Johnson PA. 2000. *Paleoculis minutus* (Diptera: Culicidae) n. gen., n. sp., from Cretaceous Canadian amber, with a summary of described fossil mosquitoes. *Acta Geologica Hispanica.* 35:119–128.

Reidenbach KR, Cook S, Bertone MA, Harbach RE, Wiegmann BM, Besansky NJ. 2009. Phylogenetic analysis and temporal diversification of mosquitoes (Diptera: Culicidae) based on nuclear genes and morphology. *BMC Evol Biol.* 9:298. doi:10.1186/1471-2148-9-298

Reisen WK. 2003. Malaria. In: Resh VH, Cardé RT, editors. *Encyclopedia of insects*. Amsterdam: Academic Press; p. 672–675.

Sallum MAM, Schultz TR, Foster PG, Aronstein K, Wirtz RA, Wilkerson RC. 2002. Phylogeny of Anophelinae (Diptera: Culicidae) used on nuclear ribosomal and mitochondrial DNA sequences. *Syst Entomol.* 27:361–382.

Shevtsova E, Hansson C, Janzen DH, Kjørandsen J. 2011. Stable structural color patterns displayed on transparent insect wings. *PNAS.* 108:668–673.

Shi G, Grimaldi DA, Harlow GE, Wang J, Wang JY, Yand M, Lei W, Li Q, Li X. 2012. Age constraint on Burmese amber based on U-Pb dating of zircons. *Cret Res.* 37:155–163.

Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J,

- Patil AP, Temperley WH, Gething PW, Kabaria CW, et al. 2010. The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and biologic précis. *Parasit Vectors*. 3:117. <http://www.parasitesandvectors.com/content/3/1/117>.
- Smith AG, Smith DG, Funnell BM. 1994. Atlas of mesozoic and cenozoic coastlines. Cambridge (UK): Cambridge University Press; p. 99.
- Stone A. 1981. Mosquitoes. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM, editors. Manual of nearctic diptera. Vol. 1, Monograph No. 27. Ottawa: Research Branch, Agriculture Canada; p. 341–350.
- Tenorio JA. 1975. *Bironella (Brugella) obscura*, a new species of mosquito from New Guinea (Diptera: Culicidae). *J Medical Entomol*. 11:734–738.
- Tenorio JA. 1977. Revision of the genus *Bironella* (Diptera: Culicidae). *J Medical Entomol*. 14:317–361.
- Zavortink TJ, Poinar GO Jr. 2000. *Anopheles (Nyssorhynchus) dominicanus* sp. n. (Diptera: Culicidae) from Dominican amber. *Ann Entomol Soc Am*. 93:1230–1235.