Cretaceous Research 30 (2009) 1066–1072

Contents lists available at ScienceDirect

Cretaceous Research

journal homepage: www.elsevier.com/locate/CretRes

Early Cretaceous protist flagellates (Parabasalia: Hypermastigia: Oxymonada) of cockroaches (Insecta: Blattaria) in Burmese amber

George Poinar, Jr.

Department of Zoology, Oregon State University, Cordley Hall, Corvallis, OR 97331, USA

ARTICLE INFO

Article history:
Received 21 January 2009
Accepted in revised form 31 March 2009
Available online 8 April 2009

Keywords:
Early Cretaceous
Burmese amber
Cockroaches
Flagellates

ABSTRACT

Two Early Cretaceous Burmese amber cockroaches contained protists related to mutualistic flagellates occurring in extant *Cryptocercus* cockroaches and lower termites. The fossil protists are described as *Devescovites proteus* Poinar n. gen., n. sp. (Parabasalia: Trichomonadida: Devescovinidae), *Paleotrichomones burmanicus* Poinar n. gen., n. sp. (Parabasalia: Trichomonida), *Burmanymphus cretacea* Poinar n. gen., n. sp. (Hypermastigia: Trichonymphida: Burmanymphidae n. fam.) and *Oxymonas gigantea* Poinar, n. sp. Additional putative protists are also illustrated. Evolutionary implications of this discovery are discussed.

1. Introduction

Fossil protists are rare but have been reported in various amber deposits dating back to the Mesozoic (Schmidt et al., 2004; Poinar et al., 1993 (now considered Cenomanian in age); Waggoner, 1993). These are reports of free-living protists trapped when the resin was deposited on small microbial micro-communities living on tree bark or on the ground under the resin-producing tree. Fossil symbiotic protists exposed outside their hosts are quite rare.

Certain flagellate lineages have developed mutualistic associations with extant xylophagous cockroaches of the genus *Cryptocercus* and lower termites and digest cellulose in return for food and shelter (Cleveland et al., 1934; Bignell, 2000; Bell et al., 2007; Brugerolle and Lee, 2000a,b; Bobyleva, 1975; Honigberg, 1970; Ohkuma et al., 2008). The present study describes Early Cretaceous flagellates associated with two separate lineages of cockroaches in Burmese amber.

2. Materials and method

The two fossil cockroaches investigated in the present study occur in separate pieces of Burmese amber. Accession # B-OR-1C (Fig. 1) is a juvenile cockroach (4.2 mm in length), which could not be identified to family due to its condition and immature state. The dorsum of the abdomen and thorax was missing and the body cavity exposed. It is in a rectangular piece of amber measuring 100 mm long by 8 mm wide by 2 mm in depth. Accession # B-OR-1D (Fig. 2) is an adult cockroach identified as a member of the family Blattellidae based on its wing venation. It is completely disemboweled and its length is estimated at approximately 7 mm. It is in an oval piece of amber measuring 19 mm in greatest length, 12 mm in greatest width and 4 mm in greatest depth. There were no other dictyopterans or termites present in either of the two amber pieces, so it is highly unlikely that the protists described here originated from any organism other than the cockroaches.

The amber was obtained from a mine first excavated in 2001 in the Hukawng Valley, southwest of Maingkhwan in Kachin State (26°20′N, 96°36′E), Myanmar (Burma). On the basis of paleontological evidence, the Noije Bum 2001 Summit Amber Site was assigned to the upper Albian of the Lower Cretaceous (Cruickshank and Ko, 2003), placing the age at 97 to 110 Ma. Nuclear magnetic resonance (NMR) spectra and the presence of araucaroid wood fibers in amber samples from the Noije Bum 2001 Summit site indicate an araucarian (possibly *Agathis*) tree source for the amber (Poinar et al., 2007). Observations, drawings and photographs were made with a Nikon SMZ-10 R stereoscopic microscope and Nikon Optiphot compound microscope with magnifications up to 1060×. Photos of the protists were viewed in Adobe Photoshop under various degrees of contrast and brightness for revealing details depicted in the accompanying drawings. Terminology and classification follows that of Brugerolle and Lee (2000a,b,c) and Patterson et al. (2000a,b).

E-mail address: poinarg@science.oregonstate.edu

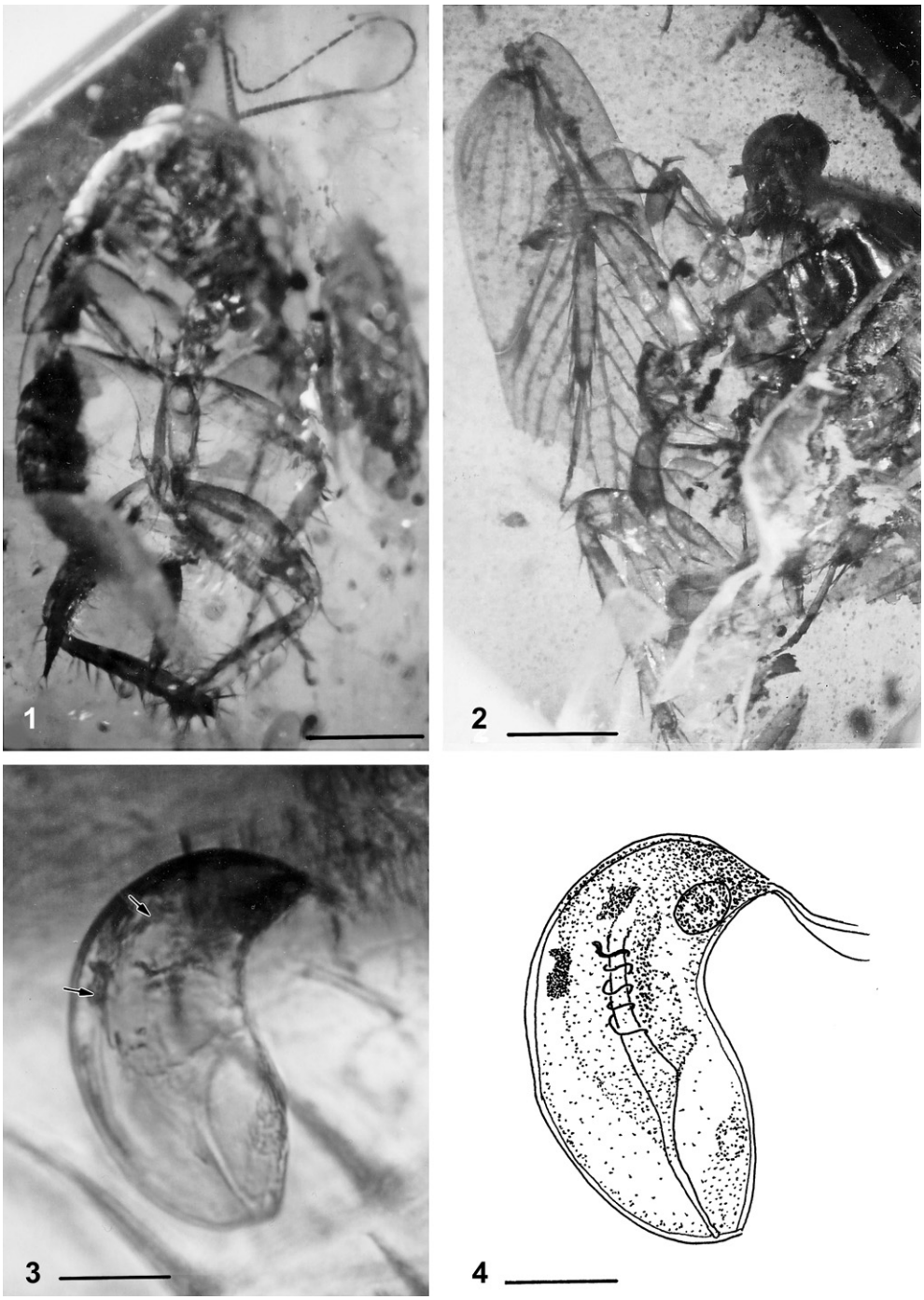
0195-6671/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cretres.2009.03.008

3. Systematic paleontology

A summary of the described fossils with their higher-level systematic positions is presented below. The systematic assignments of the protists are tentative and based solely on morphological similarities shared with extant flagellates. However, at 100 mya, there certainly were many genera and families unknown today and this makes it difficult to equate the fossil protists with present-day taxa.

3.1. Protists from specimen B-OR-1C (undetermined family of cockroaches)

Phylum Parabasalia
Class Trichomonada Kirby
Order Trichomonadida Kirby
Family Devescovinidae Doflein
Devescovites Poinar n. gen.
D. proteus Poinar n. sp.



Figs. 1–4. 1, Cockroach host of unknown family (B-OR-1C) in Early Cretaceous Burmese amber. Scale 930 μm. 2, Cockroach host of the family Blattellidae (B-OR-1D) in Early Cretaceous Burmese amber. Scale 1.4 mm. 3, *Devescovites proteus* Poinar n. gen., n. sp. (specimen 1). Arrows indicate possible wood particles. Scale 32 μm. 4, Drawing of *Devescovites proteus* Poinar n. gen., n. sp. (specimen 1). Scale 32 μm.

Family unknown
Paleotrichomones Poinar n. gen.
P. burmanicus Poinar n. sp.

3.2. Protists from specimen B-OR-1D (family Blattellidae)

Phylum Parabasalia
Class Hypermastigia Grassi & Foá
Order Trichonymphida
Family Burmanymphidae fam. n.
Burmanymphus Poinar n. gen.
Burmanymphus cretacea Poinar n. sp.

Phylum Oxymonada
Class Oxymonadea Grassi
Order Oxymonadida Grassi
Family Oxymonadidae Kirby
Genus *Oxymonas* Janicki
Oxymonas gigantea Poinar, n. sp.

3.3. Descriptions of fossil flagellates

Family Devescovinidae Doflein, 1911

Devescovites Poinar, n. gen.

Diagnosis: Body asymmetrical; three flagella arising from anterior tip; trailing flagellum not observed; nucleus located in anterior portion of body; parabasal body coiled several times around axostyle; axostylar trunk tapered at posterior end, enclosed in body.
Type species: Devescovites proteus n. sp.

Devescovites proteus Poinar n. sp.
Figs. 3–6

Description: Two specimens: specimen A (Figs. 3 and 4); length, 113 μm, greatest width, 45 μm; specimen B (Figs. 5 and 6); length, 131 μm, greatest width, 86 μm; length flagella in specimen A, 26–35 μm; length flagella in specimen B, 27–37 μm; parabasal body coiled around axostyle at least 4 times in specimen A; parabasal body obscured in specimen B; axostyle not protruding from body in either specimen.
Etymology: The genus is based on its superficial morphological similarity to extant species in the family Devescovinidae.
Holotype: Both specimen A and B in accession # B-OR-1C deposited in the Poinar amber collection maintained at Oregon State University.
Locality: Amber mine in the Hukawng Valley, southwest of Maingkhwan, Kachin State (26°20′N, 96°36′E), Myanmar (Burma).
Type host: An Early Cretaceous cockroach of undetermined family.
Comments: The fossil possesses some characters of extant members of the genus *Caduceia*, especially its shape and slender axostyle contained within the cell. While three anterior flagella and a trailing flagellum are characteristic of most devescovinids, the absence of a trailing flagellum, as on the fossil species, is a diagnostic character of the genus *Devescovites*. Possible undigested wood fragments are inside both specimens (Figs. 3 and 5). All extant members of the Devescovinidae occur in termites of the families Kalotermitidae, Hodotermitidae and Mastotermitidae (Kirby, 1942; Brugerolle and Lee, 2000b).

Family unknown
Paleotrichomonis Poinar n. gen.

Diagnosis: Small, slender trichomonadid with a single long anterior flagellum; posterior flagella absent; slender axostyle not protruding from body.
Type species: P. burmanicus n. sp.

P. burmanicus Poinar n. sp.
Figs. 7 and 8

Description: Cell small, length 32 μm, greatest width, 21 μm, anterior flagella longer than body, length, 50 μm; length axostyle, 27 μm; nucleus spherical, 5 μm in diameter.
Etymology: Paleo is from the Greek "paleus" for old and burmanicus refers to the country of origin.
Holotype: Specimen in accession # B-OR-1C deposited in the Poinar amber collection maintained at Oregon State University.
Locality: Amber mine in the Hukawng Valley, southwest of Maingkhwan, Kachin State (26°20′N, 96°36′E), Myanmar (Burma).
Type host: An Early Cretaceous cockroach of undetermined family status.
Comments: Because many of the key characters for trichomonadids are obscured in the fossil (presence and type of basal bodies, type of costa and cresta, etc.) it was not possible to assign the specimen to an extant family and its assignment to the trichonomadids is tentative. While most trichonomadids have 3 anterior flagella, *Histomonas* Tyzzer has only a single flagellum, which is similar to that of the fossil. Extant trichonomadids occur in the guts of a wide range of animals, including termites and cockroaches (Brugerolle and Lee, 2000b).

Class Hypermastigida Grassi & Foá
Order Trichonymphida
Family Burmanymphidae Poinar fam. n.

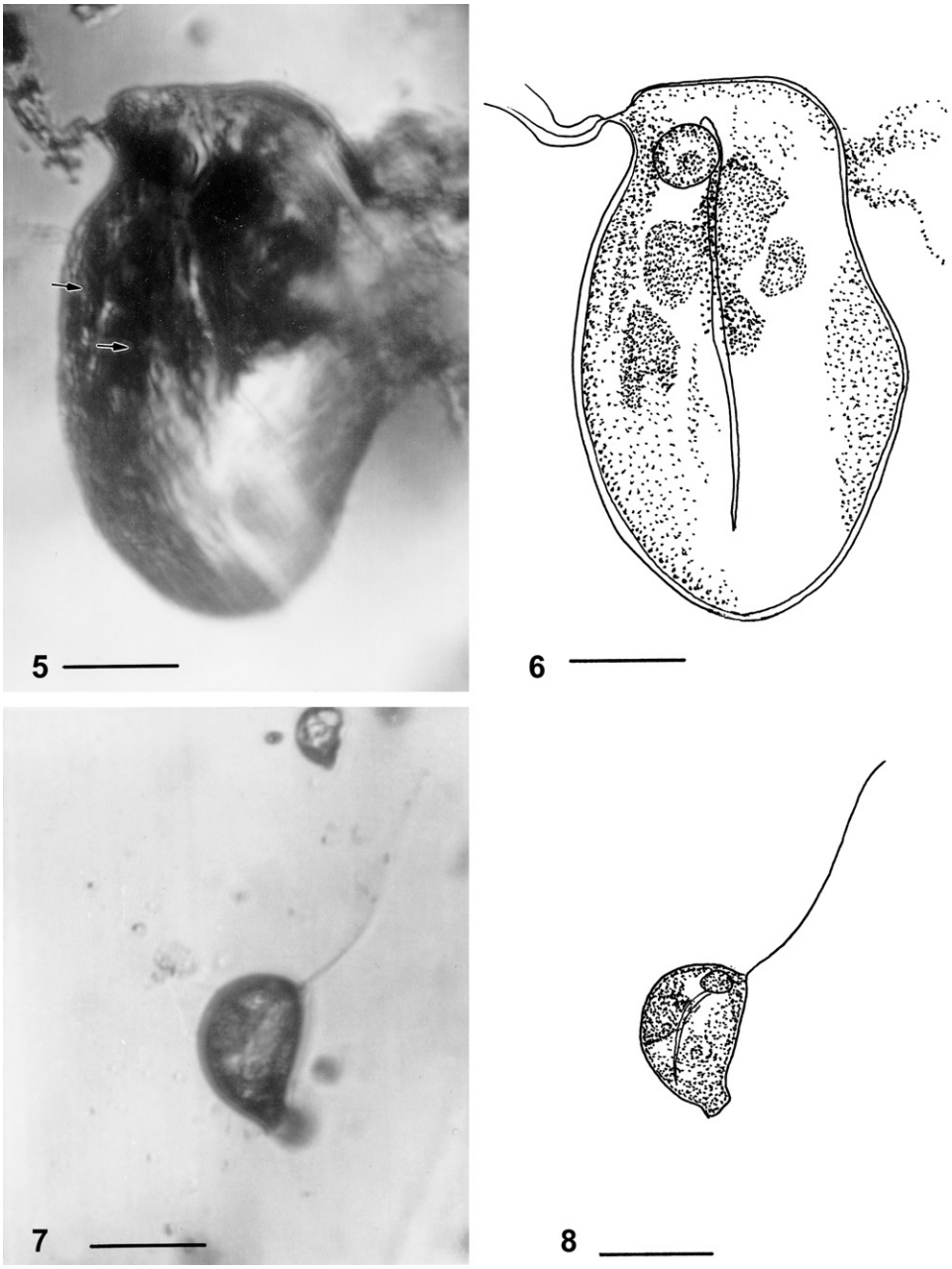
Diagnosis: Body separated into rostral and post-rostral areas; rostrum separated into two bilaterally symmetrical areas; two (possibly 3) axostyles extend from tip of rostrum laterally to base of post-rostral area; rostral flagella as long as entire cell; rostral and post-rostral flagella shorter; nucleus spherical, located in upper half of post-rostral portion of cell; parabasals obscured.
Type genus: *Burmanymphus* Poinar

Burmanymphus Poinar gen. n.

Diagnosis: With characters as described for family.
Type species: *Burmanymphus cretacea* Poinar

Burmanymphus cretacea Poinar, n. sp.
Figs. 9 and 10

Description: Cell oval with acute posterior end; total length, 170 μm; greatest width, 91 μm; length of rostrum (in middle), 25 μm; width of rostrum (at base), 54 μm; diameter of nucleus, 25 μm; length of longest flagellum, 125 μm; length of axostyles, 131 μm and 125 μm.
Etymology: The genus name is based on the locality and the species name on the specimen's age.
Holotype: Specimen in accession # B-OR-1D deposited in the Poinar amber collection maintained at Oregon State University.
Locality: Amber mine in the Hukawng Valley, southwest of Maingkhwan, Kachin State (26°20′N, 96°36′E), Myanmar (Burma).
Type host: An Early Cretaceous cockroach of the family Blattellidae.
Comments: While it is not possible to place this species in any extant group of hypermastigotes, there are some similarities with members of the Trichonymphidae and Spirotrichosomidae, both of



Figs. 5–8. *5, Devescovites proteus* Poinar n. gen., n. sp. (specimen 2). Arrows indicate possible wood particles. Scale 27 μm. 6, Drawing of *Devescovites proteus* Poinar n. gen., n. sp. (specimen 2). Scale 27 μm. 7, *Paleotrichomones burmanicus* Poinar n. gen., n. sp. Scale 21 μm. 8, Drawing of *Paleotrichomones burmanicus* Poinar n. gen., n. sp. Scale 21 μm.

which have representatives in *Cryptocercus* (Ohkuma et al., 2008). Particles resembling undigested wood fragments occur inside the cell (Fig. 9).

Family Oxymonadidae Kirby
Genus *Oxymonas* Janicki

Oxymonas gigantea Poinar, n. sp.
Figs. 14 and 15

Description: Length, 765 μm; greatest width, 300 μm; length rostellum, 311 μm; width at middle of rostellum, 71 μm; holdfast not observed; nucleus oval, length, 153 μm, width, 82 μm; three short fibers attached to base of rostellum; internal fibrous structure extends from tip of rostellum to lower part of cell; broad single axostyle extends from above nucleus to near base of cell; length axostyle, 500 μm.

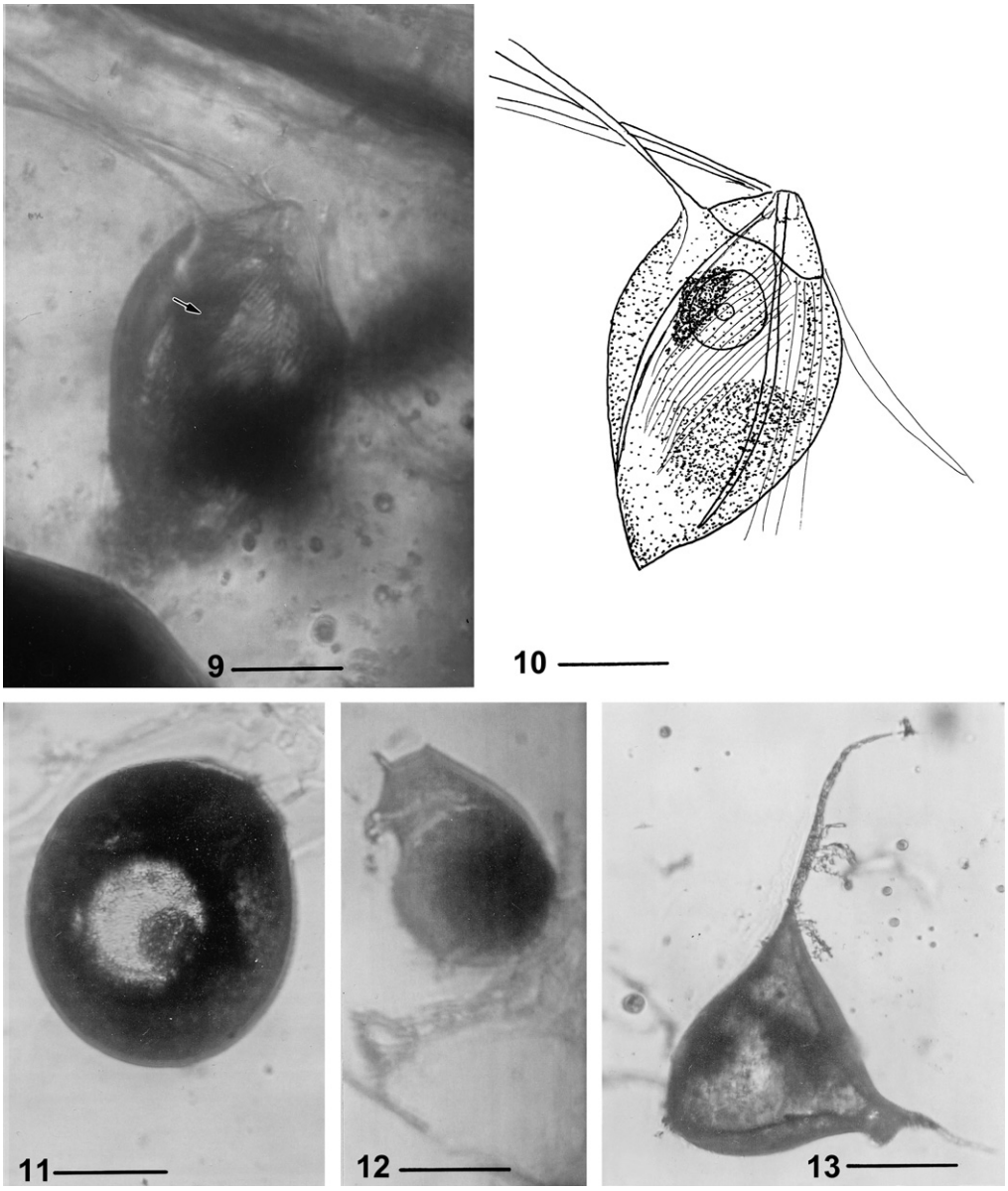
Etymology: The name "gigantea" refers to the extraordinary size of this species.

Holotype: Specimen in accession # B-OR-1D deposited in the Poinar amber collection maintained at Oregon State University.

Type host: An Early Cretaceous cockroach of the family Blattellidae.

Locality: Amber mine in the Hukawng Valley, southwest of Maingkhwan, Kachin State (26°20′N, 96°36′E), Myanmar (Burma).

Comment: The shape of the cell, including its anterior rostellum and uninucleate condition are characters of the extant genus *Oxymonas* Janicki. The most obvious difference separating the fossil from all extant members of the genus, which range from 5 μm to



Figs. 9–13. 9, *Burmanymphus cretacea* Poinar n. gen., n. sp. Arrow indicates possible wood particle. Scale 49 μm. 10, Drawing of *Burmanymphus cretacea* Poinar n. gen., n. sp. Scale 49 μm. 11, Putative protist cyst. Scale 53 μm. 12, Putative protist. Scale 44 μm. 13, Unknown cell. Scale 60 μm.

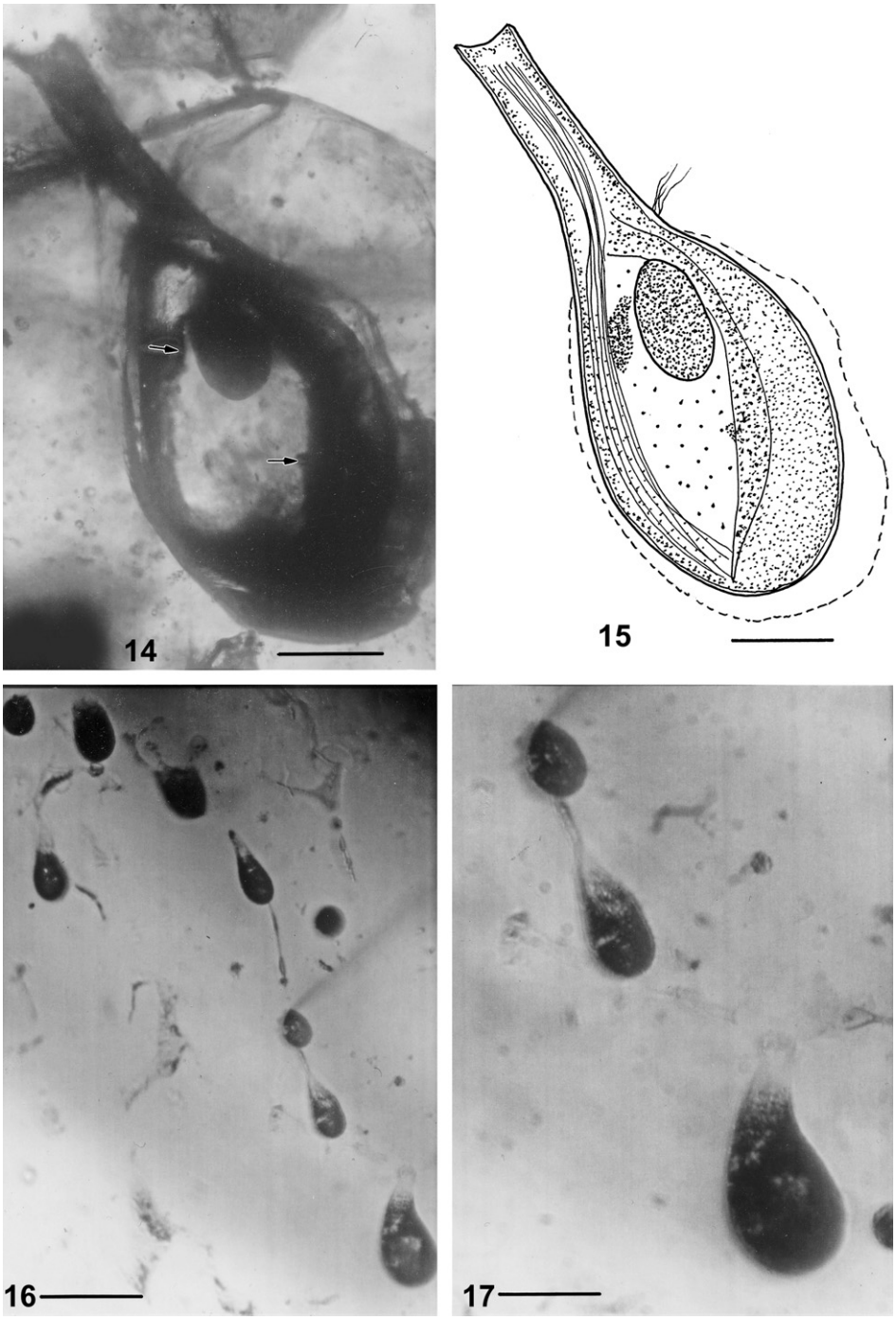
165 μm in length, is its enormous size (765 μm). There are currently some 24 extant species of *Oxymonas*, with 2 species described from *Cryptocercus* cockroaches and the remaining from kalotermitid termites (Brugerolle and Lee, 2000a). Possible undigested wood fragments occur inside the body (Fig. 14).

4. Putative protists

Adjacent to cockroach # B-OR-1C were eight specimens of putative protists ranging in length from 35 μm to 57 μm and in shape from sub-spherical (without a rostellum) to elliptical (with a narrow rostellum) to flask-shaped (with a broad rostellum)(Figs. 16 and 17). Internal structures such as nuclei, fibers, fibroid bundles and axostyles were obscured, so it was impossible to determine if these specimens represent one or more species in the Family Oxymonadidae Kirby. However it is known that the rostellum of oxymonadid species may show various degrees of extension and contraction (Cross, 1946; Brugerolle and Lee, 2000a).

Cockroach # B-OR-1C contained some additional stages that could not be identified. One was a partial putative cyst (Fig. 11) measuring 150 μm in diameter with a well-defined putative spherical nucleus 57 μm in diameter and a putative nucleolus 34 μm in diameter. There was no evidence of flagella, axostyles or parabasals. Only a single wall appeared to be present, which suggests that it may be a partial or pseudocyst rather than a complete cyst. Similar shaped cysts are formed by oxymonids (Grassé, 1952) and hypermastigids (Cleveland et al., 1934).

Another putative protist is shown in Fig. 12. The cell is 102 μm in length and 68 μm in greatest width. The anterior portion was rigid with three short straight projections at both ends and a longer one from the center. The posterior portion was less defined and could be amoeboid. A third unknown cell, shown in Fig. 13 is triangular-shaped, with the sides measuring 181 μm by



Figs. 14–17. 14, *Oxymonas gigantea* Poinar, n. sp., n. sp. Arrows indicate possible wood particles. Scale 112 μm. 15, Drawing of *Oxymonas gigantea* Poinar, n. gen., n. sp. Scale 112 μm. 16, Nine specimens of putative oxymonadids. Scale 46 μm. 17, Three specimens of putative oxymonadids showing range in shape. Scale 46 μm.

204 μm by 260 μm. Two of the corners are extended while the third is bluntly rounded.

5. Discussion

Identification of the fossil Blattellidae (B-OR-1D) was based, in part, on the shape and venation of the tegmen (Fig. 18). The relatively elongate tegmen, a heavy, straight and simple subcosta shorter than the anal field, the undifferentiated R_1 and the radial sector with simple, spaced anterior rami with only the apical ones branched, are all characteristic of the Blattellidae (Rehn, 1951). In addition, the basally fused media and cubitus, the simple, unbranched posterior cubitus that parallels the plial furrow and the distinct, apically subangulate plial furrow as well as an anal area with only a few simple veins, are additional characters of blattellid tegmina (Rehn, 1951). On the basis of internal characters, McKittrick (1964) concluded that the Cryptoceridae was a sister group to the Blattidae and both represented a phylogenetic lineage distinct from that of the Blattellidae.

While it is difficult to determine the lineage of the wingless juvenile (specimen B-OR-1C), it has arolia and pads on the ventral surfaces of tarsomeres 2–4, which are lacking in the fossil blattellid.

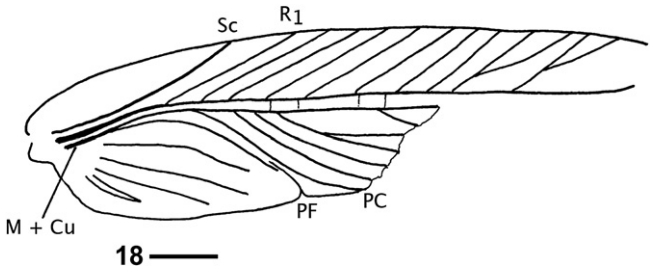


Fig. 18. Venation of tegmen of cockroach of the family Blattellidae (B- OR-1D) in Burmese amber. Scale 1 mm. Sc = subcosta, R_1 = radial vein, M + Cu = fused media and cubitus, PF = plial furrow, PC = posterior cubitus.

Specimen B-OR-1C also differs from *Cryptocercus* because the latter also lacks arolia and pads on the ventral surfaces of tarsomeres 2–4 (Cleveland et al., 1934).

Extant cockroaches contain a diverse assemblage of intestinal microbiota (Klass et al., 2008; Guthrie and Tindall, 1968; Roth and Willis, 1960; Brugerolle and Lee, 2000a,b), however none other than *Crytpocercus* spp. contains mutualists belonging to the Trichonymphida and Oxymonadida. The discovery of representatives of the Trichonymphida and Oxymonadida in lineages of Early Cretaceous cockroaches, neither of which belongs to the cryptocercid clade, is intriguing.

The findings suggest that symbiotic flagellates were more commonly encountered in the environment and among diverse cockroaches in the Early Cretaceous than at present. And if these fossil flagellates are true mutualists as they are in the Isoptera-Cryptocercidae clade today, xylophagous cockroaches may have been more widespread during the Cretaceous. A separate study showed that Early Cretaceous kalotermitid termites already had established an assortment of intestinal mutualistic protists, none of which were identical to those reported in the present study (Poinar, 2009).

It is generally assumed that the mutualistic flagellates of lower termites and cryptocercids were inherited from a common ancestor (Cleveland et al., 1934; Ohkuma et al., 2008). But if this is true, why aren't their descendants found in cockroaches other than *Cryptocercus* today? Granted that not all extant roach species have been examined for gut protists, but certainly most, if not all, of the domestic species have.

It is likely that various flagellates were, and probably still are, acquired, lost and re-acquired continuously by cockroaches. Whether they survived in potential hosts probably depended on their tolerance to the gut habitat, availability and type of food, a benign nature and a means of being transferred from generation to generation. It is likely that the fossil protists were distributed by the ingestion of spores in fecal material since coprophagy is a characteristic of most cockroaches (Bell et al., 2007).

The method of vertical and horizontal transfer of the protists could be the key to their survival in select host taxa. The practice of proctodeal trophallaxis, as exhibited in the subsocial cryptocercids and eusocial termites, is certainly a more secure method of transfer than coprophagy.

Acknowledgements

I thank Roberta Poinar, Christine A. Nalepa, and Art Boucot for discussions and, supplying literature on flagellates of cockroaches and lower termites, and Andrej Gorochov and Leonid Anisyutkin for their opinions on the systematic position of the fossil cockroach B-OR-1D (Blattellidae).

References

Bell, W.J., Roth, L.M., Nalepa, C.A., 2007. Cockroaches, Ecology, Behavior, and Natural History. The Johns Hopkins University Press, Baltimore, pp. 248.
Bignell, D.E., 2000. Introduction to symbiosis. In: Abe, T., Bignell, D.E., Higashi, M. (Eds.), Termites: Evolution, Sociality, Symbioses, Ecology. Kluwer Academic Publishers, Dordrecht, pp. 189–208.
Bobyleva, N.N., 1975. Morphology and evolution of intestinal parasitic flagellates of the Far-Eastern roach Cryptocercus relictus. Acta Protozoologica 14, 109–160.
Brugerolle, G., Lee, J.J., 2000a. Order Oxymonadida. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.) An Illustrated Guide to the Protozoa, second ed., vol. 2. Society of Protozoologists and Allen Press Inc., Lawrence, Kansas, pp. 1186–1195.
Brugerolle, G., Lee, J.J., 2000b. Phylum Parabasalia. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.)An Illustrated Guide to the Protozoa, second ed., vol. 2. Society of Protozoologists and Allen Press Inc., Lawrence, Kansas, pp. 1196–1250.
Brugerolle, G., Lee, J.J., 2000c. Order Diplomonadida. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.)An Illustrated Guide to the Protozoa, second ed., vol. 2. Society of Protozoologists and Allen Press Inc., Lawrence, Kansas, pp. 1125–1135.
Cleveland, L.R., Hall, S.R., Sanders, E.P., Collier, J., 1934. The wood feeding roach Cryptocercus, its protozoa, and the symbiosis between protozoa and roach. Memoirs of the American Academy of Arts and Sciences 17, 185–342.
Cross, J.B., 1946. The flagellate subfamily Oxymonadinae. University of California Publications in Zoology 53, 67–162.
Cruickshank, R.D., Ko, K., 2003. Geology of an amber locality in the Hukawng Valley, northern Myanmar. Journal of Asian Earth Sciences 21, 441–455.
Grassé, P.P., 1952. Ordre des Oxymonadines. In: Grassé, P.P. (Ed.), Traité de Zoologie, vol. 1. Masson et Cie, Paris, pp. 801–823.
Guthrie, D.M., Tindall, A.R., 1968. The Biology of the Cockroach. Edward Arnold Ltd., London.
Honigberg, B.M., 1970. Protozoa associated with termites and their role in digestion. In: Krishna, K., Wiesner, F.M. (Eds.), Biology of Termites, vol. 2. Academic Press, New York, pp. 1–36.
Kirby, H., 1942. Devescovinid flagellates of termites II. The genera Caduceia and Macrotrichomonas. University of California Publications in Zoology 45, 93–166.
Klass, K.D., Nalepa, C., Lo, N., 2008. Wood-feeding cockroaches as models for termite evolution (Insecta: Dictyoptera): Cryptocercus vs. Parasphaeria boleiriana. Molecular Phylogenetics and Evolution 46, 809–817.
McKittrick, F.A., 1964. Evolutionary studies of cockroaches. Cornell University Agricultural Experiment Station Memoir 389, 1–197.
Ohkuma, M., Noda, S., Yuichi Hongoh, Y., Christine, A., Nalepa, C.A., Inoue, T., 2008. Inheritance and diversification of symbiotic trichonymphid flagellates from a common ancestor of termites and the cockroach Cryptocercus. Proceeding of the Royal Society B 276, 239–245.
Patterson, D.J., Rogerson, A., Vørs, N., 2000a. Class Heterolobosea. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.)An Illustrated Guide to the Protozoa, second ed., vol. 2. Society of Protozoologists and Allen Press Inc., Lawrence, Kansas, pp. 1104–1111.
Patterson, D.J., Vørs, N., Simpson, A.G.B., O'Kelly, C., 2000b. Residual free-living and predatory heterotrophic flagellates. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.)An Illustrated Guide to the Protozoa, second ed., vol. 2. Society of Protozoologists & Allen Press Inc., Lawrence, Kansas, pp. 1302–1328.
Poinar Jr., G., 2009. Description of an Early Cretaceous Termite (Isoptera: Kalotermitidae) and its associated intestinal protozoa, with comments on their coevolution. Parasites and Vectors 2, 12. doi:10.1186/1756-3305-2-12.
Poinar Jr., G., Waggoner, B.M., Bauer, U.-Ch., 1993. Terrestrial soft-bodied protists and other micro-organisms in Triassic amber. Science 259, 222–224.
Poinar Jr., G., Lambert, J.B., Wu, Y., 2007. Araucarian source of fossiliferous Burmese amber: spectroscopic and anatomical evidence. Journal of the Botanical Research Institut of Texas 1, 449–455.
Rehn, J.W.H., 1951. Classification of the Blattaria as indicated by their wings (Orthoptera). Memoirs of the American Entomological Society 14, 1–134.
Roth, L.M., Willis, E.R., 1960. The biotic associations of cockroaches. Smithsonian Miscellaneous Collections 141, 1–470.
Schmidt, A.R., Schönborn, W., Schäfer, U., 2004. Diverse fossil amoebae in German Mesozoic amber. Palaeontology 47, 185–197.
Waggoner, B.M., 1993. *Naegleria*-like cysts in Cretaceous amber from central Kansas. Journal of Eukaryotic Microbiology 40, 97–100.