 0014-4754/93/040354-04$1.50 + 0.20

Fossil habrotrochid rotifers in Dominican amber

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Received 9 November 1992; accepted 10 February 1993

Abstract. Flask-shaped microfossils are reported from bracts of a moss in Eocene-Oligocene amber from the northern Dominican Republic. These microfossils are identical with the thecae of certain living moss-dwelling rotifers in the genus *Habrotrocha* (Bdelloidea), which have previously been reported as fossils only from Holocene peat. What may be an egg and a rotifer body fossil are associated with these thecae and further support the identification of these fossils with *Habrotrocha*; the fossils are almost identical to extant *H. angusticollis*. The parthenogenetic bdelloid rotifers have a longer evolutionary history than was previously thought; habrotrochid rotifers seem to have persisted for 35 million years with very little change in morphology or ecological role.
Key words. Habrotrochidae; Bdelloidea; fossil rotifers; Dominican Republic; Eocene-Oligocene

Rotifers, like most 'pseudocoelomate' organisms, have an extremely sparse fossil record; their small size and lack of mineralization give rotifers almost no preservation potential. The exception to this rule is the case of rotifers that build external thecae, which may be preserved in some instances. Thecae, eggs, and body fossils of rotifers have occasionally been reported from Quaternary bog sediments in Europe and North America[1, 2]. The curious '*Pararotifera enigmatica*', described as a 'pseudorotifer' from the Miocene silicified fauna of the Calico Mountains near Barstow, California[3], is almost certainly not a rotifer and is probably artifactual. The only definite pre-Quaternary fossil representatives of the Phylum Rotifera are bdelloids, possibly habrotrochids, from late Eocene amber of the Dominican Republic[4]. These were found associated with a fossil mushroom, *Coprinites dominicana*, lying close to the cap and gills and presumably living in the water film covering the mushroom.

The water film surrounding bryophytes harbors a distinctive rotifer fauna of both motile and sessile forms. A piece of Dominican amber containing a sizable fragment of moss has been found to contain numerous flask-shaped fossils attached to the leaves of the moss. These are identical with the thecae secreted by certain habrotrochid rotifers, especially the extant rotifer *Habrotrocha angusticollis* (Habrotrochidae; Bdelloidea). The fossiliferous amber is designated B-1-4 in the Poinar collection of Dominican amber at the University of California, Berkeley. The piece is roughly quadrangular, measures 30 × 30 × 8 mm, and weighs 7.3 g. Piece B-1-4 originated from the La Toca mine, located in the Cordillera Septentrional between Santiago and Puerto Plata, in the northern portion of the Dominican Republic. Dominican amber may range from Late Eocene to middle Miocene in age, but the La Toca mine is in the Altimira facies of the El Mamey Formation (Upper Eocene, based on coccolith stratigraphy), which is shale-sandstone interspersed with conglomerate of well-rounded pebbles[5]. It is also possible to obtain relative dates of an amber sample by means of NMR spectroscopic measurements of the magnitude of the *exo*-methylene peak. Using as a calibration the minimum 20 to 23 million year age of the Palo Alto mine, obtained from foraminiferal stratigraphy, this method yields tentative absolute dates of Dominican amber samples which are consistent with relative stratigraphic dates. NMR dates of northern Dominican amber range from 15 to 40 million years, with amber from the La Toca mine being among the oldest at 35 million to 40 million years old[6]. This age is confirmed by the independent age of 30 to 45 million years for the La Toca mine, based on coccoliths, reported by Cepek[7]. The fossiliferous amber studied here may thus be assigned confidently to the late Eocene. The source of this amber was a plant in the extant Gondwanian genus *Hymenaea* (Leguminosae; Caesalpinoideae), probably the extinct *H. protera*[8].

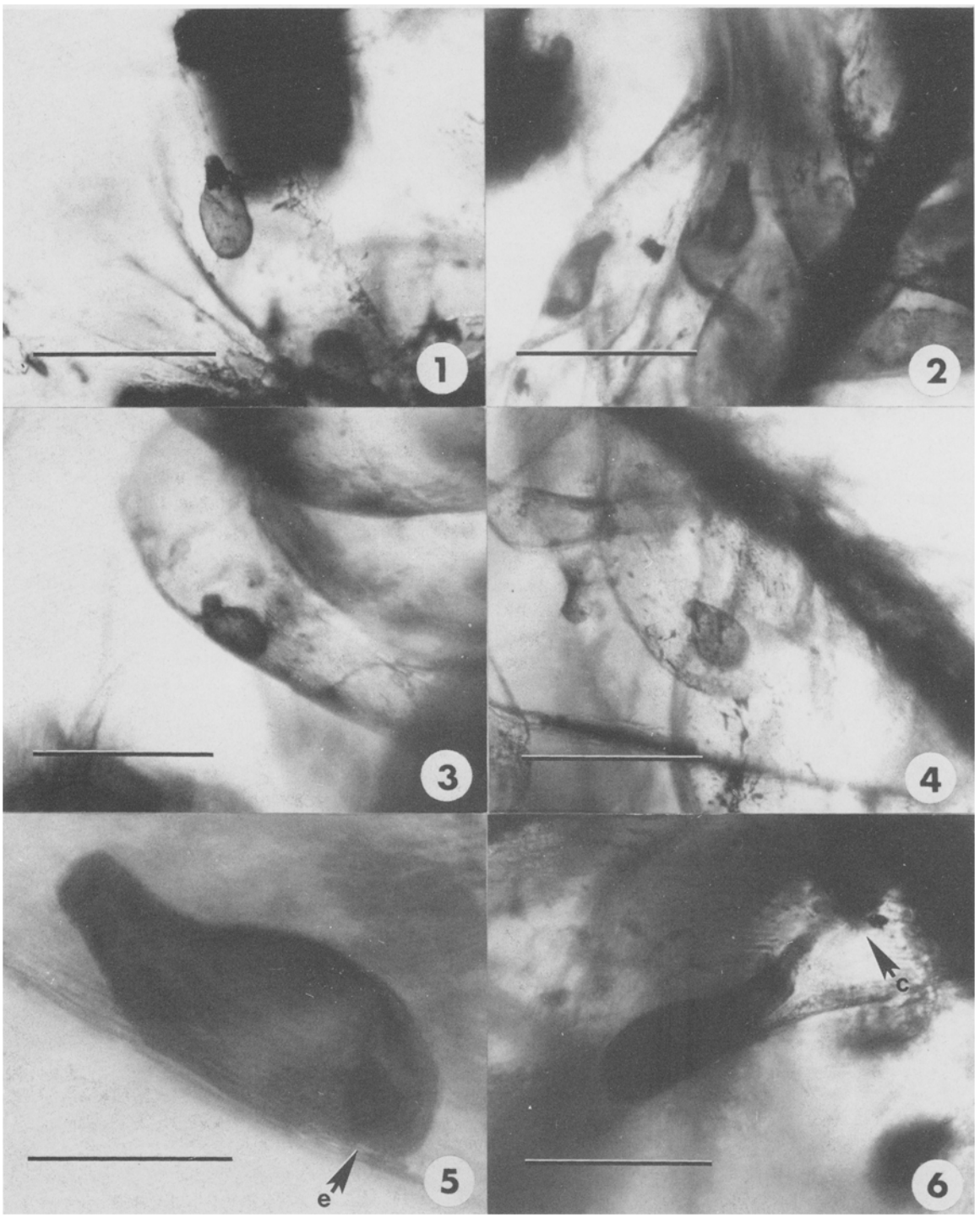
Piece B-1-4 contains a large moss thallus, which has been identified as a representative of the genus *Hypnum*, very close to the extant tropical species *H. amabile* (W. R. Buck, personal communication). It presumably was growing on or very near the trunk of a *Hymenaea* tree. Aside from the moss, the amber piece contains a small mite and two unidentified dipterans close to the moss thallus; the mite presumably lived on the moss, but the dipterans are probably only associated with the moss by chance. Bulbous, flask-shaped structures are visible on the undersides of some of the moss bracts; about 25 were seen in the piece. Although rather variable in morphology, they are generally dark brown, translucent, range from 95 to 135 μm long, and have a more or less bent, slightly constricted neck (fig. 1–6). The sur-

faces are smooth, without agglutinated material or debris; there may be faint longitudinal striations (fig. 5), but this feature is difficult to see. Most of these flask-shaped microfossils appear empty, but one contains a single ovoid structure with a lighter central spot (fig. 5). These structures differ from the fruiting bodies (capsules) produced by *Hypnum*: they are acellular, lack stalks, and are located on the underside of the bracts, not in the axils. All specimens are attached to moss bracts in the same orientation; this rules out testate amoebae, which may superficially resemble these thecae and are also common in mosses. These thecae are therefore interpreted as the thecae of an epibiont on the moss. They superficially resemble thecae made by certain ciliates such as *Folliculina*; however, *Folliculina* is generally marine, and folliculinid thecae generally have an extended, flattened ventral surface plane ('cementing edge') and often a long, annulated neck, which the fossil thecae lack. The only freshwater member of *Folliculina*, *F. bolteni*, has a lorica smaller and flatter than the fossils described here[9]. Freshwater peritrichous ciliates in the genus *Platycola* also make similar loricae; *Platycola* loricae, however, are frequently smaller and more flattened than the fossils, and they have both a 'cementing edge' and a partial coating of $Fe(OH)_3$, a more pointed posterior end, and often a much more shortened and flared neck compared to these fossil thecae[9,10]. The thecae are practically identical with those made by extant moss-dwelling bdelloid rotifers in the genus *Habrotrocha* (Bdelloidea; Habrotrochidae) (fig. 7)[1,11]. The ovoid structure visible inside one of the thecae is interpreted as an egg (fig. 5), which may be laid inside the theca in *Habrotrocha*[12]. One of the flask-shaped structures seems to contain a small cylindroid body that looks like a rotifer body fossil extending from the neck of the theca (fig. 6). Overlying debris and light diffusion by the amber make it difficult to identify, but what may be an extended corona and body are visible. In overall size and shape it resembles a living *Habrotrocha*; coronal details are hard to see, but the fossil seems to lack the cuticular ring on the wheel pedicels of *Otostephanos* and the dorsal plate of *Scepanotrocha*, the two other extant genera in the Habrotrochidae[13]. In any case, *Habrotrocha* is the only genus in the Habrotrochidae in which smooth translucent thecae are found[13,14]. The genus *Habrothrocha* has many species, but fewer than ten secrete smooth thecae like this[13,14]. Of these, the closest living rotifer to the fossil is *H. angusticollis*, probably the most common, most widespread, and best-known thecate habrotrochid; the fossil thecae all fit into its rather wide range of morphological variation[13] (fig. 8). However, although the thecae of extant *H. angusticollis* are 'rather diagnostic'[2], description of these fossils at the species level is inappropriate until better-preserved body fossils are found.

Thecae attributed to *Habrotrocha* have previously been reported from *Sphagnum* peats in Ontario dated at 2400 and 4600 years before present[1], and from peat in the Netherlands dated from 1700 to 6200 years before present[2]. This find extends the fossil record of the genus back to the late Eocene. The rotifers previously reported from Dominican amber could not be identified with certainity, since the corona and foot were retracted, but they were tentatively referred to the genera *Habrotrocha* or *Scepanotrocha* (Habrotrochidae) on the basis of cuticular ornamentation patterns and the presence of loose, rough sheaths of mucus and debris[11,13]. Although the previously described fossil rotifers from Dominican amber cannot be equated with those that built these thecae, there certainly is a precedent for the existence and success of habrotrochid bdelloids in the Eocene Dominican Republic environment.

While these flask-shaped thecae are the most common microfossils in the amber piece, very few contained any possible traces of the rotifers themselves, with the exception of one egg and one body fossil. This is not too surprising, as thecate bdelloid rotifers can and do leave their thecae 'at will'[11], presumably when conditions become unfavorable. The absence of rotifers themselves might suggest a drying out of the moss just before its fossilization in resin, or else the rotifers might have been able to get away from the noxious resin itself. This might also explain the apparent absence in the amber piece of any other rotifers and of other moss microfauna such as tardigrades and nematodes. Modern *Habrotrocha* is most common in wet mosses, especially *Sphagnum*, where the moisture content is above 90%[1]. These fossil thecae indicate a similar moisture content for the Eocene moss microenvironment, which is supported by paleoecological reconstruction of the Dominican amber forest as analogous to a tropical rainforest[15] and by the recent find in Dominican amber of a testate amoeba, *Cyphoderia*, typical of aquatic vegetation and moist mosses[16]. The fossils are unevenly dispersed throughout the moss, being absent from some areas and clumped in others; this may reflect an uneven distribution of moisture within the moss. However, extant moss-dwelling bdelloid species are not specific to species of moss[13], with the possible exception of two species of *Habrotrocha*, *H. reclusa* and *H. roeperi*, which live inside end cells of *Sphagnum* and may or may not be parasitic[17]. The fossil *Habrotrocha* thecae do not appear to have been made by rotifers parasitic on *Hypnum*, and the species of *Habrotrocha* responsible for the fossil thecae was probably a non-obligate commensal.

This find presents further corroborative evidence for parthenogenetic continuity over evolutionary time. It is theoretically possible that obligate parthenogenesis is a recent development in rotifers, but our evidence suggests this is not the case. With the exception of seisonids, which are epizooic on crustaceans, male rotifers are always smaller and anatomically simpler than females, when they are present at all[11,12]. Male-type



Figures 1–4. Fossil *Habrotrocha* thecae on moss bracts, showing range of morphological variation (bar = 200 μm). Figure 5. Closeup of one of the thecae in figure 1. Note ovoid inclusion inside theca, interpreted as an egg (bar = 50 μm). Figure 6. Closeup of a theca with a possible rotifer body fossil protruding from the aperture (bar = 100 μm). c, corona.

rotifers are not present in either this sample or in the other known instance of fossil rotifers in Dominican amber[4]. This does not absolutely rule out seasonal or induced production of males for these fossil bdelloids (as is common in other orders), but reviews of fossil evidence for animal behavior show that the behavior of fossil animals generally remains constant, including mode of sexuality and fertilization, at least to the level of the family[18]. It therefore seems reasonable to suggest that fossil bdelloids reproduced parthenogenetically[4]. The near identity of these fossil thecae with the thecae of a modern species suggests that bdelloid rotifers have

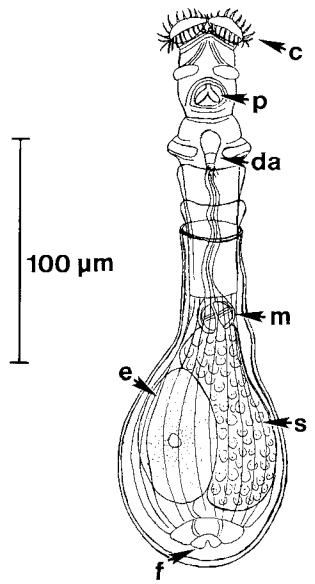


Figure 7. Living *Habrotrocha angusticollis*. c, corona; p, proboscis; da, dorsal antenna; m, mastax; s, syncytial stomach; e, egg; f, foot. (After Hyman[12] and Warner and Chengalath[1]).

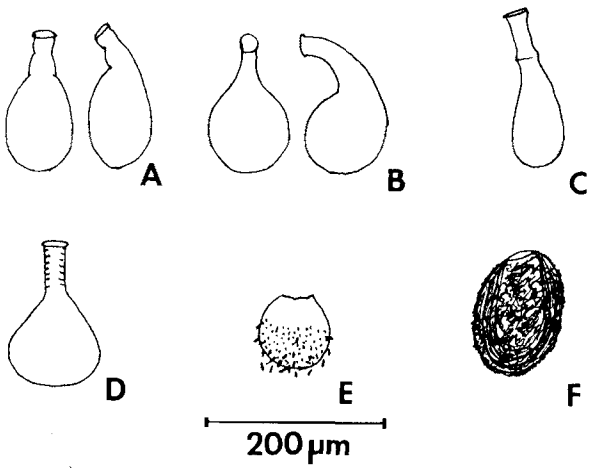


Figure 8. Thecae of various extant *Habrotrocha* species, drawn for comparison. A) *Habrotrocha angusticollis*, dorsal and lateral views. B) *H. angusticollis reversa*, dorsal and lateral views. C) *H. augusticollis attenuata*, lateral view. D) *H. ampulla*. dorsal view. E) *H. eremita*, dorsal view. Dots and lines are bacterial epibionts. F) *H. pusilla*, dorsal view. (After Donner[14] and Bartos[13]).

changed very little since the late Eocene. Parthenogenetic reproduction may be the primary reason for this stasis, since one of the consequences of loss of sexual reproduction is thought to be a reduction in variability[19].

While most parthenogenetic species of animals and plants have close sexual relatives and are probably evolutionarily short-lived, the bdelloid rotifers are 'something of an evolutionary scandal' in being a large, speciose high-level taxon composed entirely of obligate parthenogens[19]. Dominican rotifer fossils suggest that the parthenogenetic bdelloids have existed with little change for at least 35 million years; parthenogenetic rotifers are not evolutionary 'dead ends' that arose only recently. Habrotrochid bdelloids, at least, have some mechanism to avoid or compensate for the accumulation of deleterious mutations and loss of variability that would result from obligate parthenogenesis[4,19].

The rarity of rotifer fossils seems strange; although one would not expect them to be common, it might be expected that more would have been found in peat and lacustrine deposits. Based on their known occurrence, fossil habrotrochid thecae may be more common and widespread than previously suspected. Other rotifers of all classes construct various types of thecae which might preserve as fossils, or else have chitinous loricae or trophi. We hope that awareness of their occurrence brings about the discovery of more fossils of this important group.

Acknowledgements. We thank J. H. Lipps and H. Schorn for the use of photographic facilities, and two anonymous reviewers for their helpful comments. This is University of California Museum of Paleontology Contribution #1591.

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