4 Nieuwkoop, P. D., and Faber, J., Normal Table of *Xenopus laevis*. North-Holland Publishing Co., Amsterdam 1967.
5 Brun, R. B., and Garson, J., J. Embryol. exp. Morph. *74* (1983) 275.
6 Dale, L., Smith, J. C., and Slack, J. M. W., J. Embryol. exp. Morph. *89* (1985) 289.
7 Dale, L., and Slack, J. M. W., Development *99* (1987) 551.
8 Sudarwati, S., and Nieuwkoop, P. D., Wilhelm Roux Arch. devl Biol. *166* (1971) 204.
9 Smith, J. C., Development *105* (1989) 677.
10 Sokol, S., and Melton, D. A., Nature *351* (1991) 411.
11 Mitrani, E., Ziv, T., Tomsen, G., Shimoni, Y., Melton, D. A., and Bril, A., Cell *63* (1990) 501.
12 Jones, E. A., and Woodland, H. R., Development *101* (1987) 563.

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Bdelloid rotifers in Dominican amber: Evidence for parthenogenetic continuity

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Abstract. Recently discovered representatives of the Class Bdelloidea from Tertiary amber from the Dominican Republic represent the oldest known fossils of the Phylum Rotifera. Assuming that the fossil bdelloids had a similar mode of reproduction as present day members of the Class (apomictic thelytoky), then contrary to current thought, some lines of parthenogenetic organisms are not doomed to an early extinction and have evolved built-in mechanisms for genetic diversity.
Key words. Fossil rotifers; Bdelloidea; Tertiary amber; Dominican Republic.

Representatives of the Phylum Rotifera are poorly represented in the fossil record, being known only from the Holocene (8000 years and younger)[1]. A piece of amber from the Dominican Republic was found to contain a population of 22 rotifers as well as another organism that could be a rotifer but cannot be assigned with certainty to this or any other Phylum. At least 18 of the 22 rotifers could be assigned to the Order Bdelloida. All of these animals were associated with the cap of the fossil gilled mushroom *Coprinites dominicana* Poinar and Singer[2]. The total fossil assemblage represents a palaeobiocoenosis or 'fossil community'.

The amber containing the fossils originated from the La Toca mine, located between Santiago and Puerto Plata in the Cordillera Septentrional of the Dominican Republic. This mine is in the Altimira facies of the El Mamey Formation (upper Eocene), which is shale-sandstone interspersed with a conglomerate of well-rounded pebbles[3]. Differences in the magnitudes of absorption peaks in nuclear magnetic resonance spectra of the exomethylene group of amber[4] from different mines in the Dominican Republic were used to calibrate the ages of the various mines, with the age (20 million to 23 million years); (based on foraminifera counts) of the Palo Alto mine used as a standard[5]. The ages of various pieces of Dominican amber ranged from 15 million to 40 million years with that from the La Toca mine being the oldest, some 35 million to 40 million years old (lower Oligocene to upper Eocene). This age is within the independent dating reported by Cepek[6] who gave a range of 30 to 45 million years for "La Toca" mine.

The piece of amber containing the fossils (AF-9-11) had all the visual characteristics of natural Dominican amber. A series of chemical and physical tests[7] performed on a small portion of the amber piece verified that it was authentic. The piece of yellow transparent amber containing the rotifers weighed 0.5 g and was elliptical in shape, 9 mm long and 6 mm wide. It is deposited in the Poinar collection of Dominican amber maintained at the University of California, Berkeley.

All of the bdelloid rotifers were contracted so that the corona and foot were unrecognizable. Because it was impossible to observe the number of toes, the stomach lumen and the shape of the trochi and the mastax, it was not possible to place them in any existing family with certainty. The size of the bdelloid rotifers ranged from 126 to 504 μm in length (av = 266 μm) and from 76 to 200 μm in width (av = 149 μm) (N = 18). The specimen shown in figure 1 (L = 360 μm; W = 189 μm) shows the tail incompletely extended and covered with small spines arranged in parallel files (fig. 2). As can be seen from a portion of the trunk surface that is free from detritus, the trunk was also covered with transverse rows of posteriorly directed small spines (fig. 3). Similar patterns of cuticular ornamentation occur in representatives of the genera *Habrotrocha* and *Scepanotrocha* of the family Habrotrochidae and *Macrotrachela* and *Rotaria* of the family Philodinidae.

Figure 4 shows the specimen that could also be a rotifer but does not resemble any extant forms. It is unique in the foot and trunk possessing large spines hinged at their bases. Although a few bdelloids have trunk spines, none

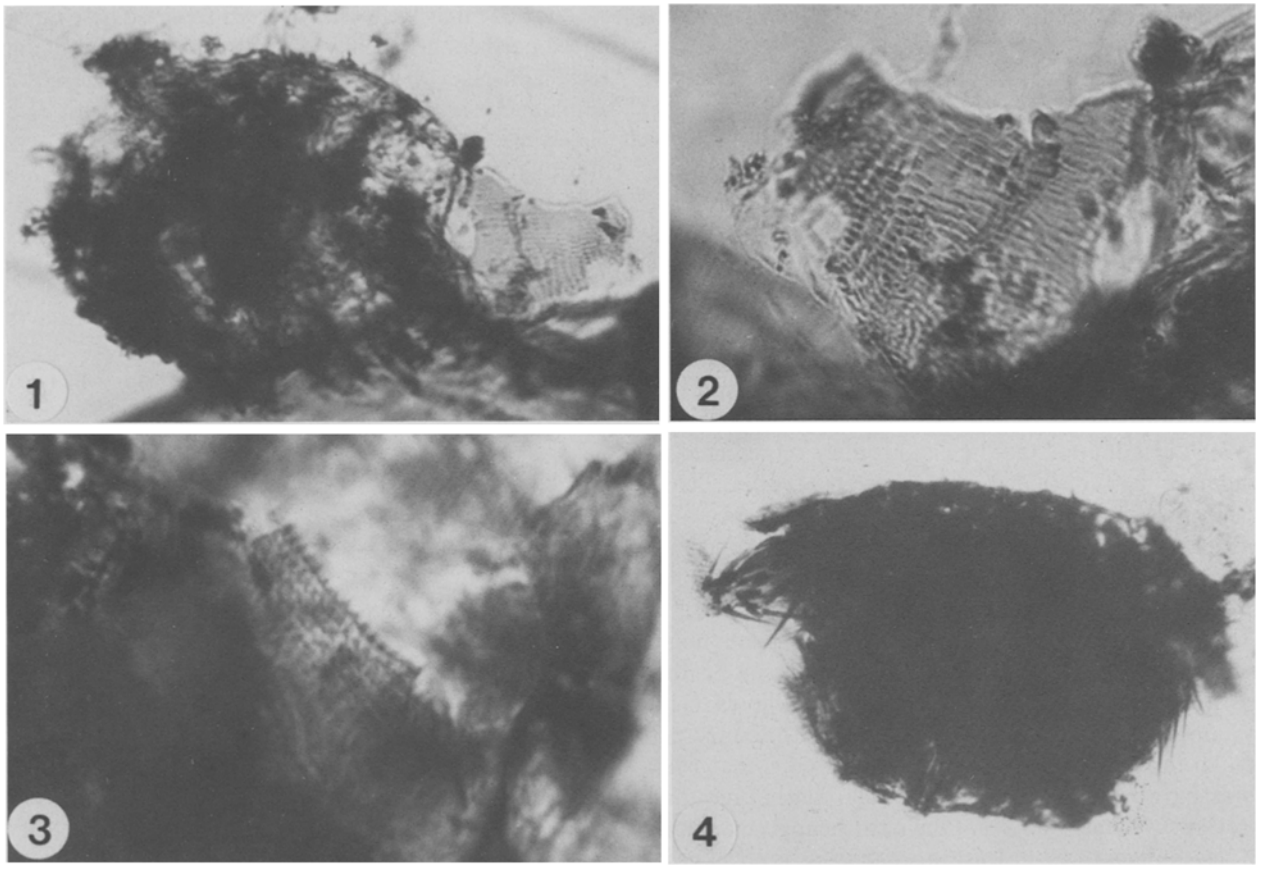

Figure 1. Lateral view of a fossil bdelloid rotifer in Dominican amber. A large portion of the trunk is covered with debris, probably adhering to a mucous deposit, which extant bdelloids are known to secrete.
Figure 2. Close up of the tail region of the fossil bdelloid rotifer shown in fig. 1.
Figure 3. Detail of the trunk ornamentation of the bdelloid rotifer shown in fig. 1.
Figure 4. Unknown organism, which may be an extinct or a yet undiscovered type of rotifer.

has spines that are hinged as appear in this fossil specimen. Its length is 388 μm; the partially expanded foot is 87 μm long and the foot spines vary from 40 to 50 μm. The spines appear to have been movable through a range of 180° as suggested by their present positions. The spines could have been used for attachment to or for removing organic particles from a substrate.

Bdelloid rotifers are ubiquitous animals that occur in fresh water, soil and the water films that cover terrestrial bryophytes[8]. Although none has been reported from the surface of mushrooms, this moist habitat would be adequate for bdelloid development. Two of the 22 representatives present were lodged between the gills of *Coprinites dominicana*. The remainder were just under the gills or adjacent to the cap. Because the original habitat was tropical, it is likely that at least the undersurface of the cap was covered with a film of moisture, allowing the rotifers to move about between the gills. In this location the fossil forms probably fed on microorganisms and organic particles (possibly fungal spores), similar to the type of food utilized by extant bdelloid rotifers[9].

Bdelloid rotifers as a class[10] differ from other rotifer classes in two major respects[8]. One of these is their ability to undergo anhydrobiosis, which allows them to colonize favorable habitats during wet periods and then enter a quiescent desiccated stage when moisture is unavailable. Such a pattern of existence would agree with temporary development on the surface of mushrooms in a humid climate followed by survival in a desiccated state during dry spells between periods of mushroom emergence.

The second character that distinguishes bdelloids from other rotifers is their obligatory parthenogenetic mode of reproduction[8]. In fact, the bdelloids are unique in the animal kingdom in being the only Class that is entirely obligately parthenogenetic. There are two other classes in the Phylum Rotifera. Members of the Class Seisonidea are amphimictic whereas representatives of the Class

Monogonanta exhibit cyclical parthenogenesis where asexual reproduction predominates but sexual reproduction can occur[11]. In parthenogenetic reproduction, the reliability of producing new generations takes precedence over obtaining genetic variation inherent in sexual reproduction. Parthenogenesis occurs in certain members of the closely related Phylum Nematoda and exists mainly in individuals living in relatively stable environments and therefore not requiring high degrees of genetic variability within populations[12]. Bdelloids do not fit that category, however, because the terrestrial forms are in a constantly changing environment. Maynard-Smith stated that 'a group which does wholly abandon sexual reproduction has a limited evolutionary future'[13]. Although he admitted that bdelloid rotifers represented an anomaly he concluded that, despite the bdelloids, parthenogens are doomed to an early extinction because of lack of evolutionary potential. Other authors have stated that the entire loss of meiotic sexuality has not been tolerated[14].

Up until now, it appeared that these statements might well have been correct even for the bdelloids since with a fossil record of less than 8000 years[1], the bdelloids might be considered a relatively recent group with limited potential for survival. We now know, however, that bdelloids have existed for a considerably longer time than previously thought and that their parthenogenetic mode of development has, at least in some lines, been successful. After extensively reviewing the fossil record for evidence of behavior and coevolution, Boucot[15] concluded that the data show that, on the basis of functional morphology, the behavior of fossil animals, including the mode of fertilization and sexual development, remains constant. He reported that it is reasonable to assume that the behavior of fossil species will be the same as extant forms, almost always down to the taxonomic level of family and certainly at the higher categories. Therefore we conclude that the fossil bdelloids reported here reproduced parthenogenetically. Of course the possibility that certain lineages of fossil bdelloid rotifers possessed an amphimictic or cyclic type of reproduction cannot completely be ruled out. However, the large body of data attesting to the behavioral fixity of fossil organisms does not support this contention. Success of the parthenogenetic bdelloids could possibly be explained by possession of a very plastic genotype, as suggested by Lynch[16] or by still unknown means of exchanging genes as speculated by Maynard-Smith[13].

1 Warner, B. G., and Chengalath, R., J. Paleolimnology *7* (1988) 141.
2 Poinar, G. O. Jr, and Singer, R., Science *248* (1990) 1099.
3 Eberle, W., Hirdes, W., Muff, R., and Pelaez, M., The Geology of the Cordillera Septentrional, pp. 619–632. Proc. Ninth Caribb. Geol. Conf., Santo Domingo, August 1980.
4 Lambert, J. B., Frye, J. S., and Poinar, G. O. Jr, Archaeometry *27* (1985) 43.
5 Baroni-Urbani, C., and Saunders, J. B., The Fauna of the Dominican Republic Amber: The Present Status of Knowledge, pp. 213–223. Proc. Ninth Caribb. Geol. Conf., Santo Domingo, August 1980.
6 Schlee, D., Das Bernstein-Kabinett. Stuttg. Beitr. Naturk., Ser. C No. 28, 1990.
7 Poinar, G. O. Jr, Amber – true or false. Gems Miner. *534* (1982) 80.
8 Ricci, C., Hydrobiologia *147* (1987) 117.
9 Hyman, L. H., The Invertebrates, vol. III, McGraw-Hill Book Co., New York 1951.
10 Nogrady, T., Synopsis and Classification of Living Organisms, vol. 1, McGraw-Hill Book Co., New York 1982.
11 Wallace, R. L., and Snell, T. W., Rotifera, in: Ecology and Classification of North American Freshwater Invertebrates, pp. 187–248. Eds J. H. Thorp and A. P. Covich. Academic Press, New York 1991.
12 Poinar, G. O. Jr, and Hansen, E., Helminthological Abstr., Series B, *52* (1983) 145.
13 Maynard-Smith, J., The Evolution of Sex. Cambridge Univ. Press, Cambridge 1978.
14 Margulis, L., and Sagan, D., Origins of Sex. Yale Univ. Press, New Haven 1986.
15 Boucot, A. J., Evolutionary Paleobiology of Behavior and Coevolution. Elsevier, Amsterdam 1990.
16 Lynch, M., Q. Rev. Biol. *59* (1984) 257.

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A potent attractant of zoospores of *Aphanomyces cochlioides* isolated from its host, *Spinacia oleracea*

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Abstract. A highly potent attractant of zoospores of *Aphanomyces cochlioides*, a causal fungus of the root rot disease of spinach (*Spinacia oleracea*), was isolated from spinach roots, and its structure was determined by spectroscopic evidence and chemical synthesis as cochliophilin A (5-hydroxy-6,7-methylenedioxyflavone, **1**). A chromosorb particle prepared by soaking in solution of **1** showed a potent attracting activity toward the zoospores using concentrations of **1** above 10^{-9} or 10^{-10} M.
Key words. Aphanomyces cochlioides; *Spinacia oleracea*; zoospore; attractant; cochliophilin A.