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Cylindroiulus truncorum (Silvestri): A New Milliped for Virginia (USA), with Natural History Observations (Julida: Julidae)

Jorge A. Santiago-Blay

Department of Paleobiology, MRC-121
National Museum of Natural History
10th and Constitution Avenue
Smithsonian Institution
P.O. Box 37012
Washington, DC 20013-7012

Richard L. Hoffman

Virginia Museum of Natural History
Martinsville, Virginia 24112

Joseph B. Lambert and Yuyang Wu

Department of Chemistry
Northwestern University
2145 Sheridan Road
Evanston, Illinois 60208-3113

INTRODUCTION

In the fall 2000, author SB cleared the underbrush of an Eastern White Pine (*Pinus strobus* L.) grove in his backyard located in an urban area of Salem, Virginia (USA) by cutting and removing the lower branches. About a year later, he revisited the same trees and noticed copious resinous exudations originating from the branch stumps, particularly on five of the trees. There, he observed about twenty millipeds, later identified as *Cylindroiulus truncorum* (Silvestri, 1896; species group reviewed by Korsós & Enghoff, 1990), attached to the resin, 1-2 meters above ground (Fig. 1). Voucher specimens of *Cylindroiulus truncorum* are deposited at the Virginia Museum of Natural History (Martinsville, VA).

This common, soil-dwelling Palearctic milliped has not been documented for Virginia although it was recorded at Colorado Springs, Colorado (Chamberlin, 1923 as *Diploiulus truncorum*) and, subsequently, from hothouses in more northern states of the United States (Chamberlin & Hoffman, 1958). There is also one record from St. John's, Newfoundland, Canada (Palmén, 1952; Shelley, 1988). The report of *C. truncorum* for Raleigh, North Carolina, about 320 km SSE of Salem (Shelley, 1978) is the southernmost known occurrence of this species in the United States. This milliped has also been documented for Brazil (Chamberlin & Hoffman, 1958; Hoffman, 1999).

Natural History Observations

Berlese extractions from *P. strobus* leaf litter were conducted in November 2001 and yielded a maximum of about 50 *C. truncorum* per 0.25 m^2 (= 200 *C. truncorum* per m^2). In his many years of studying soil invertebrates and running numerous Berlese samples, particularly in southwestern Virginia, RLH has seldom encountered millipeds under pine litter. Subsequent Berlese extractions were run from mid-December 2001 to mid-February 2002 with generally disappointing results, although the 2001-2002 winter was unusually mild. Other millipeds found in *P. strobus* litter were the julidans *Blaniulus guttulatus* (Fabricius, 1798) (Blaniulidae) and *Ophyiulus pilosus* (Newport, 1843) (Julidae), as well as the polydesmid *Polydesmus superus* (Latzel, 1884), all introduced Palearctic millipeds. Only individuals of *C. truncorum* were found

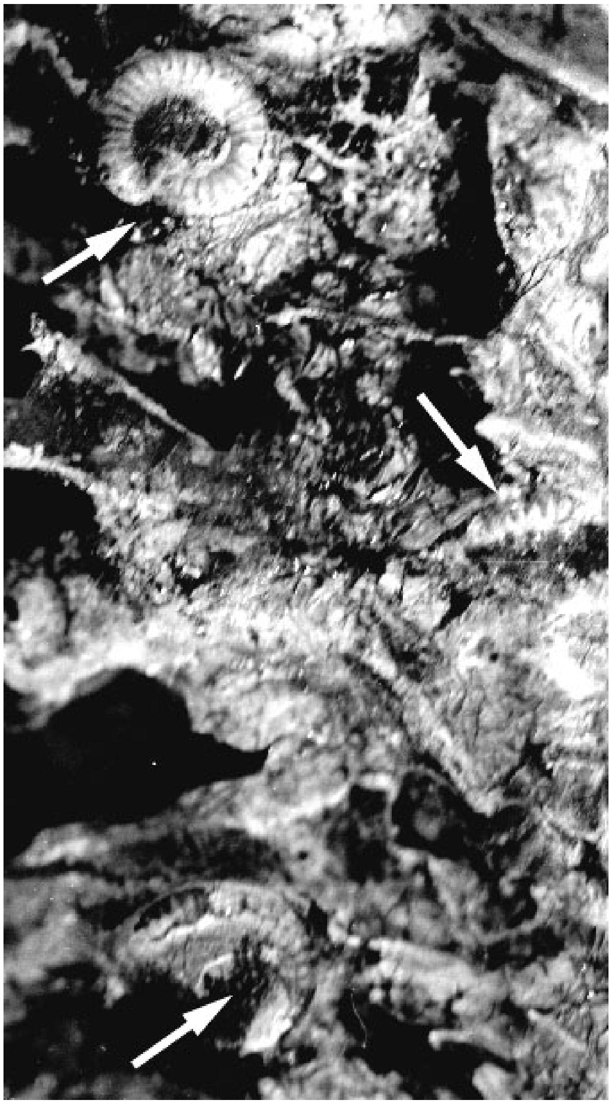

Fig. 1. Three *Cylindroiulus truncorum* (arrows) attached to *Pinus strobus* resin.

in *P. strobus*; this species was not observed in non-resinous parts of *P. strobus* trunks (almost all observations occurred during daylight). In addition, no *C. truncorum* was found on other nearby exudate-bearing trees 15-30 m from the *P. strobus* grove, including one *Ailanthus altissima* (Mill.) Swingle (tree-of-heaven, Simaroubaceae) and one *Picea pungens* Engelm. (blue spruce, Pinaceae). Interestingly, three *C. truncorum* were found adhered to resin in one *Picea abies* (L.) Karst. (Norway spruce, Pinaceae).

The presence of a monospecific sample of millipeds on two confamilial resin-producing tree genera (*Pinus* and *Picea*) at first suggested to us that a long-distance chemical attractant may be responsible for this behavior. Long-distance communication has been reported for millipeds (Hopkin & Read, 1992), but milliped attraction to plant resins apparently has never been reported. We devised two experimental approaches to test whether there is positive chemotaxis of *C. truncorum* to the resin: artificial "trees", in nature, and "cafeteria style" resin preference tests, in the laboratory. In both cases, the null hypothesis (H_o) was that there is no difference between a milliped getting and not getting immersed in the resin. The probability of a significant difference favoring the resin is any p value ≤ 0.05.

The artificial trees consisted of small (generally 11.3 x 1.0 x 0.2 cm) polished pieces of wood, such as those used to stir paint or as handles of frozen desserts (Economy Craft Sticks, Forster, Inc., Wilton, Maine). The "trees" were generously coated with exudate from *P. strobus*, *P. abies*, or rubbed with relatively dry *P. pungens* resin; a fourth "tree" was left uncoated and served as a presumed negative "tree" control. A total of 20 artificial trees was planted on 9 February 2002, 3-4 cm deep into the soil. The artificial trees were placed about 25 cm away from five conspicuously resinous *P. strobus* trunks, in groups of four (approximately equally spaced, one tree per exudate treatment and a control). Reciprocal experiments were not performed on the other neighboring exudate-bearing trees because the number of millipeds on those trees was much lower (or zero). Hence, we assumed that *C. truncorum* soil populations were smaller than in the grove's litter. After 21 days, no millipeds were found attached to the artificial trees.

In the cafeteria-style resin preference tests, small (about 0.5 cm diameter) pieces of resin were placed in uncovered glass Petri dishes (9.5 cm in diameter). All millipeds used were retrieved from freshly collected *P. strobus* leaf litter; only clearly moving *C. truncorum* were used for the experiments. All tests were allowed to run for 15-30 min and the position of the millipeds with respect to the resin was recorded at the end of each test. Individuals of *C. truncorum*, which tend to walk close to the walls of the Petri dish (thigmotaxis), were tested only once. In one set of preliminary experiments, resins from *P. strobus*, *P. abies*, and *P. pungens* were placed close (< 0.5 cm) to the walls of the Petri dish. In these experiments, two of the three tested millipeds became glued to the *P. strobus* resin. We suspected that *C. truncorum* were trapped on *P. strobus* resin because of their proximity to the resin and not necessarily because of positive resin chemotaxis. Consequently, we devised two more tests designed to reduce the effects of thigmotaxis by placing the resin farther away from the wall of the Petri dishes. When *P. strobus* resin was placed approximately 3.0 cm from the wall of the Petri dishes, none of 12 millipeds was found attached to the resin (Sign Test, $p < 0.0005$, http://fonsg3.let.uva.nl/Service/Statistics/Sign_Test.html, Sokal & Rohlf, 1995). When *P. strobus*, *P. abies*, and *P. pungens* resins were placed in the same Petri dish with a blank control, only 4 of 12 millipeds were found adhered on any of the resins, always *P. strobus* (Sign test, $p = 0.39$). Hence, we reject the hypothesis of positive resin chemotaxis for *C. truncorum*.

Sakwa (1974) showed that some simple sugars, at times made available in nature through microbial action, can be phagostimulatory to some European millipeds, including two species of julids. Using the carbon-13 isotope solid state nuclear magnetic resonance spectroscopy (C-13 SS NMR; Lambert et al., 1999; Lambert & Poinar, 2002; Lambert et al., 2002), authors JBL and YW analyzed a *P. strobus* resin sample from one of the trees where *C. truncorum* were immersed in the resin. In this technique, a pulverized sample is exposed to a strong magnetic field making all magnetic nuclei, such as carbon-13, absorb energy, or resonate. Normally, each chemically distinct carbon nucleus in the molecules resonates at a different frequency. In the spectrograph, the horizontal axis is a measure of the magnetic interactions between any particular atom in the molecule and the magnetic field of the experiment. These interactions are interpreted in terms of the chemical bonds to the atom. Many different carbon atoms in the resin molecules experience different interactions with the magnetic field and hence fall at different frequencies along the horizontal axis. The units of delta on this axis are parts per million (ppm) of the magnetic field, which is slowly changing as the experiment evolves to place each kind of carbon at the resonance frequency. The vertical axis is a measure of the intensity of the interaction and generally is determined by the number of each specific type of carbon atom in the resin molecules. A sample of adamantane is run to calibrate the horizontal axis, then the unknown sample is run. The final spectrum has no direct dependence on the adamantane sample, other than the numbers on the horizontal axis. For instance, carbon atoms bonded to two oxygen atoms (O-C-O), characteristic of the anomeric carbon of carbohydrates (sugars), resonate at about 100 ppm. The absence of this peak from the spectrum of the *P. strobus* sample indicates that there are no detectable sugars in our sample (Fig. 2). Interestingly, the C-13 SS NMR shows peaks at about 70 ppm that are interpreted as possible alcohols. In laboratory observations, millipeds glued to *P. strobus* resin were not feeding on it and they appeared to be struggling to escape from the resin.

We infer that *C. truncorum* move up and down the trees, like other arthropods such as alticine (Coleoptera: Chrysomelidae) larvae (N. Virkki, pers. comm.). After the original field observations were completed, author SB collected other julids, probably *Cylindroiulus* sp. (identified by RLH), on other resin-producing conifers [*Chaemacyparis nootkatensis* (D. Don) Spach (specimen number 58-377-A) and *C. lowsoniana* (A. Murray bis) Parl. (specimen number 54-1647-A) (Cupresaceae), collected on 29 March 2002 at the

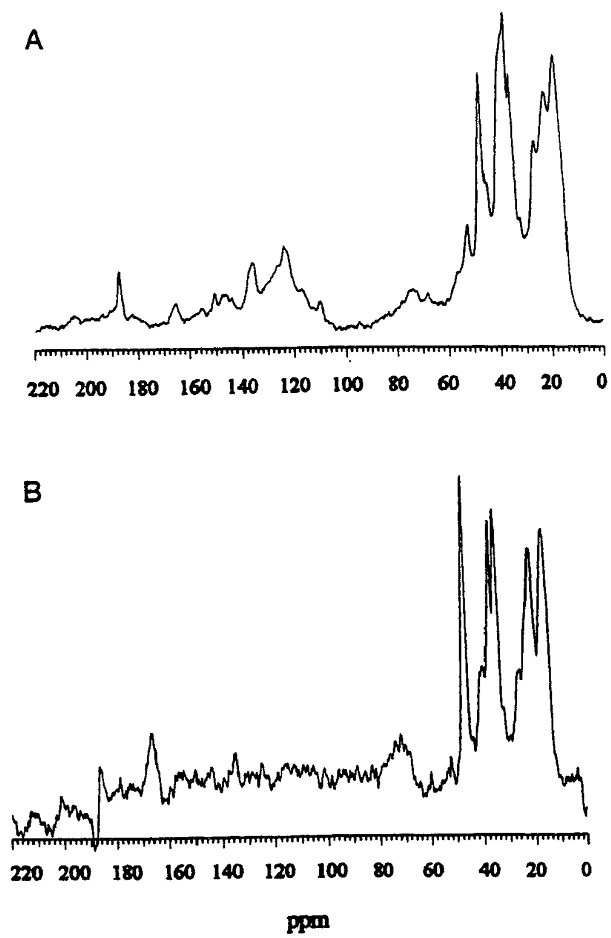

Fig. 2. Spectra of *Pinus strobus* resin using C-13 Solid State Nuclear Magnetic Resonance Spectroscopy. Note the absence of peaks representing the anomeric carbon (O-C) of sugars at about 100 ppm and the presence of peaks at about 70 ppm suggesting the presence of alcohols. A. Normal Decoupling. B. Interrupted Decoupling.

Holden Arboretum, Kirtland, Ohio] suggesting that this phenomenon is widespread. Voucher specimens of the probable *Cylindroiulus* sp. and of the *C. nootkatensis* resin are deposited in the Department of Entomology at the California Academy of Sciences (San Francisco). The causes of this behavior remain unknown although movement of julids, including circadian vertical movements (Haacker, 1967) as well as yearly movements of *C. truncorum* within the soil (Geoffroy & Célérier, 1996), but not on trees, have been reported. Other species of *Cylindroiulus* have been reported climbing trees on Madeira (Portugal) (Enghoff, 1983). Some millipeds, such as *Cutervodesmus adisi* Golovatch (Polydesmida: Furmannodesmidae), move from the soil to trees as a survival strategy to escape flooding (Adis et al., 1996), which is clearly not the situation in the case of the *C. truncorum* from Salem (VA).

Exudate production of resins, gums, latexes, and other substances is widespread in plants. Such substances have been shown, or suspected, for at least 510 genera in 137 vascular plant families (Santiago-Blay et al., 2002; Santiago-Blay, unpublished ongoing compilation). Natural entombment of organisms in modern exudates, especially resins, may be a useful model to better understand fossilization in ancient plant materials, such as solidified resin or amber. First, the exudates need not fall on organisms for entombment to occur. Herein, we have reported observations that only seem interpretable by inferring haphazard walking of the millipeds towards the resin and their inability to free themselves. Second, after about only one year, some of the millipeds were almost totally covered by an already hardened resin, suggesting that fossilization may be faster than previously suspected (Poinar, 1992). Situations like the ones described in this paper may represent the "fossils of the future" (C. Mauffe, pers. comm.). Nevertheless, the time needed for full resin polymerization remains unknown (Langenheim, 1995). Third, our observations add to the data that demonstrates the biotic bias of the amber fossil record. For example, assuming that the current abundance of individuals of different subfamilies of the Chrysomelidae is reasonably similar to that in the ancient Dominican amber forest (some 20-40 Ma; Poinar, 1992), eumolpine leaf beetles are the most commonly found chrysomelids in amber, while other leaf beetle subfamilies are disproportionately less represented (e.g., Santiago-Blay, 1994).

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LITERATURE CITED

Adis, J., S. I. Golovatch, & S. Hamann. 1996. Survival strategy of the terricolous millipede *Cutervodesmus adisi* Golovatch (Fuhrmannodesmidae, Polydesmida) in a blackwater inundation forest of Central Amazonia (Brazil) in response to the flood pulse. Pp. 523-532 *In* J.-J. Geoffroy, J.-P. Mauriès, & M. Nguyen Duy-Jacquemin (eds.), Acta Myriapodologica. Memoirs du Muséum National d'Histoire Naturelle. 169. Paris, France.

Chamberlin, R. V. 1923. An Algerian julid in America. Proceedings of the Biological Society of Washington 36: 191-192.

Chamberlin, R. V., & R. L. Hoffman. 1958. Checklist of the millipeds of North America. Bulletin 212. United States National Museum. Smithsonian Institution, Washington, DC. 236 pp.

Enghoff, H. 1983. Adaptive radiation of the millipede genus *Cylindroiulus* on Madeira: habitat, body size, and morphology (Diplopoda, Julida: Julidae). Revue d'Ecologie et de Biologie du Sol 20: 403-415.

Geoffroy, J.-J., & M.-L. Célérier. 1996. Cycles d'activité comparés de populations de diplopodes édaphiques dans un écosistème forestier tempéré. Pp. 533-554 *In* J.-J. Geoffroy, J.-P. Mauriès, & M. Nguyen Duy-Jacquemin (eds.), Acta Myriapodologica. Memoirs du Muséum National d'Histoire Naturelle. 169. Paris, France.

Haacker, U. 1967. Tagesrhythmische Vertikalbewegung bei Tausendfüßlern (Myriapoda, Diplopoda). Naturwissenschaften 54: 346-347.

Hoffman, R. L. 1999. Checklist of the Millipeds of North & Middle America. Special Publication Number 8, Virginia Museum of Natural History, Martinsville, VA. 584 pp.

Hopkin, S. P., & H. J. Read. 1992. The Biology of Millipedes. Oxford University Press, Oxford, England. 233 pp.

Korsós, Z., & H. Enghoff. 1990. The *Cylindroiulus truncorum*-group (Diplopoda: Julidae). Entomologica Scandinavica 21: 345-360.

Lambert, J. B., & G. O. Poinar, Jr. 2002. Amber: the organic gemstone. Accounts of Chemical Research 35: 628-632.

Lambert, J. B., C. E. Shawl, G. O. Poinar, Jr., & J. A. Santiago-Blay. 1999. Classification of modern resins by solid state nuclear magnetic resonance spectroscopy. Bioorganic Chemistry 27: 409-433.

Lambert, J. B., Y. Wu, & J. A. Santiago-Blay. 2002. Modern and ancient resins from Africa and the Americas. Pp. 64-83 *In* K. A. Jakes (ed.), Archeological Chemistry. Materials, Methods, and Meaning. ACS Symposium Series 831, American Chemical Society, Washington, DC.

Langenheim, J. H. 1995. Biology of amber-producing trees: focus on case studies of *Hymenaea* and *Agathis*. Pp. 1-31 *In* K. B. Anderson & J. C. Crelling (eds.), Amber, Resinite, and Fossil Resins. ACS Symposium Series 617, American Chemical Society, Washington, DC.

Palmén, E. 1952. Survey of the Diplopoda of Newfoundland. Annales Zoologici Societatis Zoologicae-Botanicae Fennicae "Vanamo" (Helsinki, Finland) 15: 1-31.

Poinar, G. O., Jr. 1992. Life in Amber. Stanford University Press. Palo Alto, CA. 350 pp.

Sakwa, W. N. 1974. A consideration of the chemical basis of food preference in millipeds. Pp. 329-346 *In* J. G. Blower (ed.), Myriapoda. Symposia of the Zoological Society of London. Number 32. The Zoological Society of London. Academic Press. 712 pp.

Santiago-Blay, J. A. 1994. Paleontology of leaf beetles. Pp. 1-68 *In* P. Jolivet, E. Petitpierre, & M. L. Cox (eds.), Novel Aspects of the Biology of Chrysomelidae (Coleoptera). Series Entomologica (Bari). Kluver Academic Publishing. Dordretcht, The Netherlands.

Santiago-Blay, J. A., J. B. Lambert, & Y. Wu. 2002. Chemical analyses of fossil and modern plant exudates using C-13 SSNMR. P. 428 *In* Geological Society of America. Annual Meetings and Exposition Abstracts. Science at the Highest Level. October 27-30, 2002. Denver, CO. (Abstract).

Shelley, R. M. 1978. Millipeds of the eastern Piedmont region of North Carolina, U.S.A. (Diplopoda). Journal of Natural History 12: 37-39.

Shelley, R. M. 1988. The millipeds of eastern Canada (Arthropoda: Diplopoda). Canadian Journal of Zoology 66: 1638-1663.

Sokal, R. R. & F. J. Rohlf. 1995. Biometry: the Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, New York, NY. 887 pp.