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A living bdelloid rotifer from 24,000-year-old Arctic permafrost

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In natural, permanently frozen habitats, some organisms may be preserved for hundreds to tens of thousands of years. For example, stems of Antarctic moss were successfully regrown from an over millennium-old sample covered by ice for about 400 years[1]. Likewise, whole campion plants were regenerated from seed tissue preserved in relict 32,000-year-old permafrost[2], and nematodes were revived from the permafrost of two localities in northeastern Siberia, with source sediments dated over 30,000 years BP[3]. Bdelloid rotifers, microscopic multicellular animals, are known for their ability to survive extremely low temperatures[4]. Previous reports suggest survival after six to ten years when frozen between –20° to 0°C[4–6]. Here, we report the survival of an obligate parthenogenetic bdelloid rotifer, recovered from northeastern Siberian permafrost radiocarbon-dated to ~24,000 years BP. This constitutes the longest reported case of rotifer survival in a frozen state. We confirmed the finding by identifying rotifer actin gene sequences in a metagenome obtained from the same sample. By morphological and molecular markers, the discovered rotifer belongs to the genus *Adineta*, and aligns with a contemporary *Adineta vaga* isolate collected in Belgium. Experiments demonstrated that the ancient rotifer withstands slow cooling and freezing (~1°C min^{-1}) for at least seven days. We also show that a clonal culture can continuously reproduce in the laboratory by parthenogenesis.

Samples were collected in the middle reaches of the Alazeya River in northeastern Siberia (Russian Federation, 69.338889°N, 154.996944°E; Figure S1A) at a depth of 3.5 m below ground surface employing core extraction and sample processing techniques that prevented contamination from upper layers. The core contained ice-rich loam from the Late Pleistocene Yedoma formation (also called the Ice Complex). The shape, good development and wide distribution of ice wedges, and occasional finding of well-preserved mammal mummies support syncryogenetic formation of the Ice Complex, i.e. that layers of sediments were frozen relatively quickly after their formation and have never melted[7]. Parallel studies from several laboratories have shown that 2 μm particles, the size of a bacterium, cannot move through ice or ice-cemented ground[8–10]. Thus, no significant vertical movement could have occurred in the studied sediments, and therefore the isolated microbes were likely trapped in permafrost at the same time as the radiocarbon-dated organics. The Accelerator Mass Spectrometry (AMS) analysis dated the material as 23,960–24,485 years old (calibrated age of low-temperature combustion humin fraction, σ 95%; University of Arizona AMS Laboratory, sample AA109004).

Initial cultures from the permafrost core were maintained for about one month and yielded, among other microscopic organisms, numerous living rotifers (Figure 1A and Video S1). Several secondary rotifer cultures were established, each from a single individual, as bdelloids reproduce by obligate parthenogenesis. Specimens

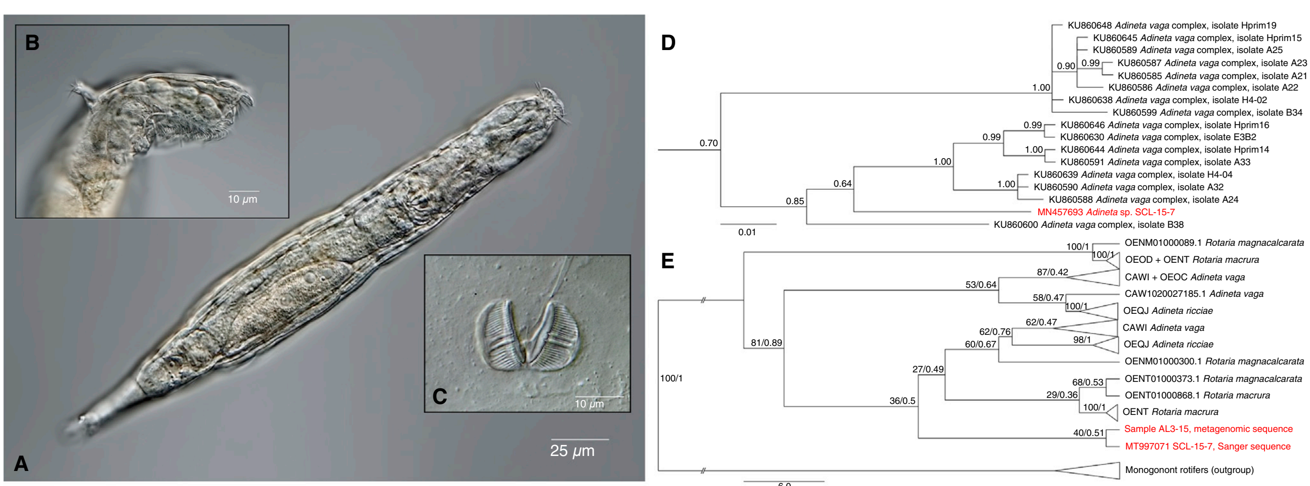

Figure 1. *Adineta* sp. isolated from permafrost, site of sampling, results of bioinformatics and experimental procedures.
(A) *Adineta* sp. from permafrost, total view of an active individual. (B) Lateral view of the head. (C) Trophi. (D) COX1 phylogenetic tree for *Adineta* sp., a fragment of 50 percent majority rule consensus tree from Bayesian analysis showing the closest genetic relatives of strain SCL-15-7 (numbers indicate branch posterior probabilities, branch lengths proportional to the average number of substitutions per site under a GTR+I+G model). (E) Bayesian tree of actin gene fragments from all available bdelloid contigs, the sequence from AL3/15 metagenome, and the Sanger sequence from the living culture. Maximum likelihood tree (not shown) gave the same topology. Triangles join several contigs from the projects indicated by accession numbers. Numbers are bootstrap percentage/posterior probability. (A), (B), and (C) taken on a Canon Eos 600-D APS-C camera under a modified Reichert Diastar microscope with Zeiss planapo DIC optics and custom-built flash. Images were post-processed in Adobe Photoshop ® for contrast and color balance.

from the secondary culture SCL-15-7 were then used for all further analyses. Morphological features linked the ancient rotifer to the genus *Adineta* (Figure 1A–C). The identical COX1 gene sequences from three individuals (GenBank: MN457693) were compared against available COX1 sequences of contemporary *Adinetae* using Bayesian phylogenetic analyses combined with GMYC species delimitation. Molecular phylogenetic analysis corroborated the morphological attribution (Figure 1D) and demonstrated that clonal culture SCL-15-7 constitutes a new species of the cryptic species complex *Adineta vaga* (described elsewhere). BLAST alignment showed that the discovered *Adineta* sp. has the closest affinity to *A. vaga* collected in Belgium (*A. vaga* complex, isolate Hprim14, GenBank: KU860644; maximal total z-score = 907, E = 0.0, identity 96.37%).

To confirm that rotifers originated from the permafrost core, we searched for their sequences in a metagenome obtained from the same core fragment (NCBI SRA: SRR13615827). Our analysis demonstrated the presence of an actin gene fragment (two paired reads) belonging to a bdelloid rotifer. The same fragment was Sanger-sequenced from live rotifers (GenBank: MT997071). Due to ambiguous bases in both Sanger and metagenomic sequences (Supplemental information), they were not identical, yet demonstrated closer proximity to each other than to any contigs in the NCBI Genome database (Figure 1E). Most of the nucleotide differences were also shared by different scaffolds of certain *Adineta* genome assemblies, suggesting the existence of different actin paralogues. As only one pair of reads was found in the metagenome, the analyzed fragment was covered just once, necessarily capturing one gene variant. These results strongly suggest that the isolate originated from the permafrost layer and not sample contamination.

To follow the process of freezing and recovery of the ancient rotifer, we randomly selected 144 individuals from the strain SCL-15-7 and froze them at –15°C for one week. Surviving individuals were counted one hour post-thawing, and the process of recovery was documented for one individual per plate (Video S1). Data on survival of the ancient *Adineta* sp. were compared to those of contemporary *Adineta* species from Svalbard, Alaska, Western and Southern Europe, tropical Asia and Africa, and North America (10 species, 404 individuals in total), frozen using the same protocol (Figure S1G). Not all analyzed *Adineta* species survived the chosen regime of freezing (Supplemental information). The ancient *Adineta* sp. was not significantly more freeze-tolerant than contemporary species (Generalized linear mixed-effects model (GLMM) of the ‘Species’ effect: AIC/AIC_{null} 502.17/497.15, p = 0.25), including the strain from Alaska (X^2 = 0.63587, df = 1, p = 0.4252). The difference in freeze resistance between *Adineta* species from the Arctic and other regions was also not significant (GLMM of the ‘Region’ effect (‘Arctic’ or ‘Temperate’) with the ‘Species’ effect considered random: AIC/AIC_{null} 500.29/499.15, p = 0.36). Notably, the ancient *Adineta* sp. was more freeze resistant than its genetically closest relative *Adineta* cf. *vaga* SP1, although the difference was marginal (X^2 = 2.8572, df = 1, p = 0.091). Clearly, the ancient rotifer is capable of surviving a relatively slow freezing process that allows ice crystals detrimental for cells to form (the duration of complete freezing of a well with a rotifer 45 ± 4 min). In combination with its occurrence in permafrost, this suggests that the discovered *Adineta* sp. has effective biochemical mechanisms of organ and cell shielding necessary to survive low temperatures. Our discovery is of interest not only for evolutionary biology but also for practical purposes of cryobiology and biotechnology.

SUPPLEMENTAL INFORMATION

Supplemental information includes experimental procedures, one figure and one video and can be found with this article online at https://doi.org/10.1016/j.cub.2021.04.077.

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