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Microarthropod Voucher Specimens

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Strategic importance

Microarthropods – predominately mites and their relatives – directly contribute to sustainable forest productivity. They are vital in decomposition processes, nutrient cycling and soil formation. These small organisms release nutrients through the primary breakdown of organic matter and consequently are fundamental to forest productivity. An estimated 10,000 species of microarthropods in more than 700 genera and 250 families occur in Canada (Figure 1). As most are microscopic, their large numbers and diversity are often overlooked in forest management (Figure 2). Although the ecological roles of many microarthropods are unknown, they are nevertheless important indicators of use to foresters and biologists in understanding forest ecosystem biodiversity and productivity. (Biodiversity includes the variability within species, i.e., genetic diversity, the variability between species, and the variety of ecosystems.)

Consistent and reliable identifications are essential for understanding the diversity of microarthropods. When samples are poorly collected, prepared or mounted, or when voucher collections are not deposited in accessible locations for future reference, research studies do not contribute fully to increasing our knowledge of microarthropod diversity. Forestry professionals, students and researchers can help build and improve the availability and scope of these valuable collections, and their associated ecological information, by following the techniques described below.

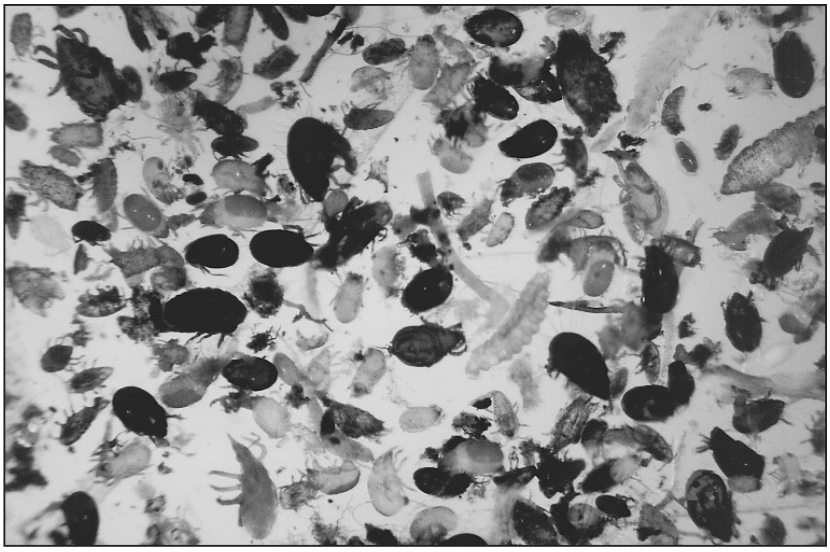

Figure 1. In a typical temperate forest canopy sample such as this, Oribatid mites represent more than 60% of the total microarthropod fauna.

Studying microarthropods

The microscopic size and large numbers of individuals and species, in conjunction with incomplete taxonomic knowledge, make the study of microarthropods difficult. There are few complete keys to North American species and only a small percentage (53% of the microarthropod and 15% of the mite fauna) have been described (named) to date. Consequently, a comparative approach to species identifications, using benchmark or voucher specimens that have been reliably identified, is essential. This approach also allows the tracking and comparison of spatial, temporal and ecological data across landscapes and between diverse studies (e.g. ecosystem disturbances due to forest practices and changing environmental conditions).

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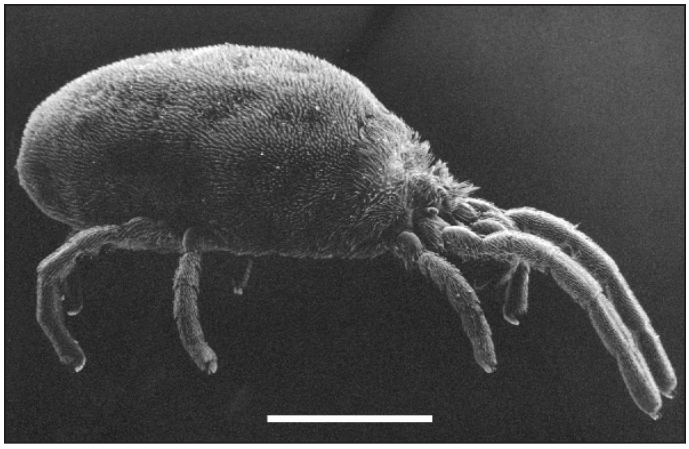


Figure 2. Red velvet mites (Prostigmata) are among the giants of the mite fauna and can often be seen hunting on the ground or on tree trunks (bar = 1000 µm).

Voucher collections typically consist of at least three or four good microscopic slide mounts of each new and previously described species found in a study and all pertinent collection and ecological data associated with each specimen. These vouchers should document the range of habitats, morphological variations, life stages, and seasonal occurrences.

A reliably identified voucher collection serves as a benchmark or standard to which ecologists, students and foresters can compare subsequent specimens and make future identifications. These collections also allow retroactive re-evaluations of species identifications when taxonomic knowledge changes, thus offering the opportunity to continually validate completed research. If a species is "split" (into two or more species or sub-species) or synonymized with another species, the specimens are available for re-identification and no data is lost. Consequently, it is important to have each voucher specimen properly prepared (Figure 3).

Collecting fauna samples

The substrate dictates sampling methods and collection techniques:

- Soil — use a soil corer.
- Sand — dig and put in pails or bags.
- Bark, lichens, mosses, and litter — pick by hand and place in a plastic bag.
- Branches — bag, hand cut and lower or carry to the ground.

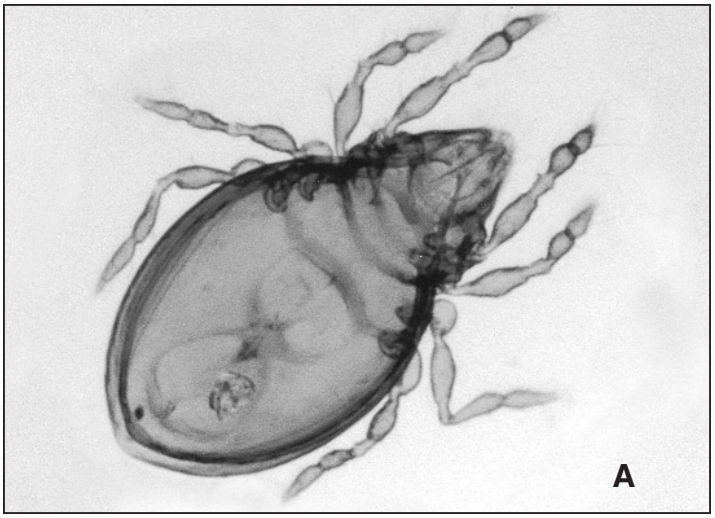
Keep all samples as intact as possible without compacting or squashing them. As most microarthropods have a low tolerance for light, heat and desiccation, keep samples cool and out of direct sunlight. Store between 4 and 5°C until the specimens can be extracted (up to 5-7 days).

Three basic methods are available for separating microarthropods from the substrate:

1. Active extraction techniques (e.g., heat and light extractors such as the modified Lussenhop or Berlese funnels) are suitable for soils, litter and some moss mats;
2. Washing is suitable for branches, lichens and mosses; and
3. Heptane flotation is suitable for sandy soils and other substrates low in organic matter.

Collecting microarthropods from canopies

Forest canopy habitats contain complex micro-ecosystems that extend over large areas. These aerial habitats include the bark of branches and stems, needle surfaces, cones, mosses, lichens and accumulated debris. The organisms that inhabit them are largely unstudied.



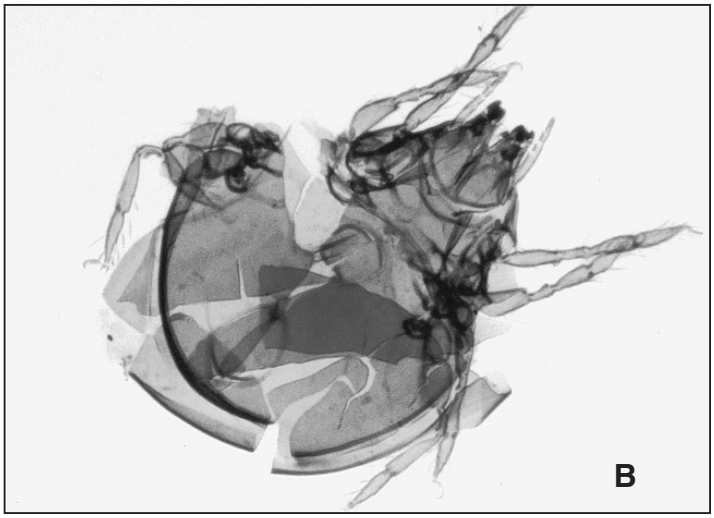
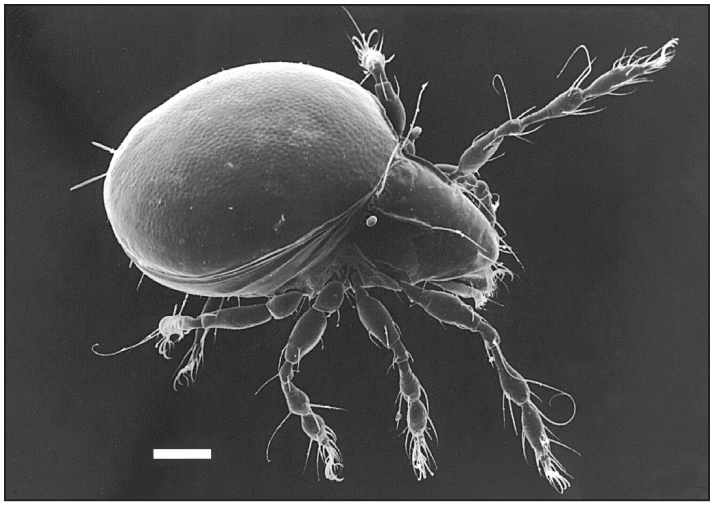


Figure 3. Poorly prepared microscope specimens are more difficult to identify than properly prepared ones. A. proper mount: **Cultroribatula** *sp.; B. crushed specimen:* **Sphaerozetes** *sp.*



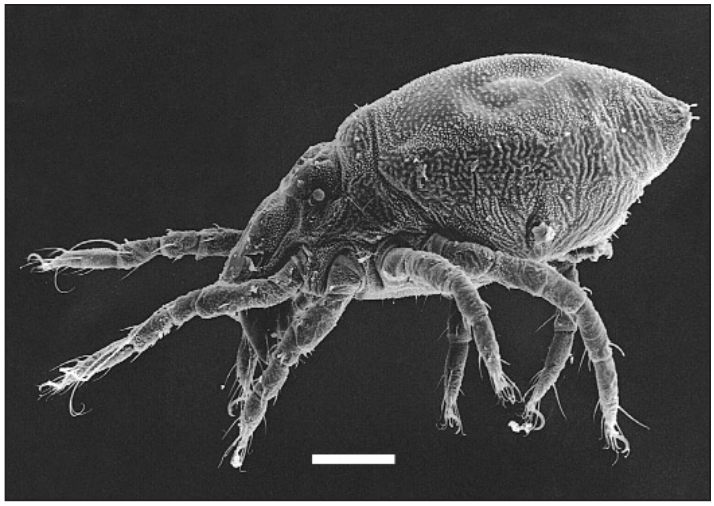


Figure 4. The immature stage (l) of an Oribatid species often has significantly different characteristics from the adult form (r) as seen in this **Dendrozetes n. sp.** *(bar = 100 µm)*

Oribatid mites make up more than 60% of the microarthropod life in temperate forest canopies. They are the most abundant microarthropods in this habitat and the major component of its biodiversity. Recognizing species from forest canopies is difficult because most species have not yet been described. Currently, the only mite studies in Canadian temperate old-growth canopies are in the Montane Alternative Silvicultural Systems (MASS), Carmanah, Rocky Point, Mt. Maquilla and Mt. Cain sites on Vancouver Island in British Columbia. These studies have led to the discovery of multiple genera and species previously unrecorded from North America.

To sample a branch from the canopy, enclose the branch with a plastic bag, cut it from the tree, and label and seal the bag before lowering it to the ground. Microhabitats (such as lichens, mosses, suspended soil layers, and bark) present in canopies must be carefully hand picked or cored (using a corer) and bagged. Label each sample with the following information: tree species, position within the canopy, latitude and longitude, nearest town, collector, date collected and other pertinent information. The size of the sample will depend upon the tree species, age of the tree, and the purpose of the collection.

Washing Techniques

Within one week of collection, use the branch washing technique summarized below to extract specimens from each sample. Washing is the recommended extraction technique for branches as it effectively captures about 90% of the microarthropods including adult, immature and resting stages (Figure 4).

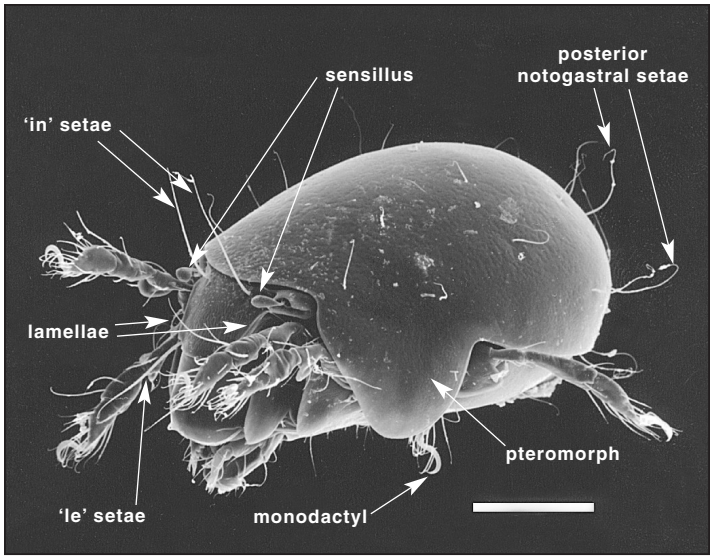
Prior to washing, measure and record branch size and structure. Measurements should include: branch length from tip to base; width across the widest part of the branch when placed on a flat surface; branch age; and the numbers of branchlets, vegetative buds, and male and female cones.

The steps for branch washing are:

1. Carefully cut each branch sample into pieces small enough to fit into a bucket;
2. Fill the bucket with just enough water to cover the branch pieces;
3. Place five or six sodium hydroxide pellets in the bucket with the branch sample;
4. Let the branch pieces soak for 48 hours;
5. Place each branch piece on a coarse screened sieve (e.g. #A 12/64") and rinse thoroughly over the wash bucket to recover microarthropods still on the branches. Place all branch pieces and the debris remaining in the sieve, in an appropriately labelled paper bag for drying;
6. Pour the rinse water through a medium screen (e.g. #18) to remove smaller debris, retaining the rinse water; rinse the small debris in the sieve again; then add the sieve contents to the paper bag with the branch pieces;
7. Pour the wash water (including the rinse water from steps 5 and 6) through a fine screened (100 µm or finer) sieve; rinse thoroughly;
8. Back rinse the contents of the fine-screened sieve (containing the microarthropods) with 70% alcohol into a properly labelled Nasco WHIRL-PAK® bag, and store the sample at 4-5 °C until the microarthropods can be sorted from the remaining debris;
9. Dry the branch samples to a constant weight at 60 °C and separately record branch, needle and cone dry weights.

The steps for washing lichens and mosses are:

1. Place the entire lichen or moss sample into a bucket and cover with water;
2. Place 1-2 sodium hydroxide pellets into the bucket with each sample;



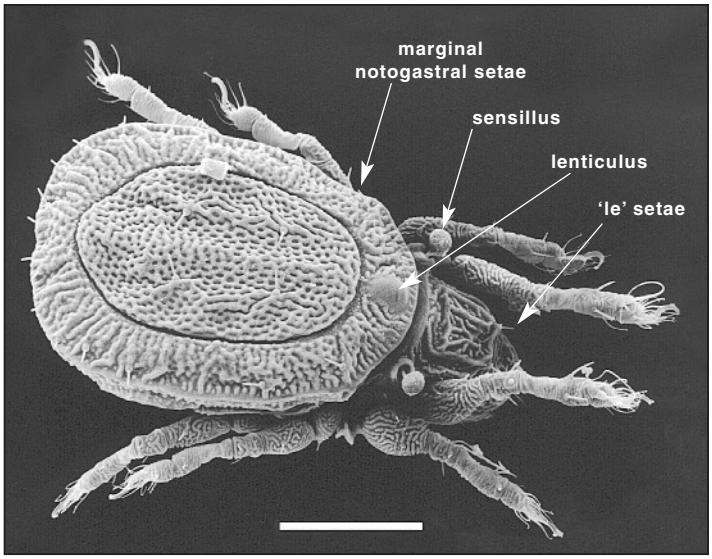


Figure 5. Body shape, colour, size and structural features can be used to group Oribatid mites into morpho-species. **Mycobates** ***n. sp. (l) and a*** **Scapheremaeus palustris** ***(r). (bar = 100 µm)***

3. Let the sample soak for 48 hours;
4. Rinse the larger lichen or moss pieces over a medium (#18) screen sieve; retain the rinse water; and place the sieve contents in a properly labelled paper bag for drying;
5. Pour the wash water through a medium screen (#18) sieve; rinse thoroughly; retain the rinse water;
6. Remove the larger debris from the medium screen; rinse it over the fine mesh screen; and place in the paper bag;
7. Pour the rinse water from stages 4 and then 5 through a fine mesh screened (100 µm or finer) sieve;
8. Add the remaining contents of both sieves, which now contain the finer debris and mircoarthropods, into a WHIRL-PAK® bag with 70% alcohol;
9. Dry and record the entire lichen sample. If wood debris is included, record the weights of the debris (bark and wood) and lichens separately.

Specimen sorting

You now have washed samples stored in 70% alcohol in whirl paks. To separate the specimens from the debris associated with these samples use the following procedure:

1. To split large samples:
   (a) Pour contents of WHIRL-PAK® bag through a sieve with a 100 µm mesh opening. Back wash the sieve contents with 70% ethanol and remove all specimens as per step 2 below;
   (b) Split the resulting rinse water into two equal parts with a plankton splitter;
   (c) Store split 1 in a labeled vial of 70% ethanol; and
   (d) Sort through split 2 as per step 2.
2. Under a dissecting microscope, sort through the debris of split 2 with a fine probe or insect pin. Transfer all specimens with a micro-pipette to properly labeled vials of 70% ethanol and store.

For samples split once, multiply the number of specimens obtained from split 2 by two and add the number of specimens from the sieve to get an estimated total for each sample.

Grouping microarthropod specimens

Once specimens have been extracted, group them by morphospecies (a definable specimen, or group of specimens, which are morphologically distinct from all other specimens, according to the morphological characteristics (Figure 5) visible under a dissecting microscope (up to 40 × magnification)). For oribatid mites, use the following criteria in the initial sort:

- Size;
- Color — light (unsclerotized) vs. dark (heavily sclerotized); white vs. yellow vs. red-brown vs. black-brown;
- Overall shape when viewed dorsally - from above, the specimen can appear somewhat round, elongate, or triangular;
- Overall shape when viewed laterally - from the side, the specimen can appear somewhat dorso-ventrally flattened, globose, or domed;
- Setae — Are setae visible under low magnification? Are they generally long, short, or intermediate? Are the lengths uniform across the body or are some noticeably longer or shorter than most? Are they thickened or a unique shape?
- Body texture and sculpture;

- Presence of cerotegument (superficial layers of the tegument exuded through pores of the cuticle) or debris carried on the dorsum;
- Presence of nymphal scalps (dorsal part of nymphal exuviae);
- Presence, size, and shape of pteromorphs (which look similar to "wings"; Fig. 5);
- Presence and type of lamellae (Fig. 5);
- Ventrally, the general shape of, and distance between, the genital and anal flaps; and,
- Size, shape, and length of the sensillus (Fig. 5).

To see the fine anatomical features referred to in keys, it is necessary to view the specimens under a compound microscope (possibly under oil) or a scanning electron microscope.

Preparing slide mounts of mite specimens

Microscope slide mounts are required for examination with a compound microscope and for the deposition of voucher specimens. Choose clean, complete representatives of each morphospecies. Clear heavily sclerotized specimens in lactic acid for one day to one month (more heavily sclerotized specimens take longer to clear).

Experts generally request at least four good dorsal and ventral, and one or two lateral mounts of each sex. Where sex cannot be determined, three to four additional dorsal and ventral mounts should be prepared. The same number of dorsal and ventral mounts should also be prepared for each immature stage. Retain a few specimens of each life stage, if available, in 70% ethanol. The preferred slide mountant for mites (and Collembola) is Hoyer's Medium (crystalline gum arabic, distilled water, chloral hydrate, and glycerine, as per Krantz (1978)).

The steps for mounting a microarthropod specimen in Hoyer's are:

1. Rinse all specimens cleared in lactic acid thoroughly in three or four changes of distilled water to keep crystals from forming inside and around the specimens once mounted.
2. Place a drop of Hoyer's Medium in the center of a clean 1 × 3-inch microscope slide.
3. Lift the specimen from the sorting dish with a fine wire loop or dental pick and carefully manipulate the specimen to the bottom of the drop of Hoyer's. Arrange it on a vertical axis so the mouthparts are directed posteriorly.
4. Apply a 12-mm, #0 coverslip for specimens less than 3 mm in length.
5. Label the right side of the slide with an identifying number, or the locale, habitat, and sampling date, or both.
6. Place the slide in an oven at 45°C for 48 hours to one week (or longer if the media is tacky at the edge of the coverslip).
7. Hold the slides for one week at room temperature, preferably in a dessicator, to allow the extra-thin coverslip to return to its normal flat state.
8. Apply a ring of Glyptal®, a waterproofing paint, around the edge of the coverslip with a No. 1 camel-hair brush to make the slide permanent. Slides that are not properly sealed will quickly deteriorate, making the slide mount unusable.
9. Place proper identification labels on the slide. At a minimum, the right-hand identification label should state the location, date collected, collector's name, habitat, substrate, and reference number, if applicable.
10. Group your slide specimens by morphospecies.
11. Attempt to identify the specimen as far as possible - this may only be to family level.
12. When submitting your slides to an expert for identification, ask if they prefer three or four good representatives of each morphospecies or all specimens.
13. Once the specimen has been identified, affix the left-hand label which should include identification (to species where possible) and the name of the determiner.
14. Upon completion of your study, deposit your voucher collection in a well established, accessible and maintained collection.

Submitting voucher specimens

A collection of properly prepared, accurately identified, and correctly catalogued microarthropod specimens is essential for providing a means to make comparisons in future ecological and taxonomic studies. A comprehensive collection of voucher specimens is the only effective way to maintain the long-term validity of research.

The collection of vouchers should include representatives of all species or morphospecies recovered in a study irrespective of the level of identification.

When a taxonomist publishes a species description, one specimen is designated as the "type" specimen. It is the specimen upon which the species description is based and is the standard or ultimate benchmark for that species. All microarthropod "types" designated from specimens collected in Canada should be deposited in the Canadian National Collection (CNC). A complete, working voucher collection (of undescribed and described species) should also be deposited locally. While the CNC has the largest collection of microarthropods in Canada, the Pacific Forestry Centre of the Canadian Forest Service has the largest working collection of forest-related soil and canopy microarthropods in western Canada.

Conclusion

Conducting effective analyses of forest organisms and ecosystems depends on making accurate identifications of the species that inhabit forest ecosystems. Consistency in identifications depends on the availability of published keys and descriptions, and on properly identified voucher specimens. Specimens that cannot be matched to vouchers or published descriptions require expert examination.

Identification of microarthropods to species is fundamental in discussions of biodiversity, in recognizing community structures and patterns, and in recognizing trends within ecosystems. The techniques we describe are applicable in the study of canopy communities, as well as in other ecosystems.

Additional reading

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For Arachnida (includes all mites) contact:

Dr. Valerie Behan-Pelletier,
Unit Curator of Arachnida;

For Collembola contact:

Dr. Robert Skidmore.

Dr. Behan-Pelletier and
Dr. Skidmore can be contacted at:

The Canadian National Collection
of Insects and Arachnids (CNC),
K.W. Neatby Building,
960 Carling Avenue,
Ottawa, ON, K1A 0C6
phone: 613-759-1774

For all other microarthropods, contact:

Dr. Jacques des Chenes,
Director for Microarthropods,
Canadian National Collection (CNC)
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phone: 613-759-1799

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