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Microcetus lappus gen. nov., sp. nov.: New Species of Ciliated Protozoon from the Bovine Rumen

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A new species of small, ciliated protozoon, *Microcetus lappus* gen. nov., sp. nov., from the rumen of Norwegian Red cattle is described. *M. lappus* possesses a novel cytopharyngeal apparatus of two rod-shaped structures, one situated on the dorsal side of the buccal cavity and one on the ventral side, suggesting that it belongs to a previously undescribed taxon.

Ruminants normally harbor a ruminal microbial population containing a mixture of ciliated protozoa composed of entodiniomorphid species and other species with cilia located over part or all of the body surface. The most common species of uniformly ciliated protozoa are *Isotricha intestinalis*, *Isotricha prostoma*, and *Dasytricha ruminantium* (8, 15), which are members of the order Trichostomatida. Domestic ruminants have occasionally been described which have ruminal ciliate populations containing other species with cilia located over part of the body surface, including *Buetschlia* spp. (4, 17), *Charonina ventriculi* (6, 13), *Oligoisotricha bubali* (9, 10, 12), and *Parabundleia ruminantium* (11). All of these species have been found in cattle. We report here a previously undescribed species of protozoon, with nearly uniform surface ciliation, from the rumen of Norwegian Red cattle.

MATERIALS AND METHODS

Rumen contents. Rumen contents were obtained by aspiration via permanent rumen cannulae from two Norwegian Red cattle from Bodø, which is located on the arctic circle in Norway. The animals were fed a ration of high-quality timothy hay and 1 kg of concentrates daily. The rumen fluid was fixed immediately with 2% glutaraldehyde.

Preparation of samples. The rumen contents were filtered through two layers of muslin and examined with a light microscope. Many of the protozoa were aggregated with particulate matter; therefore, methylcellulose (final concentration, 1% [wt/vol]) was added, and the suspension was mixed by vortexing for 30 s. Subsamples were stained with methyl green (15) and iodine (2) to visualize macronuclei and intracellular polysaccharides, respectively.

Suspensions of ciliates for examination by scanning electron microscopy were prepared by twice vortexing the fixed preparation for 30 s, filtering the suspension through nylon mesh (maximum pore size, 45 μm), and centrifuging the filtrate for 5 min at 250 × *g*. The pellet was suspended in distilled water and was washed by repeated centrifugation at 250 × *g* for 3 min until the supernatant fluid was clear. The cells were dehydrated (16), critical-point dried (1), splutter coated with gold in a Polaron coating apparatus mounted on a double-sided adhesive tape on a standard aluminium stub, and examined with a JSM 2 scanning electron microscope.

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Determination of population densities of protozoa. The population densities of ruminal ciliates in the twice-filtered, methylcellulose-treated samples were determined by microscopy (2). Protozoa were identified by their morphology (15). Cell dimensions were measured with a light microscope fitted with a micrometer eyepiece calibrated against a hemacytometer grid.

RESULTS AND DISCUSSION

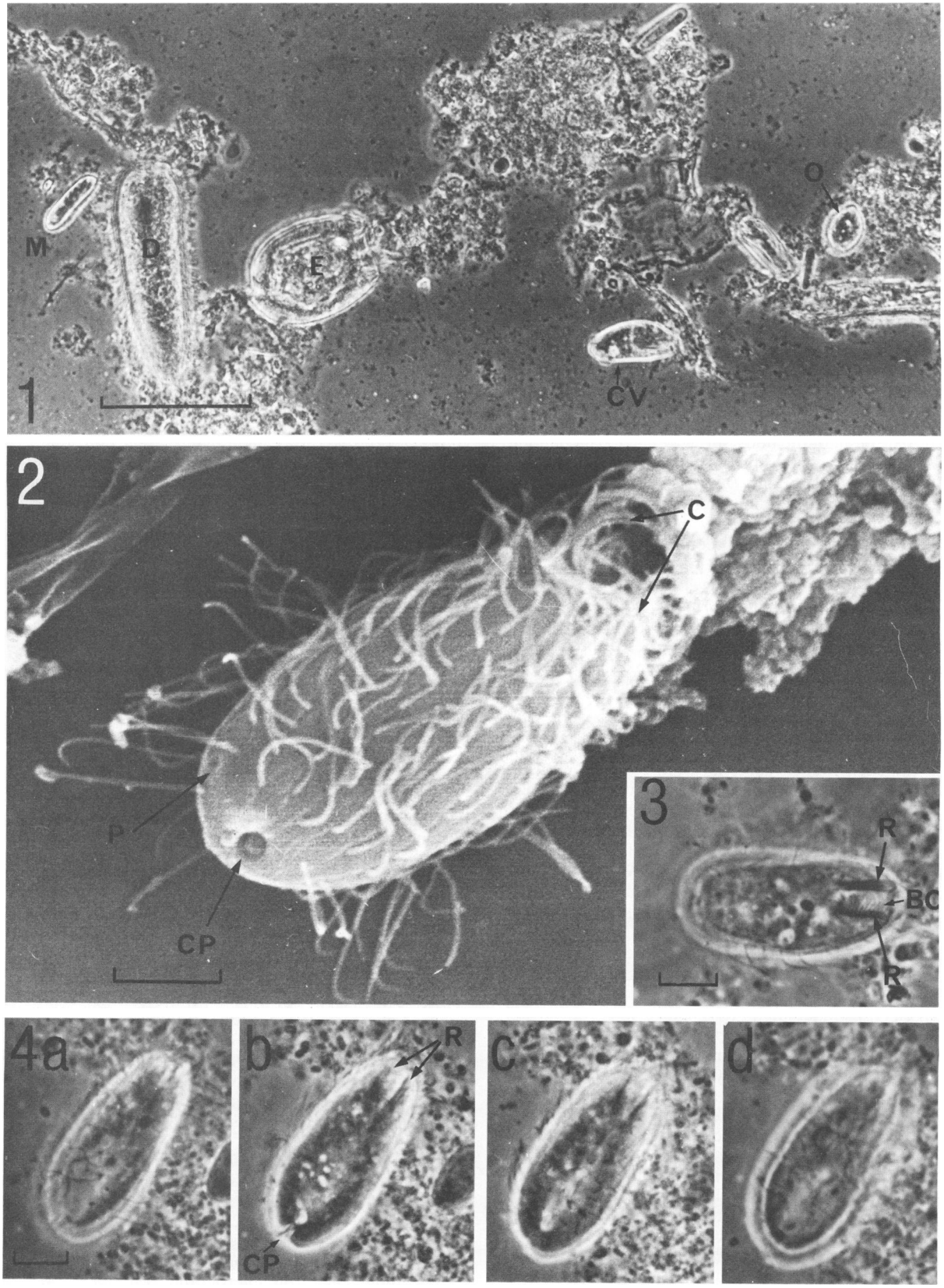
Examination of the ruminal contents by light microscopy revealed an unfamiliar species of protozoon with cilia located over much of the cell surface (Fig. 1 through 3). Its dimensions were similar to those of *Oligoisotricha* spp. and smaller than those of the other trichostomatid protozoa (*Isotricha* spp., *D. ruminantium*, and *C. ventriculi*) present in the same samples. The new species was also morphologically distinct from other small ruminal ciliate species described in the literature. The description of the new species is given below.

***Microcetus* gen. nov.** Cells ovoid to elongate, flattened bilaterally. Buccal cavity apical. Cytoproct normally subterminal on the ventral surface, or terminal. Somatic cilia sparse, occurring over about 90% of the cell surface, forward pointing, absent from the immediate vicinity of the cytoproct. Buccal cilia present on the dorsal side of the buccal cavity. Two cytopharyngeal rods present, one on the dorsal side of the buccal cavity and one on the ventral side. Macronucleus variously positioned within the cell, but usually near the center. Contractile vacuole present.

***Microcetus lappus* sp. nov.** With the characteristics of the genus. Length, 23.6 ± 5.2 μm, and width, 12.7 ± 5.4 μm (n = 40). The proximal end of the dorsal pharyngeal rod is usually curved inward; the ventral pharyngeal rod is straight. The rows of somatic cilia form an angle of about 20° to the long axis of the cell. The macronucleus is spherical to ovoid. The contractile vacuole is usually positioned near the cytoproct.

The genus name *Microcetus* (meaning a very small whale) was derived from the similarity of the protozoon observed with the light microscope to a stylized drawing of a whale by a child. The species name, from the Latin word lappa (a burr), was derived from the similarity of the cell observed in the scanning electron micrographs to a plant burr.

A scanning electron micrograph (Fig. 2) of *M. lappus* shows the somatic ciliation and external features. The buccal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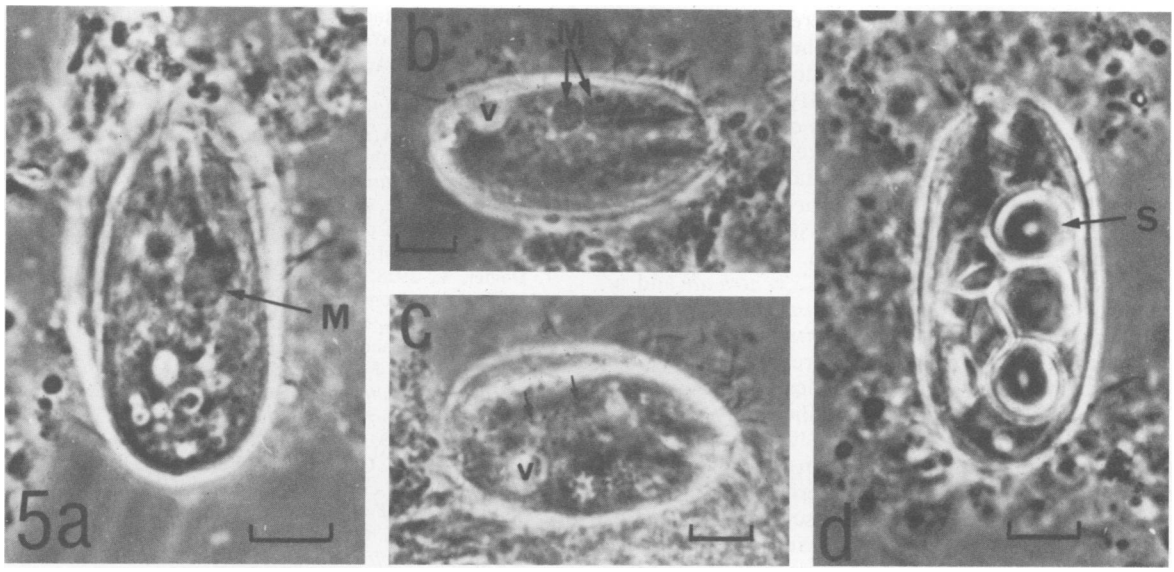

FIG. 5. Phase-contrast light micrographs of *M. lappus*, showing the macronucleus (M) as normally observed (a) and after division (b); the contractile vacuole (V) is also visible. The spiral alignment of the cilia (arrows) (c) and engulfed starch grains (S) (d) are also shown. Bar = 5 μm.

cavity, buccal cilia, and cytopharyngeal apparatus were revealed by light microscopy (Fig. 3).

Light micrographs taken at different focal planes showed that the cytopharyngeal apparatus consisted of two longitudinal rods (Fig. 4a to d), with no evidence of a more complex rhabdos or cyrtos structure. The cytopharyngeal rods could be seen with both transmitted-light and phase-contrast optics. The cytoproct can be clearly seen in Fig. 4b in a characteristic subterminal position. Staining with methyl green revealed the macronucleus (Fig. 5a), which was normally ovoid, but in a few cells (probably nearing division) two adjacent spherical macronuclei were seen (Fig. 5b). In about 20% of the cells a structure which we believe to be a contractile vacuole was found in the posterior region; the structure is just visible in Fig. 5b and c. Many cells contained starch grains, probably of dietary origin (Fig. 5d), and other intracellular bodies which were identified by their morphology as large ruminal bacteria, suggesting that particle engulfment is important in the nutrition of this species.

The external characteristics of *M. lappus* are similar to those described by Dogiel (7) for *Isotricha bubali*, later named *O. bubali* (9), but *O. bubali* does not possess cytopharyngeal rods and has a striated vestibulum. *M. lappus* is easily distinguished from *Buetschlia* spp. (4, 17), *C. ventriculi* (6), and *P. ruminantium* (11) by the possession of somatic ciliation over most of the cell surface and by its cytopharyngeal apparatus.

It is likely that the *O. bubali* reported (5) to occur in cattle in the United States was, in fact, *M. lappus*, because two cytopharyngeal rods can be clearly seen in the photomicrographs of these ciliates and the posterior of the cell is smoothly rounded. Although these structural features are clearly at variance with the original description of *O. bubali* (7), the investigators did not comment on these anomalies.

M. lappus occurred in both of the Bodø cattle examined. The total ciliate population density in the two animals was 3.4×10^4 ml^{-1} and 2.25×10^4 ml^{-1} (mean values of eight estimates); of these, *M. lappus* represented 12 and 15%, respectively. We also examined the ruminal contents of five cattle from near Tromsø (latitude, 70° N), and the new species was not present. Therefore, *M. lappus* is not uniformly distributed in Norwegian Red cattle, and we are now attempting to determine its geographical distribution.

The taxonomic position of the genus *Microcetus* is as yet unknown. Further work with freshly stained material to indicate the silver-line system and micronucleus and with transmission electron microscopy to determine the nature of the cytopharyngeal rods and the origin and structure of the buccal ciliation is necessary before any conclusions can be reached regarding the taxonomy of *Microcetus*. No taxonomic group currently exists which embraces protozoa possessing the type of cytopharyngeal apparatus found in *Microcetus*. The nearly uniform somatic ciliation, the nearly apical cytostome preceded by a vestibulum, and the presence of oral ciliation suggest that *Microcetus* should be placed in the class Kinetofragminophora de Puytorac et al. 1974, according to recent classification systems (3, 14). Within this class, two subclasses, the Vestibulifera and the

FIG. 1. Phase-contrast light micrograph of rumen contents of Norwegian Red cattle, showing the newly described ciliate in relation to other rumen ciliates. M, *M. lappus*; O, *Oligoisotricha* sp.; D, *D. ruminantium*; E, *Entodinium longinucleatum*; CV, *C. ventriculi*. Bar = 50 μm.

FIG. 2. Scanning electron micrograph of *M. lappus*, showing cilia (C), the cytoproct (CP), and what is probably the external pore of the contractile vacuole (P). Bar = 5 μm.

FIG. 3. Phase-contrast light micrograph of *M. lappus*, showing the prominent cytopharyngeal rods (R) and buccal ciliation (BC). Bar = 5 μm.

FIG. 4. Phase-contrast light micrographs taken at different focal planes through a cell of *M. lappus*, showing only two cytopharyngeal rods (R), the cytoproct (CP), and sparse somatic cilia. Bar = 5 μm.

Hypostomata, possess cytopharyngeal strengthening structures. The cytopharynx of members of the Vestibulifera is usually strengthened by several nematodesmata to form a rhabdoslike structure; members of the Hypostomata typically possess a cytopharyngeal apparatus of the more complex cyrtos type (a cone-shaped, basketlike structure, sometimes curved, composed of nematodesmata). Both types are clearly different from that found in *Microcetus*. Because of its characteristic cytopharyngeal apparatus, *Microcetus* may represent a previously undescribed taxon.

Two other species of small ciliates, *C. ventriculi* and a species putatively identified as *Oligoisotricha* sp., were present in the ruminal contents of the Norwegian Red cattle at Bodø. Whereas *C. ventriculi* has been recorded as occurring in ruminants in a variety of geographical locations (6, 8, 13), *O. bubali* has been positively identified only in water buffalo (9, 10) and in cattle in Japan (12). In all of our preparations to date the putative *Oligoisotricha* sp. has been strongly associated with particulate material, thus preventing the use of scanning electron microscopy. The *Oligoisotricha* sp., like *O. bubali* (7), possesses a striated vestibulum and lacks the cytopharyngeal rods of *M. lappus*, but unlike *O. bubali*, the posterior end of the cells is smoothly rounded. This organism, like *M. lappus*, may be a new species.

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