A NEW MINDENIELLA FROM SUBMERGED, ROSACEOUS FRUITS [1]

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THE MONOTYPIC genus *Mindeniella*, placed in the Blastocladiales by its discoverer (Kanouse, 1927), has, more recently, been assigned to the Leptomitales, in the family Rhipidiaceae (Sparrow, 1943). As proposed by Sparrow, four genera in the family were recognized: *Sapromyces* K. Fritsch, *Araiospora* Thaxter, *Rhipidium* Cornu, and *Mindeniella* Kanouse. The latter genus is immediately separated on the basis of the direct origin of the reproductive organs from the basal cell of the thallus.

Mindeniella spinospora Kanouse, if one may judge from the literature, is limited in its distribution, having been collected only in the immediate vicinity of Ann Arbor, and East Lansing, Michigan. The Huron River, near Ann Arbor, has been most prolific in yields of *M. spinospora.* A recent collection of decaying, submerged rosaceous fruits (*Malus* sp.), from the Huron River, was made by Professor F. K. Sparrow, and very kindly given to the author. From these fruits a species of *Mindeniella* has been recovered which, from the shape of its basal cell, as well as from other distinctive morphological features such as size of the zoospores, is apparently a new species.

It is of interest to note at this point that the present fungus had been collected in September, 1948. This collection was completely overlooked, however, inasmuch as the few specimens of the fungus were sparsely scattered in pustules among plants of *Rhipidium americanum,* and were unobservedly preserved on slides along with the more abundant *Rhipidium.* A third collection of the fungus, on May 18, 1950, has been taken from submerged decaying apples found in Fleming Creek, 1 mi. north of Geddes Road, near Ann Arbor, Michigan.

By procedures similar to those employed by Dr. Kanouse (1927), it has been possible to retain and multiply the original collection in gross culture in the laboratory for some months. This has made practicable a study of the fungus over an extended period of time, and has enabled us to determine which of the morphological features of the plant are consistently stable. The periodic study of subcultures has substantiated the results of observations on the original material. Unifungal subcultures have been repeatedly obtained by removal of a single zoosporangium just prior to discharge, and introduction of this structure into sterile, distilled water containing a carefully washed, ether-swabbed apple as the substratum. Bacterial-free cultures were not obtained, although frequent washing of the infected bait, with sterile, distilled water and periodic changes of the water in the culture dishes decreased bacterial growth.

The abundance of material of the species to be described has fostered preliminary studies on isolation of the fungus into pure culture, utilizing the isolation technic and synthetic media of Emerson and Cantino (1948). Despite repeated attempts at isolation, none of the propagative methods employed have been successful.

Fortunately, we have obtained for comparative purposes, living material of *Mindeniella spinospora* Kanouse, collected in the courtyard pool of the Natural Science Building on the campus of the University of Michigan. Comparison of the living material of *M. spinospora* with the present fungus reveals certain features common to both. These are: (1) the strong cellulose reaction in the basal cell wall; (2) the presence of both smooth and spiny-walled zoosporangia; (3) the formation and subsequent action of the zoospores, and (4) the pedicellate nature of the zoosporangia and resting spores, and the presence of cellulin plugs in these pedicels. Other morphological characteristics however, serve to separate the two species.

Averages, percentages, and the like, reported herein, are on the basis of 500 observations or measurements of the morphological features concerned.

MINDENIELLA **asymmetria** sp. nov. — Corpus vegetativum clavatum plerumque supra partem mediam horizontaliter deflexum, sed interdum plusminusve irregulare; secus axin 175–515 μ longum; parte horizontaliter deflexa 181–290 μ longa, 91–143μ crassa; parte basali ramosa, ramis rhizoidalibus etiam ramosis acutissimus ad substratum affixa; membrana crassa laminosa. Sporangia 1–14, propre apicem partis horizontalis affixa, breviter pedicellata, ovoidea vel subpyriformia, raro clavata, 47–88 μ (saepissima 60–70 μ) longa, 32–65 μ (saepissima 40–50 μ) diametro, laevi vel in parte quarta apicali spinis 20–30 praedita; membrana crassa; zoosporis lateraliter biflagellatis, reniformibus, per papillam apicalem extrusis cito natantibus, 5–9 μ (plerumque 6–8 μ) longis, 3–7 μ (plerumque 4–6 μ) diametro. Sporae perdurantes cum sporangiis intermixtae vel in corporibus separatis sedentes, pedicellatae in parte supra mediam spinis acutissimis prominentibus numerosis praeditae, sphaericae, 38–87 μ diametro, plerumque 56–70 μ. Reproductio sexualis hucusque ignota.

Ex malis secus ripis fluminis Huronensis submersis prope Ann Arbor, Michigan, legit F. K. Sparrow, 13 October 1949. Specimina typica in Herbario Universitatis Michiganensis conservata.

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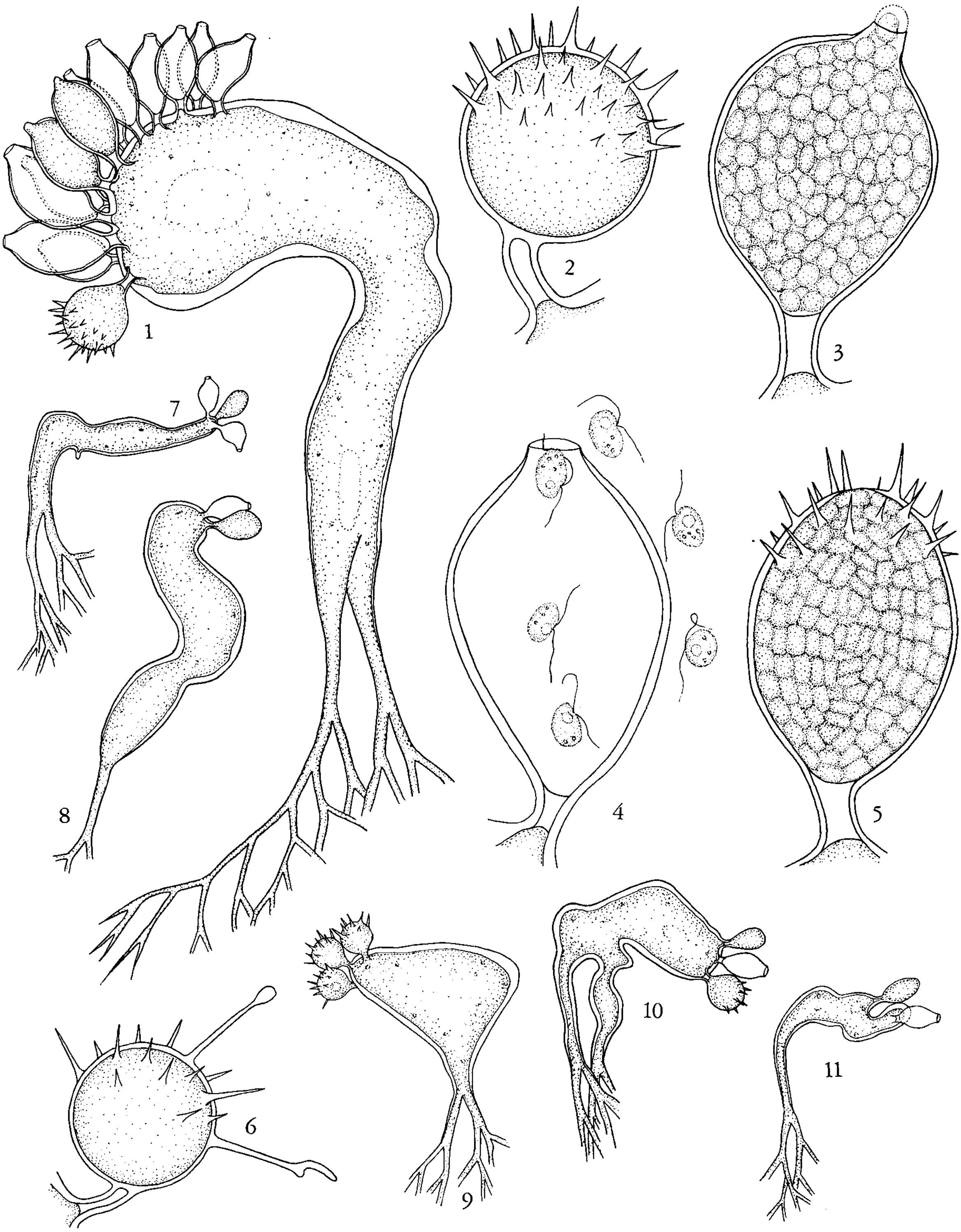
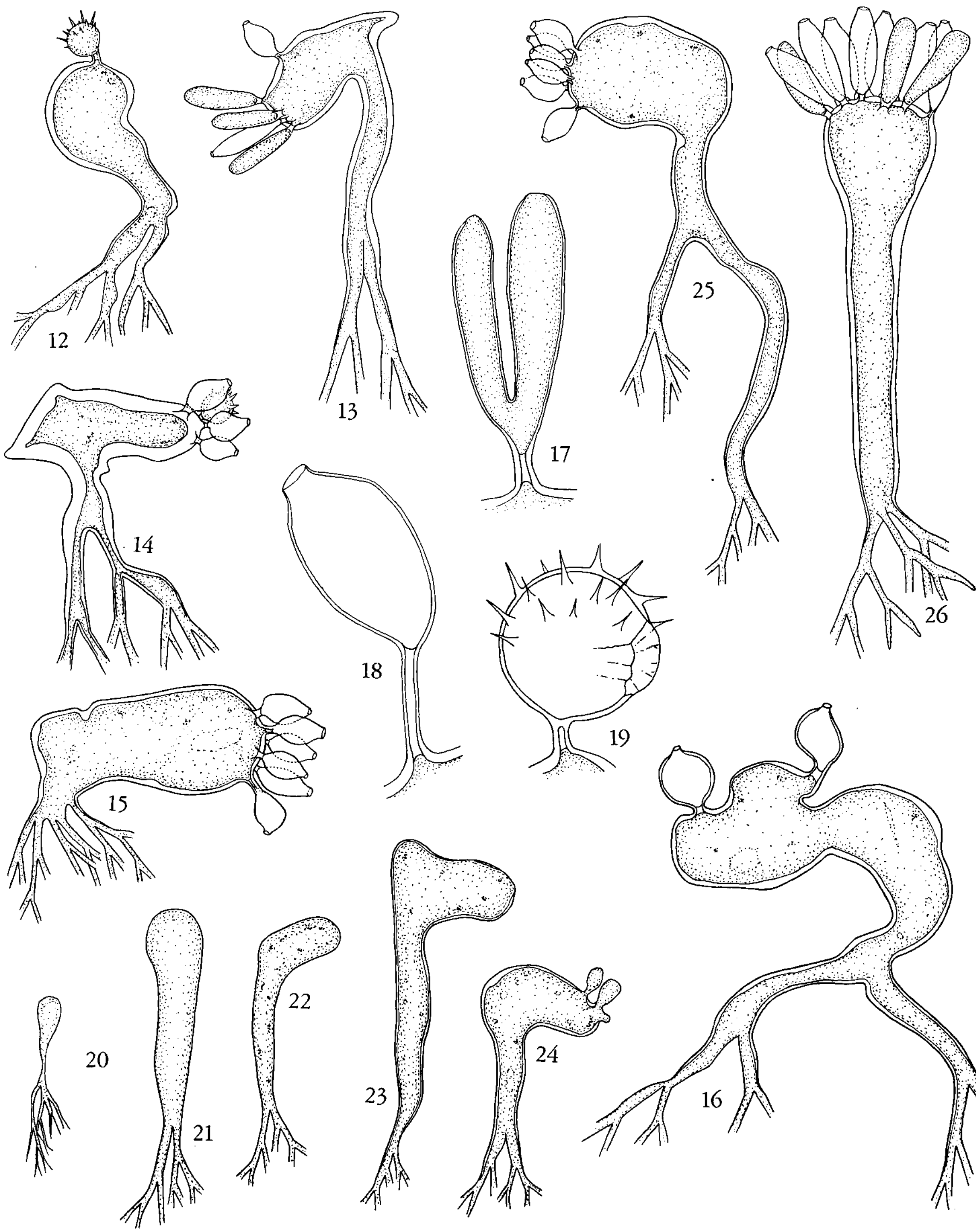


Fig. 1-11. *Mindeniella asymmetria.*—Fig. 1. Habit of plant, showing features characteristic of the species. ×210.—Fig. 2. Spherical, pedicellate resting spore with thick wall, and the numerous, solid, sharp-pointed spines. ×560.—Fig. 3. Short-ovoid zoosporangium just prior to spore discharge. ×560.—Fig. 4. Small, discharging zoosporangium, showing details of the zoospores. ×960.—Fig. 5. Short-ovoid spiny zoosporangium with zoospore initials. ×960.—Fig. 6. Small resting spore showing variations in the spines. ×960.—Fig. 7-11. Variations in basal cell shape. All show some degree of bending of the basal cell. ×85.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Basal cell bent-clavate, slightly irregular in outline, often once-branched in the lower portion; 175–515 μ long in the longitudinal axis, 181–290 μ long in the horizontal axis, 91–143μ in diameter across the bent portion, predominately 400 × 250 × 125 μ; attached to the substratum by much-branched, sharply pointed rhizoids; wall variable in thickness in the same thallus, apparently laminate. Zoosporangia 1–14, borne at or near the distal end of the horizontal portion of the basal cell on slender, variable-length pedicels closed with cellulin plugs; predominately short-ovoid or short-pyriform, rarely long-clavate, and then usually once-branched at the base; 47–88 μ in length by 32–65 μ in diameter, predominately 60–70 μ × 40–50 μ; clavate zoosporangia averaging about 115 × 25 μ; wall moderately thick, predominately smooth, but occasionally with 20–30 or more solid, sharp-pointed spines in the upper one-fourth of the sporangium wall. Zoospores reniform, biflagellate, escaping from the zoosporangium through an apical exit papillus, and swimming away at once; 5–9 μ long by 3–7 μ in diameter, predominately 6–8 μ × 4–6 μ, and averaging 7 × 5 μ. Resting spores intermingled with the zoosporangia or on separate thalli; pedicellate, with a thick wall, and with prominent, solid, sharp-pointed spines in the upper one-third or one-half of the spore wall; spherical; 38–87 μ in diameter, predominately 56–70 μ; spines 11–36 μ in length. Sexual reproduction unknown.

From apples (*Malus* sp.), submerged along shore of Huron River, near Ann Arbor, Washtenaw County, Michigan, by F. K. Sparrow, October 13, 1949. Preserved specimens from the type material are deposited in the Herbarium of the University of Michigan.

The most obvious and distinctive feature of *Mindeniella asymmetria* is to be found in the peculiar inverted boot-shaped or "chicken-leg" appearance of the basal cell (fig. 1). The pustules of the fungus on the original collection of apples yielded 443 plants. In addition, 1281 plants from the several subcultures of the original material have been studied. Of the total number of plants observed, all possessed to some degree the asymmetrical growth-appearance of the basal cell. Variations in general configuration of the bent basal cell have been observed in about 20 per cent of the plants studied (fig. 7–16), the remainder of the plants being quite similar to that illustrated in fig. 1. Only one plant (fig. 12) has been observed in which the basal cell approximated the growth-form of *M. spinospora,* but even in this isolated instance, the basal cell was markedly asymmetrical.

Certain other morphological differences between *Mindeniella asymmetria* and *M. spinospora* are to be noted. The zoosporangia of *M. spinospora* are distinctly larger than those of the present species, particularly in the longitudinal dimension. The blunt-rounded, sparingly-branched rhizoids of *M. spinospora* are distinct from the very extensive, much-branched, long-pointed rhizoids of *M. asymmetria.*

Comparison of the resting spores of *M. asymmetria* with the figures of comparable structures in Dr. Kanouse's paper (1927, pl. 34, fig. 17, 18, 24, 25, 26) is the basis for further separation of the known and the present species. As illustrated by Dr. Kanouse, the entire surface of the resting spore wall of *M. spinospora* is spiny. Furthermore, Dr. Kanouse stressed this in her description of *M. spinospora.* This is not the case in our fungus, since, at most, only the upper half of the resting spore wall is studded with spines. The resting spores of *M. spinospora* have not been observed in our living material which we have used for comparative purposes.

An important distinction between the known species of *Mindeniella* and the present one is that of zoospore size. The zoospores of *M. asymmetria* are substantially smaller than the zoospores observed by Sparrow and Cutter (1941) in their material of *M. spinospora.* The zoospores from only two discharging zoosporangia of *M. spinospora* have been observed by the writer. However, the range and predominant size of forty-eight of these zoospores agrees remarkably well with the size as recorded by Sparrow and Cutter. Zoospore size in the type material of *M. spinospora* is unfortunately lacking, since Dr. Kanouse did not observe these structures in her material.

The spiny zoosporangia (fig. 5) of *M. asymmetria* resemble, at least with respect to the pres-

Fig. 12-25. *Mindeniella asymmetria.* Fig. 26. *Mindeniella spinospora.*—Fig. 12. Plant with basal cell shape suggestive of the basal cell of *M. spinospora.* ×85.—Fig. 13. A somewhat recurved basal cell on which are borne four clavate zoosporangia. Note that these are unbranched. ×85.—Fig. 14. A variation in basal cell shape showing extreme thickness in the basal cell wall. ×85.—Fig. 15. Plant with a nearly sessile basal cell. ×85.—Fig. 16. Plant with irregular, basally branched basal cell. ×183.—Fig. 17. A branched, clavate, immature zoosporangium. ×365.—Fig. 18. Empty, short-ovoid zoosporangium showing the extreme length in pedicels. ×365.—Fig. 19. An empty resting spore with an irregular, lateral break in the spore wall. ×365.—Fig. 20-24. Plants in various stages of development.—Fig. 20. The very small, symmetrical basal cell characteristic of the very young plants. ×257.—Fig. 21. Later stage in growth of basal cell, prior to bending. ×183.—Fig. 22. Initiation of bending in the basal cell. ×183.—Fig. 23. Completion of bending and formation of thick wall except at points where reproductive organs are to form. ×183.—Fig. 24. Reproductive organ initials on a small plant. ×183.—Fig. 25. Plant of *M. asymmetria* illustrating characteristic growth form, for comparison with Fig. 26. ×183.—Fig. 26. Mature plant of *M. spinospora.* ×85. All figures made with aid of camera lucida, from living plants.

ence and number of spines, the spiny resting spores (fig. 2). We have never observed less than twenty spines on the spiny zoosporangia of our species, while in *M. spinospora*, two to eight is the usually encountered number. A final difference is that of gross size, the plants of *M. asymmetria* being consistently smaller than those of *M. spinospora.*

That *M. asymmetria* may represent only a physiological variation of *M. spinospora* is not to be overlooked. However, several factors are evidence that such is not the case. The following are the most significant of these factors: first, the fact that *M. asymmetria* has been collected from three different localities in as many years; second, the rather large number of plants propagated in culture conditions of a different nature than the natural environment of the fungus, and the stability of the distinctive morphological features throughout these numerous subcultures; third, the differences in zoospore size between *M. asymmetria* and *M. spinospora,* a feature found to be consistently stable in other aquatic fungi.

Since it has been impossible to propogate the fungus under controlled conditions where systematic observation of growth of one plant could be seen and followed, knowledge of development of the thallus is necessarily incomplete. Distinctive stages in the growth of *M. asymmetria* have, however, been observed (fig. 20–24).

The very young basal cells (fig. 20, 21) are symmetrically clavate, and resemble in general configuration, the mature basal cell of *M. spinospora.* As the basal cell elongates and begins its bending (fig. 22), the protoplasm becomes dense and granular. In the younger stages of development, and during the bending process, the basal cell wall is very thin, but as bending nears completion, the wall thickens except at points of origin of the reproductive structures (fig. 23). The zoosporangia or resting spores appear, first, as bud-like protrusions from the basal cell (fig. 24), which soon fill with protoplasm from the basal cell, elongate or expand, and are subsequently delimited from the basal cell by a thin wall. Formation of the cellulin plugs in the pedicels of the reproductive structures, and the stages in the development of spines on the resting spores or on the zoosporangia has not been observed.

The formation of zoospores and their subsequent discharge from the zosporangium is not at all different from that of *M. spinospora* (Sparrow and Cutter, 1941). Following a short period of active swimming, the zoospores become quiescent, lose their flagella, and assume an oval shape which may be evidence of an encysted stage. Development of the zoospores beyond this stage, and germination of these structures has not been observed.

It is impossible to throw further light on the problem of the nature of the resting spores characteristic of the genus, based on observation of *M. asymmetria.* Numerous empty resting spores were found, however, particularly in plants from older pustules. Despite diligent search, only one such empty resting spore was detected in which there was a fracturing of the wall. This appeared as a lateral, irregular break in the wall (fig. 19), and it would be premature to speculate that this was the method of discharge of spore contents in all the resting spores. No antheridial structures were found, nor were structures which could be termed oogonia observed.

The generic position of *Mindeniella* has been thoroughly discussed by Sparrow and Cutter. The present species only adds to the evidence that the genus belongs, quite properly, in the Leptomitales.

SUMMARY

A new species in the heretofore monotypic genus *Mindeniella* is described as *Mindeniella asymmetria.* The fungus is characterized by the bent-clavate or bent-arbusculate basal cell; much-branched, extensive, sharply-pointed rhizoids; pedicellate zoosporangia and resting spores; the presence of cellulin plugs in these pedicels, and a strong cellulose reaction in the thick basal cell wall. *Mindeniella asymmetria* is distinguished from *Mindeniella spinospora* Kanouse on the basis of its peculiarly bent basal cell, smaller zoospores, and the predominance of short-ovoid zoosporangia which are distinctly smaller than those of *M. spinospora.* The fungus grows on submerged, decaying apples.

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