On Kibisidytes marinus, n.gen., n.sp., and some other Rhizopod Protozoa found on surface films.

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With Plates 5 and 6.

THE observations set out in this paper began with the finding of small brown-shelled organisms with pseudopodia on cover-slips left in marine aquaria[1] for other purposes. The same were discovered later on the surface film. My attention was caught because at first sight they looked like testacean rhizopods, of which none, so far as I know, have ever been recorded from the sea. When similar forms were found in fresh water the interest deepened and further study led to the recognition of several distinct organisms already known to science in fresh water, and a previously undescribed form in the sea.

THE FRESH-WATER FORMS.

On the surface films of some fresh-water infusions[2] I found a small testacean rhizopod which may resemble very closely

[1] Stocked from Millport and kept in Glasgow. Also, thanks to the courtesy of Mr. Elmhirst, on the surface films of small tanks at the Millport marine laboratory. Dr. Allen also kindly allowed me to search the surface films in the aquarium at Plymouth (in July 1932); but I found none of the rhizopods there, although in some cases many of their usual companions were present.

[2] Water from a marsh near Hamilton, Lanarkshire, to which tap-water and boiled wheat grains were added, kept at room-temperature; and in the scum, very rich in bacteria, on the surface of a large tank in which are kept Lepidosiren paradoxa at 21–22° C. The surface film organisms are collected by the well-known and convenient method of floating a cover-slip on the surface for a few seconds or longer, afterwards transferring it to a slide or watchglass for inspection, and dropping it on to the surface of a fixative for permanent preparation.

Microgromia elegantula as described by Penard in 1904. In such specimens the shell is thin and smooth, but differs from his (three specimens only) in that it is soft and easily deformable. In preparations mounted in Canada balsam it is as a rule much wrinkled, and when empty shells are taken on the point of a micro-dissection needle they collapse altogether. This character is not mentioned by Valkanov (1903), who seems to have been studying the same organisms, nor does he refer to the size, which I have found to be very variable in this species. Shells may measure anything between 10μ and 30μ across, the creature inside very often being a good deal smaller. The frequency and range of the various sizes appear to be very different in different populations, and at different times in a long persisting culture.

Valkanov refers to the fact that the shell of Microgromia may be covered with an irregular brown crust or crusts—when, he says, there is much iron in the water. These encrustations vary a great deal in thickness and pattern (cf. figs. 1 and 2, Pl. 5). I have never seen the larger shells (20–30μ) very heavily encrusted. Foreign bodies may get incorporated in the outer parts of encrusted shells. In alcohol these outer parts may be decolorized and disappear, sometimes in a few moments, and sometimes after a longer interval; in preparations mounted in Canada balsam they may appear more or less blurred in outline and with a tinge of blue after Ehrlich's haematoxylin. The original thin brown shell is sometimes still visible inside. These smaller encrusted shells break into pieces when pressed with a micro-dissection needle as if they were rigid and brittle. It was not found possible to ascertain whether there was a soft lining to the shell or not; no trace of such was seen in the experiments.

The shell is flattened on to some comparatively firm substratum, the surface film in most cases studied by me. I have never seen a Microgromia change its position, and believe such movement to be very rare or extremely sluggish if it ever occurs at all after the animal has chosen a site. It is difficult to imagine how those which are as it were plastered down by encrustations extending on to the substratum beyond the base of the shell could possibly move.

One or two contractile vacuoles are present, contracting slowly,[1] and often by stages, about once a minute. Solid food, such as bacteria and other vegetable structures, is drawn into the shell by the fine granular pseudopodia, which may be very long—up to ten times the diameter of the shell. Food bodies have also been seen to crumple up while entangled in the pseudopodia outside the shell as if digestion took place there also on occasion. The pseudopodia are branched and sometimes anastomose, but I have never, even on a crowded coverslip, seen the least tendency towards protoplasmic union between separate individuals such as is described in Microgromia socialis (Archer, 1869; Hertwig, 1874).

Valkanov (1931) mentions the chromidium[2] which surrounds the nucleus as shown in figs. 1 and 3, Pl. 5, and describes the mitotic division of the latter (1930). I have seen no nuclei in division, but shells of all sizes commonly contain two individuals, one often smaller than the other, presumably the result of binary fission.

Along with Microgromia there sometimes occurred organisms (see figs. 4 and 5, Pl. 5), which at first appeared to be variants of the same species. These had more than one opening to the shell, commonly three or four. The resemblance is strengthened by the fact that their shells have about the same range of size and also may be encrusted in the same way as those of Microgromia, thus producing a corresponding series of forms.[3] These individuals may be referred to Cien-

[1] Cf. Cienkowski, 1876, p. 34, 'eine contractile, aber nicht plötzlich collabierende Vacuole'.

[2] Does not stain with Feulgen's stain used for 28 hours, after hydrolysis for 5 and 10 minutes respectively.

[3] These encrustations occur also on the brown shells of some choanoflagellates and on certain algal spores occurring in the same habitat. This would suggest that the formation of the encrustation depends possibly at any rate on external factors—especially as there appears to be a tendency for similar types to occur in, for example, Microgromia and choanoflagellates growing together in one culture. This, if substantiated, would have to be taken into account in a study of an inherited tendency to make one or another sort of shell.

The choanoflagellates have not been precisely identified. There seem to kowski's Microcometes paludosa (Cienkowski, 1876, p. 46), to which attention has recently been called by Valkanov (1931), who adds several particulars to the original account, including a description of the nuclear division, which differs from that of Microgromia described by him a year earlier.

The pseudopodia of Microcometes extend out through any or all of the shell openings; they are poorly developed, and granular like those of Microgromia. The protoplasm is not so transparent, so that the nucleus is not to be seen in the living animal, even in the largest specimens. The food inclusions are similar to those of Microgromia; and contractile vacuoles have been observed. On staining it is seen that no chromidium is present and that the nucleus looks rather different from that of Microgromia, having more conspicuous staining granules in the peripheral zone (see fig. 5, Pl. 5).

Two daughter organisms have been seen in one shell; and in one culture the larger forms encysted in considerable numbers as described by Cienkowski, inside the original shell, the spherical cyst measuring about 12μ in diameter.

The Marine Rhizopod.

The marine rhizopod seems to be different from any of the fresh-water forms (figs. 6–13, Pl. 6). It never reaches a very large size in my experience. Its protoplasm is opaque, so that

be at least two different kinds, possibly both species of Salpingoeca (see Pascher and Lemmermann, 1914, p. 77).

They are roughly of the same size, the shell being $5–10\mu$ across, and flattened on to the substratum as are those of the rhizopods referred to in this paper. The single opening is however usually almost apical, so that it appears in my surface film preparations as a hole seen through the base of the shell. This circumstance makes it more difficult to obtain a good view of the flagellate itself. In side view one may distinguish the two kinds. The first has a more or less distinct rim to the brown shell, beyond which projects a delicate colourless funnel through which the collar and flagellum are extended on a hyaline protoplasmic peduncle which may stretch to a length of 10μ. The 'funnel' does not appear to be retractile as is, I suppose, the lower collar of a Diplosigopsis, though this is not explicitly stated in the original description (Francé, 1897). In the second kind the opening of the brown shell has as a rule no well-marked rim and there is no 'funnel', the base of the peduncle bearing the collar being surrounded simply by a little mucus.

it is difficult to make out any structures within it during life—even the bacteria in the food vacuoles are rather vague in appearance. No contractile vacuole has been detected. The pseudopodia are very fine, even when the shell opening is comparatively wide (see fig. 7, Pl. 6), and are as a rule soon lost to view among the bacteria, &c., in the surface film. They are never very profuse. Bacteria seem to form a large part of the creature's food and have been observed passing in irregular succession along the pseudopodia into the shell. The shell varies in thickness, &c., in the same way as that of Microgromia. Its inner surface is not so smooth as in Microgromia, and the single opening varies much more in width, being exceedingly narrow in some cases, narrower even than is shown in fig. 8, Pl. 6, and at other times as much as 6–7μ across (see fig. 7, Pl. 6). In preparations stained with Ehrlich's haematoxylin it is seen that no chromidium is present (fig. 9, Pl. 6), although granular protoplasm staining with Heidenhain's haematoxylin may occur in the neighbourhood of the nucleus (figs. 10–13, Pl. 6); and that the nucleus differs from that of Microgromia in having a more variable karyosome. There are generally a few staining granules distributed throughout the nuclear space.

No stages in division have been observed, but the occasional presence of two organisms in one shell suggests that binary fission takes place there.

The relationships of this organism, which does not seem to have been described before, are obscure; and it is only for the sake of temporary convenience that one might place it among the Protomyxidia, along with other forms which cannot at present be included in any of the other groups. It is proposed also to name it provisionally Kibisidytes[1] marinus.

The diagnosis which follows is not conventionally correct in its arrangement; but in the case of such an organism, of unknown affinities and with no known near relations, it is impossible to say which of its characteristics are of generic and which of specific rank.

[1] κίβισις, a wallet. I am indebted to Prof. G. A. Davies for the information that Perseus wore one 'with bright tassels of gold hanging from it'.

Diagnosis.

Kibisidytes marinus, n.gen., n.sp., is a small (10–14μ) amoeboid organism occurring in the sea, attached to the surface film or to some solid object by its shell. This is of a somewhat variable pouch-like form, brown in colour, and usually more or less heavily encrusted with some brown material. The body of the creature often does not fill the space in the shell, and the pseudopodia are very delicate, branched, often appearing beaded, and not very extensive outside the single opening. Solid food, mainly bacteria, is drawn into the shell. No contractile vacuole has been seen. The nucleus contains one to several staining granules irregularly arranged. Binary fission occurs in the shell.

Summary.

Notes on various small protozoa forming brown shells on the surface film of fresh-water are given, including Microgromia elegantula Penard, 1904, and Microcometes paludosa Cienk., 1876. With these is compared a hitherto unrecorded brown-shelled form from the surface film of the sea, for which the name Kibisidytes marinus is proposed, and which for the present must be classed among the Protomyxidia.

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EXPLANATION OF PLATES 5 AND 6.

Plate 5.

Fresh-water Rhizopods from surface film collected on the lower side of coverslips. Figs. 1–3 Microgromia elegantula, Figs. 4 and 5 Microcometes paludosa.

Fig. 1.—A living animal with pseudopodia extended, the shell very

slightly encrusted. *n*, nucleus with large karyosome; *cv*, contractile vacuoles (2); *chr*, position of the chromidium; *rp*, root of pseudopodia, inside shell.

Fig. 2.—A living animal in a heavily encrusted shell, pseudopodia retracted.

Fig. 3.—After staining with Ehrlich's haematoxylin and eosin to show up the extensive chromidium, *chr*, in which the nucleus is embedded.

Fig. 4.—Living animal in encrusted shell with four openings. *cv*, contractile vacuoles (2); *b*, bacteria in food vacuoles.

Fig. 5.—Large specimen after staining with Ehrlich's haematoxylin and eosin. Note absence of chromidium.

PLATE 6.

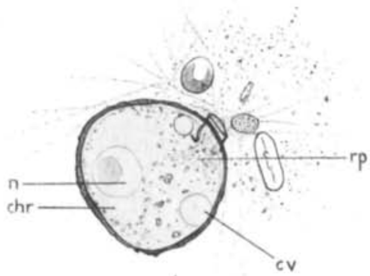
Kibisidytes marinus, n.gen., n.sp., on under side of coverslip.

Figs. 6 and 7.—Living specimens to show different types of shell.

Fig. 8.—Empty thickly encrusted shell.

Fig. 9.—Specimen from the same culture as Fig. 6, showing similar type of encrustation. Stained Ehrlich's haematoxylin and eosin. No chromidium is present. *n*, nucleus.

Figs. 10–13.—Four specimens all from the same preparation, stained Heidenhain's iron haematoxylin and eosin. *n*, nucleus with a few darkly stained granules at its periphery; *f*, solid food in vacuole.



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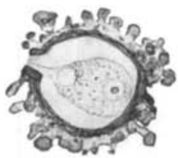

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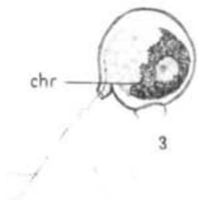

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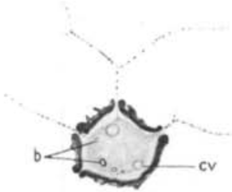

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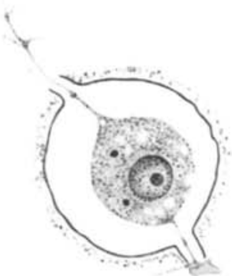

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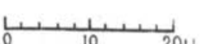

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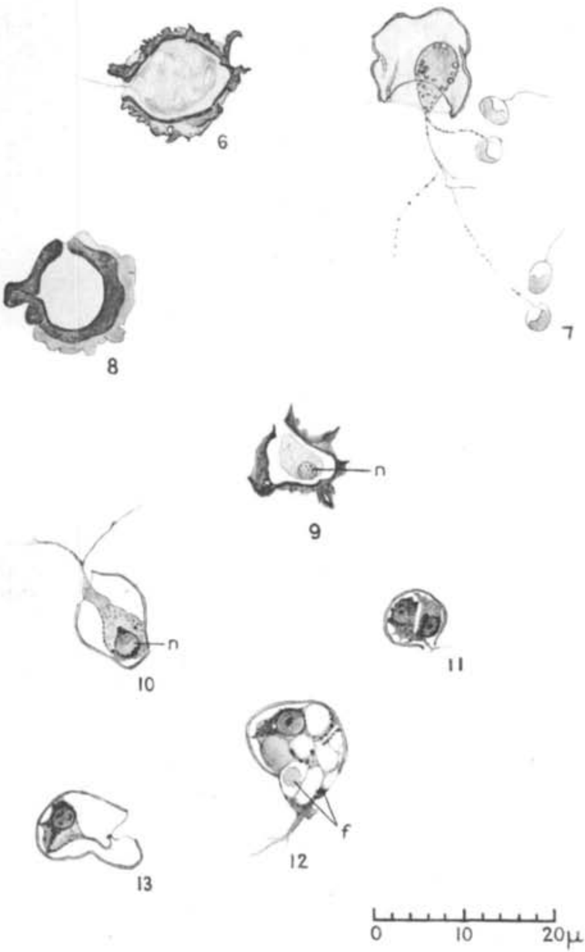

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