

ON A NEW FLAGELLATE, *HEXAMASTIX AGAMAE* N.SP., FROM THE ALIMENTARY CANAL OF THE LIZARD, *AGAMA TUBERCULATA* GRAY.

By S. N. SĀPRE, B.Sc., G.B.V.C., Research Assistant, Imperial Veterinary Research Institute, Mukteswar-Kumaun, U.P.

(Communicated by Mr. J. R. Haddow, O.B.E., F.N.I., I.V.S.)

(Received April 29, 1944.)

In May 1942, six *Agama tuberculata* were collected locally and their intestinal contents examined for protozoa. The caeca of two lizards contained flagellates belonging to genus *Hexamastix*. According to Wenyon (1926), this genus was created by Alexeieff in 1912 for a flagellate found in the intestine of the newt, *Triton toeniatus*. In 1911, it was first placed in the genus *Polymastix* from which he removed it in 1912. Wenyon thought that the flagellates of this genus resembled in every way a member of the genus *Eutrichomastix* Kofoid and Swezy (1915), except that there were six flagella. Wenyon also suggested that these might be related to the forms of *Trichomonas* with five anterior flagella. Derrieu and Raynaud (1914) held a similar view and proposed the name *Hexamastix ardin delleili* for the human form with five free flagella. Mesnil (1915), however, changed the name to *Pentatrichomonas*. According to Grassé (1926) Alexeieff defined the genus *Hexamastix* in 1914 as follows:—'Six unequal flagella which contain between them a fairly well developed axostyle and often jutting out posteriorly. Spherical nucleus anteriorly.' Grassé had recognised the following three species under the genus *Hexamastix*: *H. termitis* (Grassi, 1879) from *Reticulotermis lucifugus*, *H. batrachorum* (Alex. 1911) from batrachians, and *H. gryllotalpae* (Grassé, 1926) (= *Schedoacercomonas gryllotalpae* Grassi, 1879) from the orthopteran, *Gryllotalpa gryllotalpae*. Grassé also stated that the genus *Hexamastix* is autonomous and distinct from the genus *Trichomonas*. The organism to be described in this paper was found to differ from hitherto described species of *Hexamastix* and is named *Hexamastix agamae*. It is of interest to note that this is the first record of a flagellate of the genus *Hexamastix* from India, and the second of its type from a vertebrate host.

Technique:—Fresh preparations of the caecal contents of the lizards were made in normal saline and examined under the microscope. The flagellates were stained by Chatterjee's method as described by Ray (1944). Wet films, showing the flagellates, were fixed in Schaudinn's fluid for 15–20 minutes and subsequently stained with Heidenhain's iron-alum-haematoxylin and Dobell's (1942) ammonium molybdate-haematoxylin. It may be added here that Chatterjee's method gave excellent results and as regards the flagellar disposition, this method was solely relied upon. Haematoxylin preparations were used for studying the nuclear structure and the frontal granules.

OBSERVATIONS ON *HEXAMASTIX AGAMAE* N.SP.

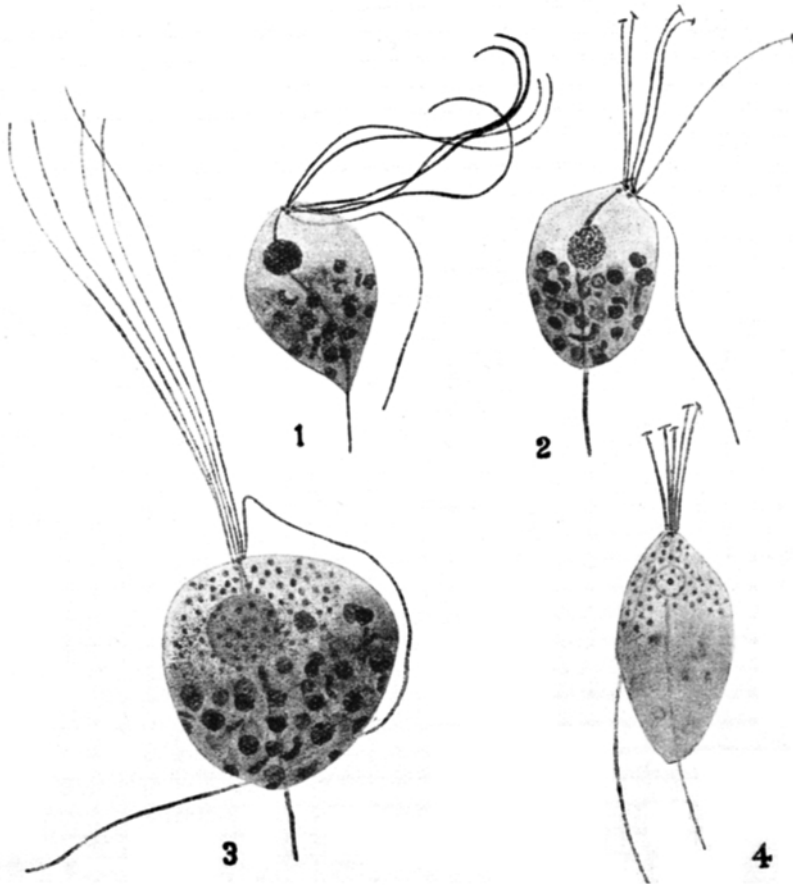
When seen alive, the organism appeared to be more or less pyriform measuring 10μ to 24μ by 5μ to 10μ . In most cases, the axostyle was seen to be projecting from the body for some distance.

The anterior group of five flagella, which were almost equal in length, were seen to beat rhythmically in one cluster. The posteriorly directed trailing flagellum showed a spiral movement. In its mode of swimming, this flagellate corresponded with *H. termitis*, *H. batrachorum* and *H. gryllotalpae*.

In fixed and stained smears the flagellates measured 7μ to 19.6μ by 5μ to 9.8μ . There was more shrinkage in films fixed in Schaudinn's fluid and subsequently stained with haematoxylin than in those stained by Chatterjee's method. In dimensions, therefore,

this organism very closely resembled *H. gryllotalpae* which is 8μ to 18μ by 5μ to 10μ . The cytoplasm was usually loaded with bacteria and other inclusions. The pellicle was prominent and individuals which were free from inclusions showed very finely granular cytoplasm. No special hyaline zone in the region of the nucleus, as described for *H. gryllotalpae*, was seen.

The anterior end was drawn out into a beak-like process and it was just beneath the pellicle of this extreme anterior end that the flagella had their origin from a group of blepharoplasts (figs. 1 and 2). Six flagella originated from three blepharoplasts while axostyle arose from the fourth one. In this respect, it differed from all three previously described species of *Hexamastix*. This arrangement of blepharoplasts was very well seen in preparations stained by Chatterjee's method. The axostyle was a very thin rod-like structure and in most cases, projected for a certain distance beyond the body. In certain cases, the diameter of the axostyle was not larger than that of the flagellum. The projecting portion of the axostyle, in some instances was half as long as the body. Unlike the axostyle of *H. termitis* and *H. batrachorum*, the endo-axostylar granules were absent in this organism. In this feature, it resembled the axostyle of *H. gryllotalpae*,



FIGS. 1 & 2. *Hexamastix agamae* n.sp., $\times 2500$.

Stained by Chatterjee's method.

FIGS. 3 & 4. *Hexamastix agamae* n.sp., $\times 2500$.

Fixed in Schaudinn's fluid and stained by Heidenhain's iron-haematoxylin.

but in no instance the anterior portion of the axostyle was swollen like the bowl of the spoon in order to lodge the nucleus in its groove.

The nucleus was spherical, with diameter 2.5μ to 3μ . Like *H. batrachorum* or *H. termitis*, the nucleus was not apposed to the blepharoplast but situated some distance from it as is the case in *H. gryllotalpae*. The karyosome was usually central in position. The nuclear membrane was thin, and unlike the nucleus of *H. gryllotalpae* was devoid of adherent perinuclear granules. The cytoplasmic area in the anterior portion of the body and sometimes also extending below the nucleus appeared to be of hyaline character and contained a large number of granules, staining deeply with haematoxylin (figs. 3 and 4). These were referred to by Grassé as frontal granules. The presence of these granules obstructed the view and the existence of a rhizoplast could not be established. The frontal granules were usually not stained by Chatterjee's method but instead the whole area took a blue stain. Dividing forms were rarely seen in preparations made directly from the gut-contents. Such forms were in late telophase and showed components of two fully formed individuals.

Diagnosis:—*Hexamastix agamae* n.sp., family *Trichomonadidae*. Size in fixed and stained smears 7μ to 19.6μ , predominantly pyriform; anterior end drawn out into a beak-like process; five anterior flagella lash about rhythmically in a single cluster; the sixth or trailing flagellum describes a spiral movement; there are four blepharoplasts—from the first three originate six flagella, while to the fourth one is attached the slender rod-like axostyle often projecting beyond the body posteriorly; the endo-axostylar granules are absent; nucleus is spherical and contains a central karyosome; it is situated a little below the blepharoplast; frontal granules present; cytostome is situated on one side of the anterior beak-like process.

Habitat:—Caecum of lizard, *Agama tuberculata* Gray.

Locality:—Mukteswar-Kumaun, U.P. (altitude 7,500 ft.).

Acknowledgments:—My thanks are due to Dr. Bains Prasad, Director, Zoological Survey of India, for kindly identifying the lizard for me and to Dr. H. N. Ray, Research Officer in Protozoology, for guidance and assistance in compiling this paper.

REFERENCES.

- Derrieu and Raynaud, M. (1914). Dysenterie chronique a flagelle nouveau. *Bull. Soc. Path. Exot.*, **7**, 571-574.
- Dobell, C. (1942). Some new methods for studying intestinal and other protozoa. *Parasitology*, **34**, 101-112.
- Grassé, P. P. (1926). Contribution à l'étude des flagellés parasites. *Arch. Zool. Exper.*, **65**, 345-602.
- Mesnil, F. (1915). A propos du flagellé nouveau décrit par MM. Derrieu et Raynaud *Pentatrachomonas* = *Hexamastix* D. et R. nec Alexieff). *Bull. Soc. Path. Exot.*, **8**, 574-575.
- Ray, H. N. (1944). A rapid method for staining intestinal flagellates. *Ind. Med. Gaz.*, **24**, 158.
- Wenyon, C. M. (1926). *Protozoology*, 1, Bailliere, Tindall and Cox, London.