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European Journal of Protistology 57 (2017) 61–72

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Morphology and ontogenesis of *Stylonychia* (*Metastylonychia*) *nodulinucleata* nov. subgen. (Ciliophora, Hypotricha) from Australia

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Received 20 July 2016; received in revised form 1 September 2016; accepted 2 September 2016
Available online 10 September 2016

Abstract

Using standard methods, we describe the morphology and ontogenesis of a possibly very rare ciliate, *Stylonychia* (*Metastylonychia*) *nodulinucleata* nov. subgen (basionym: *Stylonychia nodulinucleata* Shi and Li, 1993), isolated from soil of the Murray River floodplain in Australia. Further, we provide an English translation of the Chinese original description. Whether the Australian population is conspecific with the Chinese *Stylonychia nodulinucleata* requires a careful redescription of the latter. The new subgenus is characterized by a moniliform macronuclear strand and an ontogenetic feature, viz., that all cirri remain intact during anlagen formation. The moniliform macronuclear strand and the curved dorsal kineties resemble the stylonychid genus *Coniculostomum*. The species is easily recognized by the large size of the body and oral apparatus, the moniliform macronucleus, and the enormous caudal cirri reaching half of body length. Very likely, *Stylonychia* (*Metastylonychia*) *nodulinucleata* has a restricted distribution.

Keywords: Biogeography; Anlagen formation; Redescription; *Coniculostomum*; *Tetmemena*

Introduction

The genus *Stylonychia* Ehrenberg, 1830 is confined to freshwater with species of the *S. mytilus* complex being the most common ones. Berger (1999), in his detailed revision of the oxytrichids, recognized eleven *Stylonychia* species. However, several of them possibly belong to the genus *Tetmemena* Eigner, 1999. Since Berger's revision, *Stylonychia ammermanni* Gupta et al., 2001 and *S. harbinensis* Shi and Ammermann, 2004 have been added. Nevertheless, Foissner (2016), after reinvestigation of the type slides of *S. ammermanni*, proposed that *S. harbinensis* is very likely a synonym of *S. ammermanni*. Very recently, Foissner (2016) added two new species, namely *S. gibbera* Foissner and Heber in Foissner, 2016 and *S. notophorides* Foissner, 2016 from South America. Here, we describe a curious *Stylonychid* not reported since the original description by Shi and Li (1993), viz., *S. nodulinucleata*.

Material and Methods

Cells of *Stylonychia* (*Metastylonychia*) *nodulinucleata* were activated in December 1998 from resting cysts in an air-dried soil sample from Australia, using the non-flooded Petri dish method (Foissner 2016). For details on location and sample, see species description. Briefly, the non-flooded Petri dish method involves placing 50–500 g litter and soil in

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http://dx.doi.org/10.1016/j.ejop.2016.09.001
0932-4739/

a Petri dish and saturating, but not flooding it, with distilled water. Such a culture is analysed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, 21, and 28. For a detailed description, see Foissner et al. (2002).

Raw cultures could be established in Eau de Volvic containing some squashed wheat kernels and some millilitres of the run-off from the non-flooded Petri dish culture. Live observation, protargol impregnation and scanning electron microscopy (SEM) were performed according to Foissner (1991, 2014). Counts and measurements on silver-impregnated specimens were conducted at a magnification of 1000×. In vivo measurements were performed at magnifications of 40–1000×. Illustrations of live specimens were based on free-hand sketches and micrographs while those of impregnated cells were made with a drawing device. We did not sequence the species because this was not very common when it was rediscovered in 1998. Classification is according to Berger (1999) and Eigner (1997, 1999). Terminology is according to Berger (1999), Foissner and Al-Rasheid (2006), and Wallengren (1900).

Results

Stylonychia Ehrenberg, 1830

Improved diagnosis (includes original data): middle- to large-sized, rigid oxytrichids with two separate macronuclear nodules or a moniliform strand. On average 18 fronto-ventral-transverse cirri. One right and one left marginal cirral row. Undulating membranes in *Stylonychia* pattern. Six dorsal kineties with kinety 4 originating from kinety 3; caudal cirri narrowly or widely spaced and of same length as marginal cirri or distinctly longer. Proter anlage II originates from oral primordium or de novo; anlagen V and VI originate from or close to postoral cirrus V/4. Anlagen IV–VI of the opisthe originate from or close to cirrus V/4. Cirri IV/3 and V/4 not or partially involved in anlagen formation.

Type species: *Stylonychia mytilus* (Mueller, 1773) Ehrenberg, 1830.

Stylonychia (*Stylonychia*)

Diagnosis: macronuclear nodules separate. Cirrus IV/3 and V/4 partially involved in anlagen formation. Proter anlage II develops from oral primordium.

Species included: *Stylonychia* (*Stylonychia*) *mytilus* (type of genus), *S.* (*S.*) *lemnae*, *S.* (*S.*) *ammermanni*, *S.* (*S.*) *curvata*, *S.* (*S.*) *gibbera*, *S.* (*S.*) *notophora*, *S.* (*S.*) *notophorides*, *S.* (*S.*) *pseudograndis*, *S.* (*S.*) *putrina*, *S.* (*S.*) *pusilla*, *S.* (*S.*) *stylomuscorum* (author names and dates, see Berger 1999; Foissner 2016). Some of these very likely belong to the genus *Tetmemena* Eigner, 1999.

Stylonychia (*Metastylonychia*) nov. subgen.

Diagnosis: macronuclear nodules form a moniliform strand. All cirri remain intact during anlagen formation. Proter anlage II possibly originates de novo.

Type species: *Stylonychia nodulinucleata* Shi and Li, 1993.

Species included: *Stylonychia* (*Metastylonychia*) *nodulinucleata* Shi and Li, 1993.

Etymology: composite of the Greek prefix *meta* (associated with, next to, substituted for) and the genus-group name *Stylonychia*, referring to the similarity with *Stylonychia*. Feminine gender.

Description of an Australian *Stylonychia* (*Metastylonychia*) *nodulinucleata* Shi and Li, 1993. (Figs 1A–J, 2A–K, 3A–G, 4A–C, 5A–D, 6A–D; Table 1)

Improved diagnosis (averages are given): size in vivo about 230 × 105 μm; elongate obovate. Macronuclear strand composed of seven nodules; four micronuclei. Cirrus V/4 slightly anterior or at same level as cirrus IV/2; transverse cirri in two groups of three and two. Left marginal row composed of 26 cirri, right of 40. Adoral zone extends about 56% of body length, composed of 59 membranelles. Six dorsal kineties; kineties 1–3 distinctly curved towards right body margin anteriorly; caudal cirri widely spaced, about half of body length, right caudal cirrus optically upon sixth-last cirrus of right marginal row.

Material deposited: eight voucher slides with protargol-impregnated specimens have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), reg. no. 2015/855–862. Relevant specimens have been marked with black ink circles on the coverslip.

Description: most important features of *S.* (*Metastylonychia*) *nodulinucleata* have an ordinary variability (CV ≤ 15%; Table 1), except for the number of macronuclear nodules (CV = 18.4%), the distance between marginal cirral rows and body end (CV = 23.2%), the location of caudal cirri 1 and 2 (CV > 37%), and the gap between caudal cirri 1 and 2 (CV = 23.4%).

Size in ordinary cultures 210–250 × 90–115 μm, usually about 230 × 105 μm in vivo (Table 1); about 340 × 200 μm in exponentially growing cultures used for studying the ontogenesis (Table 1). Body moderately to elongate obovate and slightly to distinctly curved, widest in mid of adoral zone of membranelles; dorsoventrally flattened 2–3:1, anterior and posterior quarter leaf-like and thus hyaline, dorsal central quarters more or less convex, depending on nutrition state, and thus dark and opaque (Figs 1A–C, G, 2A–E, I, J, 3A–D; Table 1). Nuclear apparatus in central quarters of cell, in or slightly left of body's midline; macronucleus about 118 μm long in protargol preparations; on average composed of seven nodules forming a straight or sigmoid moniliform strand with