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Description of *Zoothamnium chlamydis* sp. n. (Protozoa: Ciliophora: Peritrichida), an Ectocommensal Peritrichous Ciliate from Cultured Scallop in North China

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Summary. The morphology, infraciliature and silverline system of an ectocommensal ciliate, *Zoothamnium chlamydis* sp. n., isolated from mantle cavity and on the shell surface of the cultured scallop, *Chlamys farreri* off the coast of Qingdao, China were studied from living and silver-impregnated specimens. The diagnosis for the new species is: marine *Zoothamnium* with alternatively branched stalk; zooids *in vivo* 50-90 x 25-60 µm, slender in shape with one layer of peristomial lip moderately everted, and bacteria covering the whole surface of the cell; one contractile vacuole apically positioned; macronucleus normally band-like, longitudinally oriented. Pellicle with conspicuous, widely spaced transverse striae. Number of silverlines from oral area to aboral ciliary wreath about 27-47; from aboral ciliary wreath to the scopula, 19-29. Zooids generally enlarged at both proximal and distal ends of branches.

Key words: marine peritrich, morphology, *Zoothamnium chlamydis* sp. n.

INTRODUCTION

Ciliated protozoa such as sessile peritrichs play an important role as ectocommensals on the body surface of aquatic organisms from both marine and freshwater habitats (Kahl 1933; Precht 1935; Nenninger 1948; Dietz 1964; Stiller 1971; Green 1974; Bierhof and Roos 1977; Corliss 1979; Valbonesi and Guglielmo 1988; Song 1991a, c, 1992a; Song and Warren 2000). For a long time, much attention has been given to sessile peritrichs of the genus *Zoothamnium*, mainly associated with amphipods, copepods and decapod shrimps. However, as to the author's knowledge, no records of *Zoothamnium*-species attached to marine scallops, have previously been made (Steuer 1932, Kahl 1935, Precht 1935, Raabe and Raabe 1959, Herman and Mihursky 1964, Fenchel 1965, Foster *et al*. 1978, Kumari and Nair 1985, Nagasawa 1986, Song 1992b, Xu *et al*. 1999).

During a survey on parasitic protozoa in molluscs culturing water in the Spring of 2000 off Qingdao, China, an unknown *Zoothamnium* species was found within the mantle cavity as well as on the shell surface of the cultured scallop *Chlamys farreri*. The present paper gives the observations and descriptions on its morphology and silverline system.

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MATERIALS AND METHODS

Host scallop *Chlamys farreri* was collected from the coast of Qingdao (Tsingtao, 36°08' N; 120°43' E), China. Ciliates were removed with a pipette and kept in culture at room temperature. Living observations were carried out using both bright field and differential interference contrast microscopy. Protargol staining according to Wilbert (1975) and Chatton-Lwoff silver nitrate method as described by Corliss (1953) were applied to reveal the infraciliature and silverline system respectively. Accounting and measurements on stained specimens were performed at a magnification of x1250. Drawings were made with the help of a camera lucida.

Terminology and systematic arrangement are mainly according to Corliss (1979), Warren (1986) and Foissner *et al.* (1992).

RESULTS

According to Corliss (1979), the current taxonomic status of this species is given as follows:

Class: Oligohymenophora de Puytorac *et al.*, 1974
Order: Peritrichida Stein, 1859
Suborder: Sessilina Kahl, 1933
Family: Vorticellidae Ehrenberg, 1838
Genus: *Zoothamnium* Bory de St. Vincent, 1826

Zoothamnium chlamydis sp. n. (Figs. 1-10)

Diagnosis: marine *Zoothamnium* with alternatively branched stalk; zooids highly variable in size *in vivo* 50-90 x 25-60 μm, slender body shape with one-layer peristomial lip moderately everted; one contractile vacuole apically positioned; macronucleus normally band-like, longitudinally oriented. Cell surface densely associated with bacteria, and pellicle with conspicuous, widely spaced transverse striae. Number of silverlines from oral area to aboral ciliary wreath (ACW) about 27-47, and from aboral ciliary wreath to scopula, 19-29. Zooids generally enlarged at both proximal and distal ends of branches.

Type specimens: one holotype (HD-00042401) and one paratype (HD-00042402) as protargol-impregnated slides are deposited in the Laboratory of Protozoology, Ocean University of Qingdao, China.

Host and site: *Chlamys farreri*, off the coast of Qingdao (Tsingtao, 36°08' N; 120°43' E), China.

Ecological features: open culturing water, temperature 5-10°C; pH 8.2-8.3, salinity 34-36 ‰.

Morphological description: body constantly slender in shape, widest at peristomial area and narrowed posteriorly with moderately everted, and rigid border (peristomial lip, PL); peristomial disc (PD) small, obliquely elevated when cell is fully extended (Fig. 1). Zooids within same colony conspicuously in two different sizes, mostly ("normal" zooids) *in vivo* about 50-60 μm long, and ratio of length: width *ca* 2:1; but zooids at proximal or distal ends of branches often enlarged, *ca* 70-90 x 50-60 μm (Figs. 2; 11, arrowheads). Formation of telotroch not observed.

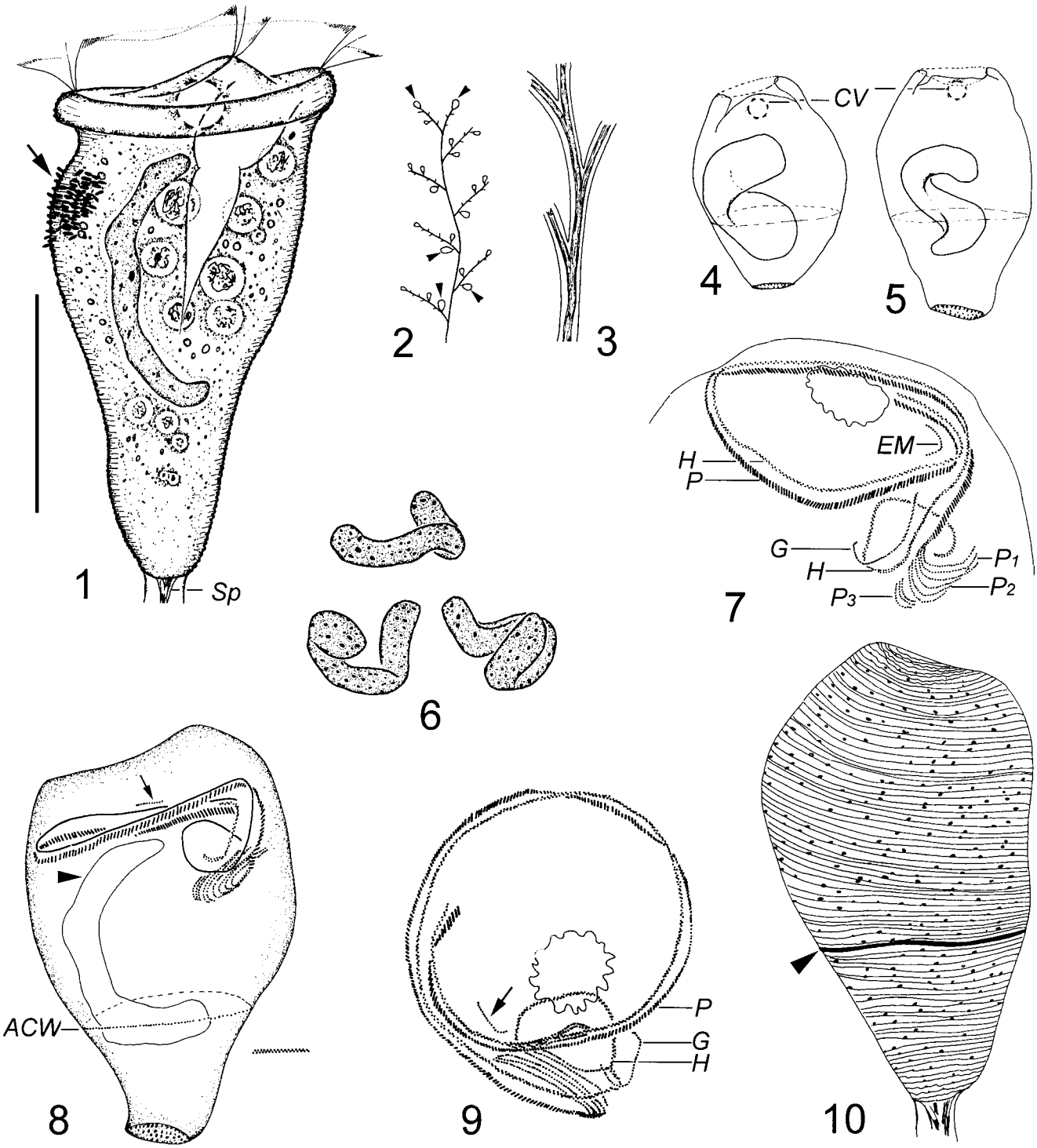
Pellicle with conspicuous transverse striations, especially in enlarged zooids even when observed under low magnification; cell surface often covered with densely associated bacteria (Figs. 1, arrow; 14).

Cytoplasm colorless and transparent, containing several large food vacuoles (4-8 μm across). One contractile vacuole (CV), 5-8 μm in diameter (Figs. 4, 5), at level of peristomial lip on dorsal wall of vestibulum, which extends about 1/2 of body length. Macronucleus (Ma) band-like, longitudinally positioned (Figs. 1; 18, arrowhead), which appears twisted or shortened in enlarged zooids (Figs. 4-6).

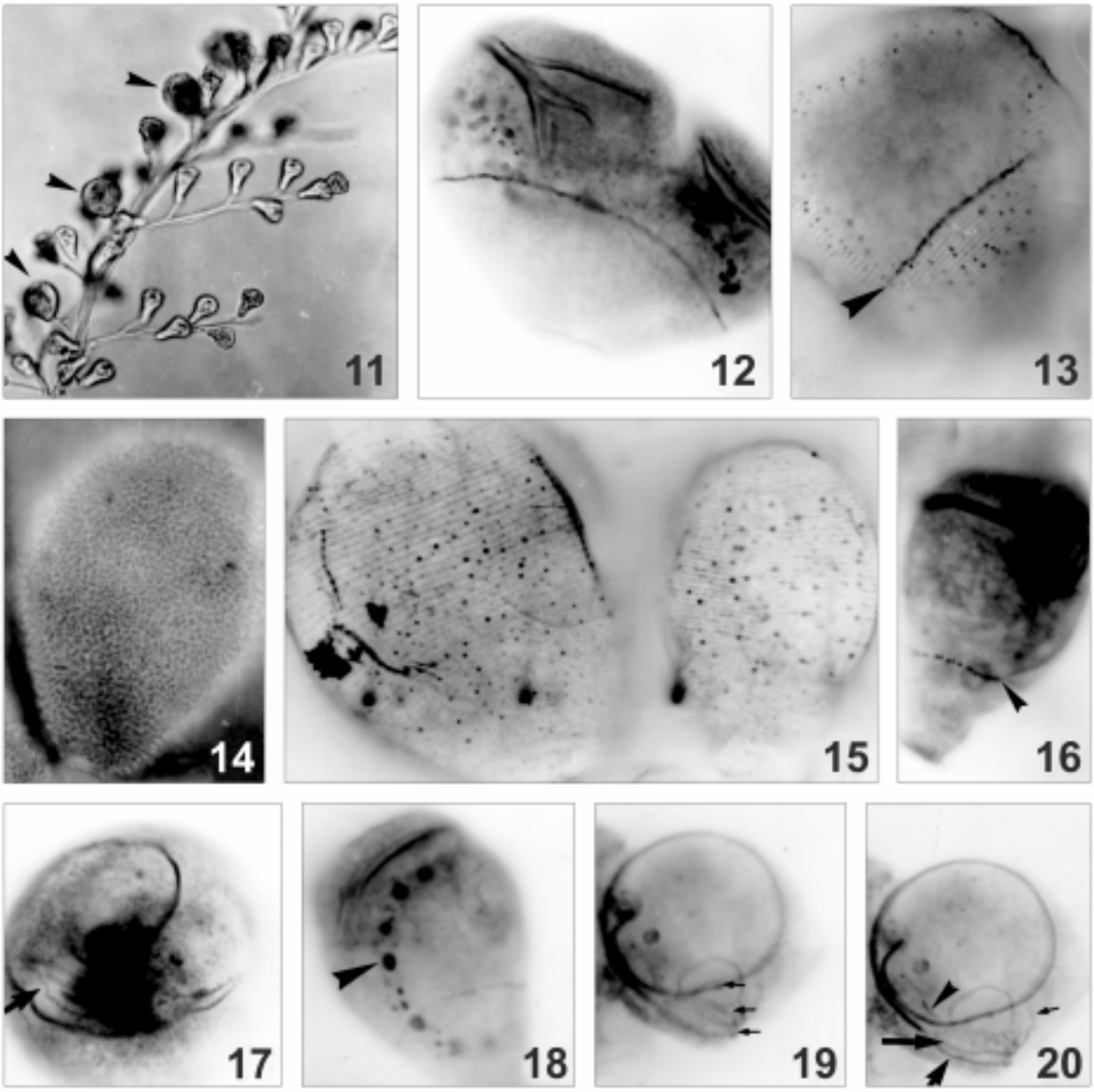
Stalk with smooth surface and thick spasmoneme (about 3 μm across) (Fig. 3). Continuous spasmoneme extending through entire stalk. Colony comparatively large (up to 0.6 mm), alternately branched, with up to 100 zooids, which are also alternatively located in branches (Figs. 2, 11). When disturbed, colony contracts as one unit.

Infraciliature and silverline system: buccal apparatus typical of genus (Figs. 7-9). Haplokinety (H; Fig. 20, long arrow) and polykinety (P; Figs. 7; 9; 20, short arrow) about 1¼ turn around peristomial disc before entering vestibulum. Polykinety forming three peniculi in lower half of vestibulum. Peniculus 1 (P_1) and 2 (P_2) about equal length, each comprising 3 rows of kinetosomes, and peniculus 3 (P_3) rather short, also composed of 3 rows of kinetosomes (Figs. 7; 19, small arrows); haplokinety passing around vestibulum on opposite wall to peniculi. Germinal kinety (G) comparatively long, located parallel to haplokinety (Figs. 7; 20, small arrow). Epistomial membrane (EM) short, near opening of vestibulum (Figs. 7; 8; 9, arrow; 20, arrowhead). Aboral ciliary wreath (ACW) composed of one row of loosely arranged kinetosomes in normal zooids (Figs. 8; 16, arrowhead), while 2-rowed in enlarged ones (Fig. 8, inset).

Silverline system as shown in Figs. 10, 15, widely striated pattern; i. e., striations widely spaced and conspicuous pellicular pores associated between silverlines. Number of silverlines from oral area to aboral ciliary wreath (ACW; Figs. 8; 13, arrowhead), 27-47 (mean 36.2); from ACW to scopula, 19-29 (mean 24.6) (Table 1).



Figs. 1-10. Morphology of *Zoothamnium chlamydis* sp. n. from life (**1-6**) and after silver impregnation (**7-10**). **1** - typical zooid, arrow shows covering of bacteria; **2** - colony, arrowheads show the enlarged zooids; **3** - stalk, note the thick spasmoneme; **4, 5** - contracted and enlarged zooids; **6** -twisted macronucleus in enlarged zooids; **7, 9** - oral apparatus, arrow in 9 indicates epistomial membrane; **8** - general infraciliature, arrow marks epistomial membrane, arrowhead shows macronucleus, inset: aboral ciliary wreath; **10** - silverline system, arrowhead shows aboral ciliary wreath. ACW - aboral ciliary wreath; CV - contractile vacuole; EM - epistomial membrane; G - germinal kinety; H - haplokinety; P - polykinety; P_{1-3} - peniculus 1-3; Sp - spasmoneme. Scale bar - 30 μm



Figs. 11-20. Microphotographs of *Zoothamnium chlamydis* sp. n. based on living and impregnated specimens. **11** - colony, arrowheads show enlarged zooids; **12** - infraciliature in a late stage of morphogenesis; **13, 15** - silverline system, arrowhead marks the aboral ciliary wreath; **14** - bacteria covering the body surface; **16** - infraciliature, arrowhead shows aboral ciliary wreath; **17** - infraciliature in a middle stage of morphogenesis, arrow indicates new oral apparatus; **18** - arrowhead refers to macronucleus; **19, 20** - oral apparatus of the same individual, long arrow shows haplokinety, short arrow indicates polykinety, arrowhead marks epistomial membrane, small arrows in Fig. 19 demonstrate peniculus 1-3 (from lower to upper), small arrow in Fig. 20 shows the germinal kinety

DISCUSSION

As commonly accepted, the genus *Zoothamnium* is characterized by a continuous spasmoneme within a branching stalk, thus leading to a contraction of the entire colony and a transverse silverline pattern (*vs. Zoothamnopsis*) (Ehrenberg 1838, Stein 1854, Claparède and Lachmann 1858, Fauré-Fremiet 1930, Kahl 1933, Bauer-Nebelsick *et al.* 1996, Song 1997). For a long time, the species identification and separation in the genus *Zoothamnium* depended on body shape and size, location of contractile vacuole, oral and nuclear

Table 1. Morphometric characterizations of *Zoothamnium chlamydis* sp. n. Data are based on silver impregnated specimens. Measurements in μm. ACW - adoral ciliary wreath; CV - coefficiency of variation; Max - maximum; Mean - arithmetic mean; Min - minimum; n - number of individuals examined; PL - peristomial lip; Sc - scopula; SD - standard deviation; SE - standard error of mean; SL - silverline system

Character	Min	Max	Mean	SD	SE	CV (%)	n
Normal zooid, body length	32	64	51.6	9.23	2.38	17.9	15
Normal zooid, body width	20	50	31.6	6.53	1.69	20.7	15
Enlarged zooid, body length	56	83	68.5	8.94	2.69	13.0	11
Enlarged zooid, body width	35	60	48.3	8.66	2.61	17.9	11
No. of SL (PL to ACW)	27	47	36.2	6.15	1.49	17.0	17
No. of SL (ACW to Sc)	19	29	24.6	3.68	0.89	14.9	17

apparatus, the habitat, features of the silverline system, and the branching pattern of the colony.

Considering the colony size and alternatively branched stalk, the peristomial lip, body size and marine habitat, 7 species should be compared with the current organism: *Zoothamnium niveum* Ehrenberg, 1838, *Z. alternans* Claparède & Lachmann, 1858, *Z. gleniscum* Claparède & Lachmann, 1858, *Z. plumula* Kahl, 1932, *Z. sinense* Song, 1991 b (Song, 1986, 1991 b), *Z. commune* Kahl, 1933 (*sensu* Song, 1991 b), and *Z. ponticum* Andrussowa, 1886.

Compared with this new species, *Zoothamnium niveum* can be distinguished by the larger size of its normal zooids (120 μm *vs*. 50-60 μm in *Z. chlamydis*), clearly lower position of the contractile vacuole (below peristomial lip *vs*. apically positioned) and presence of typical macrozooids (Ehrenberg 1838, Claparède and Lachmann 1858, Wang and Nie 1932, Kahl 1933, Wailes 1943, Bauer-Nebelsick *et al*. 1996).

Kahl (1933, 1935) redescribed three different forms of *Zoothamnium alternans*, which could not be confused with this new form regardless of the similarity in several morphological features. Among these, *Z. alternans* sensu Claparède & Lachmann, 1858 and *Z. alternans* sensu Greff, 1870 have conspicuously plumper body shapes, larger macrozooids (up to 120 μm) and cross-striated stalk. The form described by Kent (1881) possesses long cylindrical "Mikrogameten", and is hence clearly different from *Zoothamnium chlamydis*.

In terms of body shape, size and branching stalk, *Zoothamnium plumula* Kahl, 1933 is most similar to this new species. However, the latter differs distinctly from the former in having fewer silverlines (*ca* 61 *vs*. 94-98) and the position of epistomial membrane (near opening of vestibulum *vs*. at distal end of polykinety) (Perejaslawzewa 1886; Kahl 1933, 1935; Song and AL-Rasheid, unpublished).

Zoothamnium gleniscum Claparède & Lachmann, 1858 possesses cross-striated stalk, which should therefore, not be conspecific with this new species (Claparède and Lachmann 1858, Kahl 1933).

With reference to the position of contractile vacuole and the appearance of the peristomial lip, comparison should be also made with *Zoothamnium sinense* Song, 1991b and *Z. commune* sensu Song, 1991b. These two species can be distinguished by short and "plump" body shape (i.e. bell-like *vs*. elongated and slender in *Z. chlamydis*), relatively smaller body size (30-60 *vs*. 50-90 μm in length), and zooids of uniform size (*vs*. normal and enlarged ones in *Z. chlamydis*) (Song 1986, 1991b).

Considering body shape, size and branching stalk, an unknown *Zoothamnium* species by Kiesselbach (1936) might be a population of *Zoothamnium alternans* because of the presence of typical macrozooids and the long stalk for each zooid although no detailed position of CV was given.

Unlike *Zoothamnium ponticum* Andrussowa, 1886, our new species has high position of CV (*vs*. significantly below peristomial lip) and longitudinally oriented Ma. (*vs*. transversely in the former).

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