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Two new entodiniomorphid *Triplumaria* ciliates from the intestine of the wild African white rhinoceros

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Abstract

Two new species of *Triplumaria* in the order Entodiniomorphida, *T. alluvia* n. sp. and *T. grypoclunis* n. sp., are described from the large intestine of the wild African white rhinoceros. *T. alluvia* has three bud-shaped caudalia, one broad skeletal plate with a wavy left dorsal edge, and an axe-shaped tail flap. *T. grypoclunis* has three short arched caudalia, two broad skeletal plates, and a pointed and ventrally curved tail flap. These two new species have a C-shaped adoral polybrachykinety, a slender perivestibular polybrachykinety, and paralabial kineties in their retractable adoral ciliary zone. In *T. alluvia*, the perivestibular polybrachykinety is joined to both ends of the adoral polybrachykinety and paralabial kineties along the ventral side of the adoral polybrachykinety, showing the same arrangement as in *Cycloposthium* species. In *T. grypoclunis*, the perivestibular polybrachykinety is joined only to the right end of the adoral polybrachykinety and paralabial kineties along the left ventral side of the adoral polybrachykinety, showing an arrangement analogous to the *Tripalmaria* species.

Keywords: African white rhinoceros; Entodiniomorphida; Infraciliature; *Triplumaria alluvia* n. sp.; *Triplumaria grypoclunis* n. sp

Introduction

Ciliates in the genus *Triplumaria*, order Entodiniomorphida, are found in the digestive tract of several hindgut-fermenting herbivorous mammals. Fourteen *Triplumaria* species have been reported from African elephants (Latteur et al. 1970; Timoshenko and Imai 1995), Asian elephants (Eloff and Van Hoven 1980; Timoshenko and Imai 1995), Indian rhinoceroses (Hoare 1937), and African white rhinoceroses (Van Hoven et al. 1998). The affinity between *Triplumaria* and other entodiniomorphs has been insufficiently explained due to poor knowledge of their infraciliary structures. We will describe two new *Triplumaria* species found in the large intestine of the wild African white rhinoceros and discuss the affinity of *Triplumaria* with other entodiniomorphs based on the arrangement of infraciliary bands of these new species.

Materials and methods

Intestinal contents were obtained from a wild African white rhinoceros (*Ceratotherium simum*) shot by hunters in Pilanesburg Game Reserve in the North West

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Province of the Republic of South Africa (27°E, 26°S) and were the same as the sample used in Ito et al. (2006). These intestinal contents were immediately fixed in two times the volume of formalin solution (14% aq) and were stored in the dark for later study. The infraciliary bands were stained by the pyridinated silver carbonate impregnation method, following Ito and Imai (1998). The orientation of ciliates used by Dogiel (1927) was adopted: the side beneath which the macronucleus lies was termed the dorsal side and the opposite one the ventral side, defining the right and left sides. Cell measurements were made from a sample of 20 fixed cells using a calibrated micrometer. Body length was determined as the distance between the anterior and posterior ends of the body. The term, polybrachykinety, refers to infraciliary bands composed of numerous, short, parallel kineties (Fernández-Galiano et al. 1985; Ito and Imai 1998).

Results

Two new entodiniomorphid species were found in the proximal colon of the African white rhinoceros in southern Africa. Because of a retractable adoral ciliary zone, three non-retractable ciliary tufts or short arches and the broad skeletal plate beneath the body surface, they are identified as members of the genus *Triplumaria.*

Triplumaria alluvia n. sp.

Description

The body is wedge-shaped with a rounded dorsal surface and a straight ventral surface, and is laterally compressed. The body is slightly narrowed in the posterior part, forming the axe-shaped tail flap. A shallow groove runs longitudinally along the dorsal side from the anterior end of the body to the base of the tail flap. The left edge of the dorsal groove is wave-shaped whereas the right edge is rounded smoothly. The adoral ciliary zone is retractable into the anterior end of the body. Three non-retractable somatic ciliary tufts arise from bud-shaped caudalia. The anterior caudalium lies on the dorsal body surface at the level of the adoral ciliary zone and two posterior caudalia are situated at dorsal and ventral sides of the narrowed part of the body. The macronucleus is wedge-shaped, lying beneath the dorsal surface of the body, and varying in shape. The micronucleus is small and ovoid, lying on the left side of the anterior one-third of the macronucleus. The vestibulum is hourglass-shaped and its posterior part expands significantly. The cytoproct is located behind the posterior ventral caudalium. One broad skeletal plate surrounds the right, ventral and left subsurfaces of the body. The left dorsal edge of the plate is wavy and the right dorsal edge is smooth. Beneath the dorsal groove, the skeletal rod extends from the anterior end of the body to the posterior dorsal caudalium. Two contractile vacuoles lie along dorsal left side of the macronucleus (Table 1; Figs 1–13).

Habitat, type host and locality

The large intestine (proximal colon) of the African white rhinoceros (*C. simum*) in Pilanesburg Game Reserve, South Africa (27°E, 26°S).

Etymology

T. alluvia is named after the wavy dorsal left edge of the skeletal plate (L. *alluvius*, detritus deposited by running water).

Type material

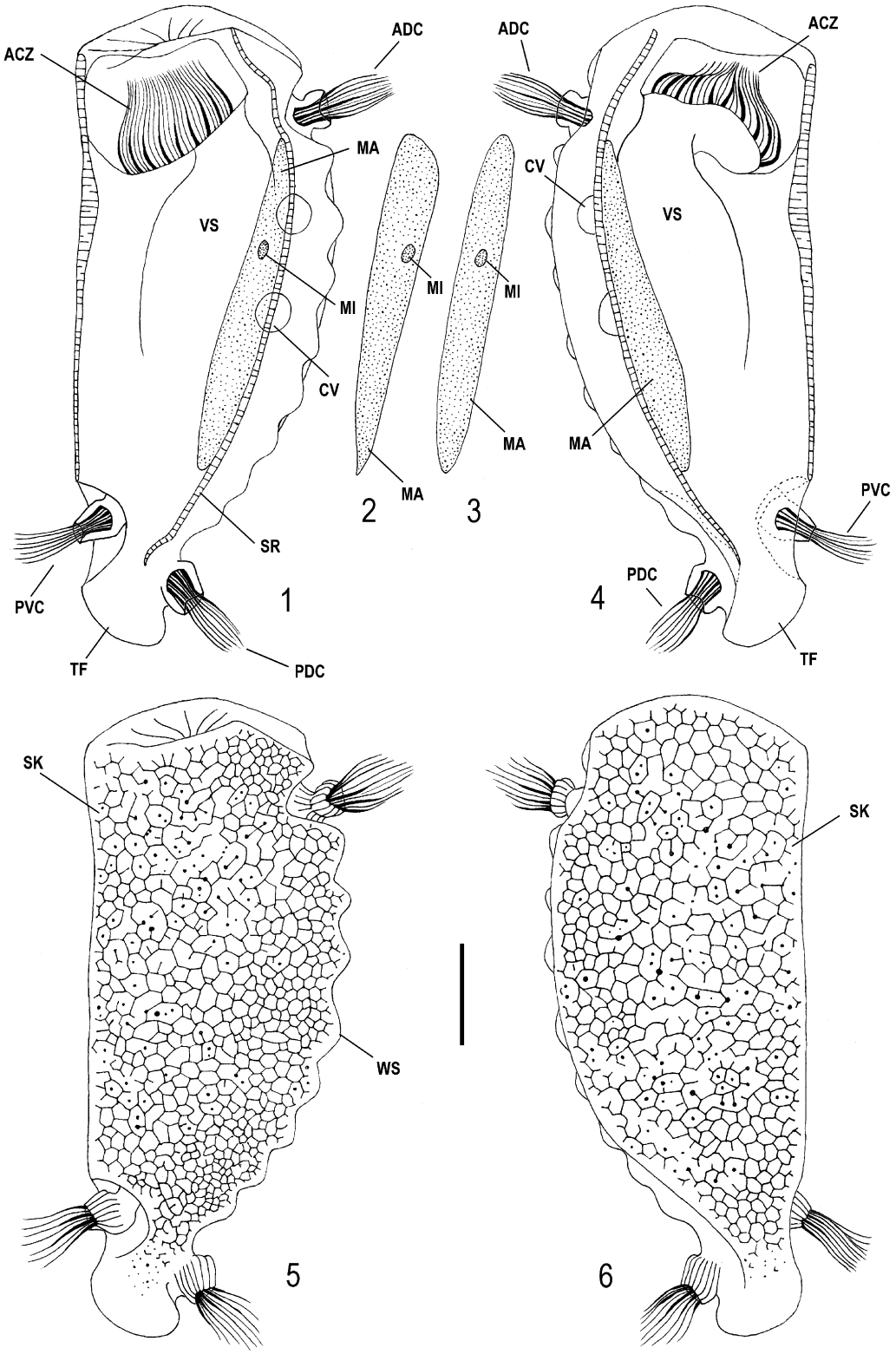
Holotype slides (NSMT-Pr189) are deposited in The National Science Museum, Tokyo, Japan.

Remarks

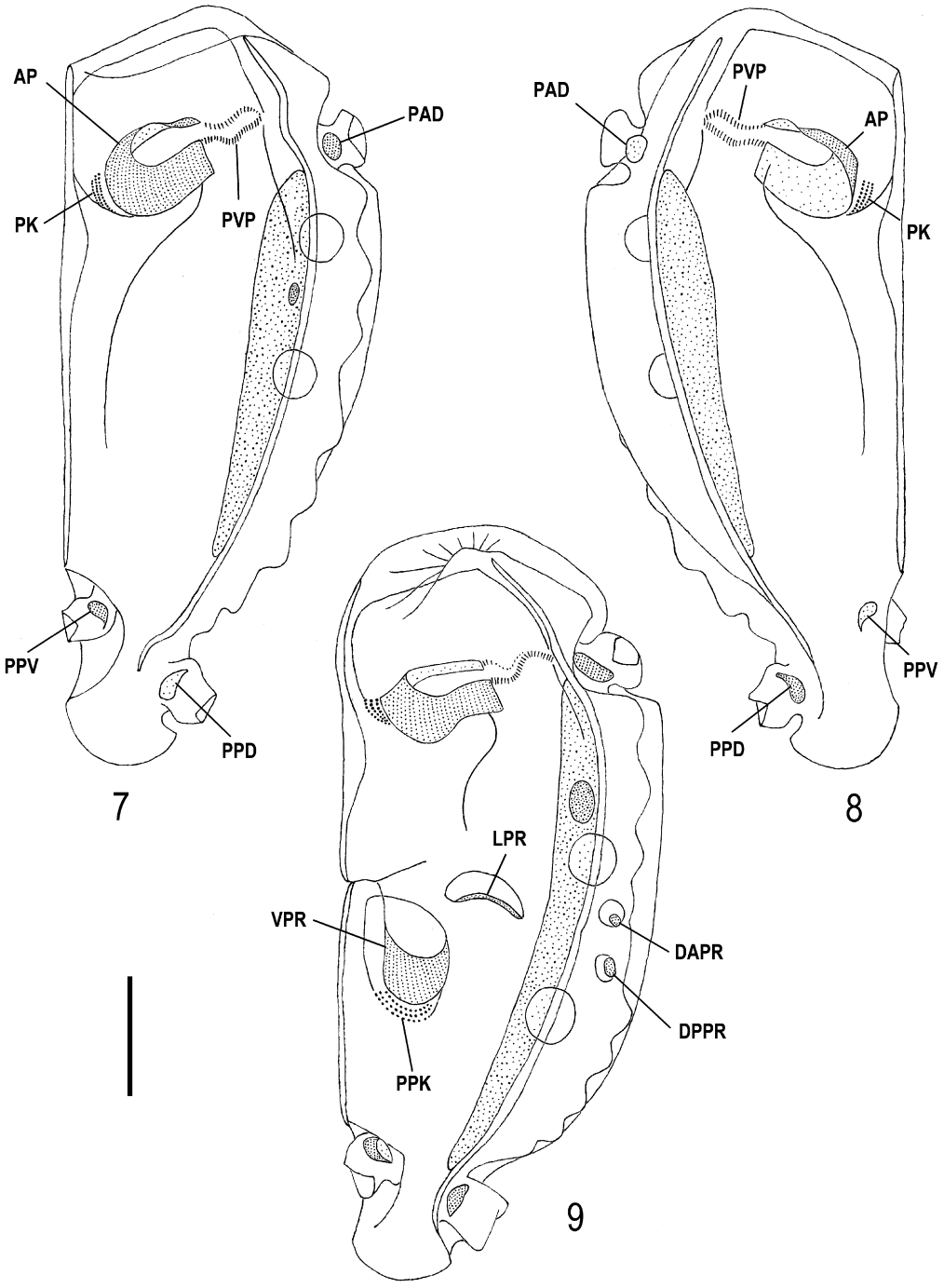
T. alluvia is distinguishable from other *Triplumaria* species by the characteristic skeletal plate with a wavy dorsal left edge and bud-shaped caudalia.

Table 1. Measurements (μm) and morphometric ratios (mean ± S.D. (minimum–maximum); $n = 20$) of *Triplumaria alluvia* and *Triplumaria grypoclunis*

	Triplumaria alluvia	*Triplumaria grypoclunis*
Body length	127.4 ± 12.6 (104.6–158.1)	106.1 ± 17.0 (68.9–137.7)
Body width	53.6 ± 7.0 (48.5–74.0)	45.4 ± 8.8 (30.6–58.7)
Body length/body width	2.40 ± 0.24 (1.93–2.74)	2.36 ± 0.18 (2.05–2.85)
Macronuclear length	67.1 ± 11.0 (48.5–91.8)	73.6 ± 11.9 (45.9–94.4)
Macronuclear length/body length	0.52 ± 0.06 (0.39–0.67)	0.69 ± 0.02 (0.67–0.72)
Distance from anterior end of the macronucleus to the micronucleus	22.1 ± 3.8 (15.3–30.6)	15.2 ± 4.2 (10.2–23.0)
Distance from anterior end of the macronucleus to the micronucleus/macronuclear length	0.33 ± 0.05 (0.27–0.45)	0.20 ± 0.04 (0.15–0.28)



Figs 1–6. Schematic figures of *Triplumaria alluvia* n. sp.: (1) cell from left side, (2, 3) macronucleus and micronucleus from left side, (4) cell from right side, (5) skeletal plate seen from left side, and (6) skeletal plate seen from right side. ACZ, adoral ciliary zone; ADC, anterior dorsal caudalium; CV, contractile vacuole; MA, macronucleus; MI, micronucleus; PDC, posterior dorsal caudalium; PVC, posterior ventral caudalium; SK, skeletal plate; SR, skeletal rod; TF, tail flap; VS, vestibulum; and WS, wavy edge of skeletal plate. Bar = 20 μm.

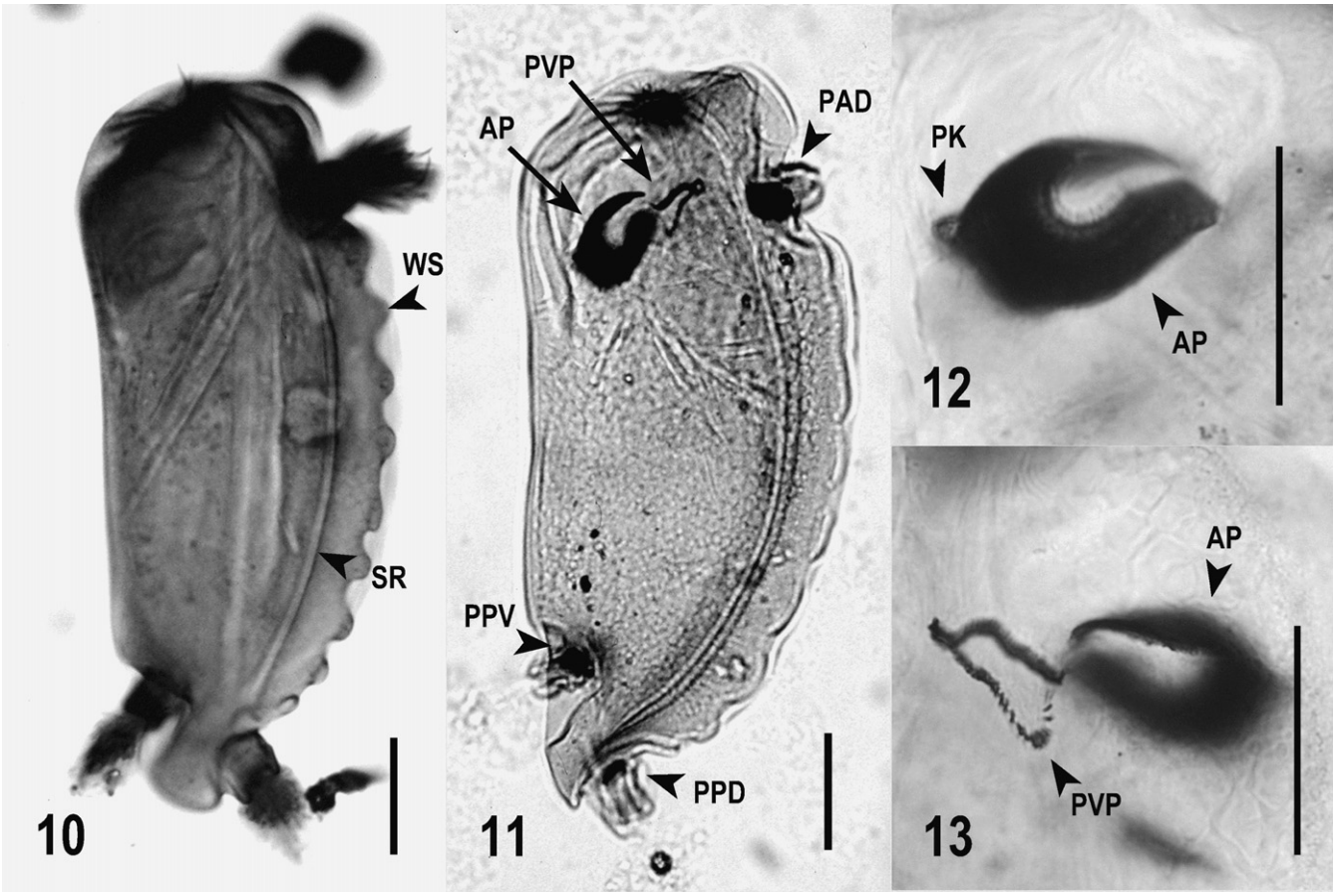


Figs 7–9. *Triplumaria alluvia* after silver impregnation: (7) cell from left side, (8) cell from right side, and (9) cell during binary fission seen from left side. AP, adoral polybrachykinety; DAPR, dorsal anterior primordium; DPPR, dorsal posterior primordium; LPR, left primordium; PAD, polybrachykinety of anterior dorsal caudalium; PK, paralabial kineties; PPD, polybrachykinety of posterior dorsal caudalium; PPK, primordium of paralabial kineties; PPV, polybrachykinety of posterior ventral caudalium; PVP, perivestibular polybrachykinety; and VPR, ventral primordium. Bar = 20 μm.

Triplumaria grypoclunis n. sp.

Description

The body is nearly rectangular with a rounded dorsal surface and a straight ventral surface, and is laterally compressed. The body is narrowed in the posterior part, forming the pointed and ventrally curved tail flap. A groove runs longitudinally along the dorsal right side from the anterior end of the body to the extremity of the tail flap. The adoral ciliary zone is retractable into the anterior end of the body. Three non-retractable somatic ciliary arches arise from obliquely short caudalia. The



Figs 10–13. Micrographs of *Triplumaria alluvia* after pyridinated silver carbonate impregnation: (10–12) from left side and (13) from right side. AP, adoral polybrachykinety; PAD, polybrachykinety of anterior dorsal caudalium; PK, paralabial kineties; PPD, polybrachykinety of posterior dorsal caudalium; PPV, polybrachykinety of posterior ventral caudalium; PVP, perivestibular polybrachykinty; SR, skeletal rod; and WS, wavy edge of skeletal plate. Bar = 20 μm.

anterior caudalium lies on the dorsal body surface at the level of the adoral ciliary zone. The posterior ventral caudalium lies opposite to the posterior end of the macronucleus. The posterior dorsal caudalium lies in the groove of the tail flap on the posterior right side of the body. The macronucleus is elongated and wedge-shaped, lying beneath the dorsal surface of the body. The micronucleus is small and ovoid, lying on the dorsal left side of anterior one-fifth of the macronucleus. The vestibulum extends posteriorly to expand slightly. The vestibular opening has a slit at the left side. The cytoproct is located behind the posterior ventral caudalium. Two broad skeletal plates lie beneath the right and left surfaces of the body. The skeletal rod extends alongside the macronucleus. Four contractile vacuoles lie beneath the dorsal side of the body (Table 1; Figs 14–26).

Habitat, type host and locality

The large intestine (proximal colon) of the African white rhinoceros (*C. simum*) in Pilanesburg Game Reserve, South Africa (27°E, 26°S).

Etymology

T. grypoclunis is named after the curved tail flap (Gr. *grypo-*, curved; L. *clunis*, rump).

Type material

Holotype slides (NSMT-Pr190) are deposited in The National Science Museum, Tokyo, Japan.

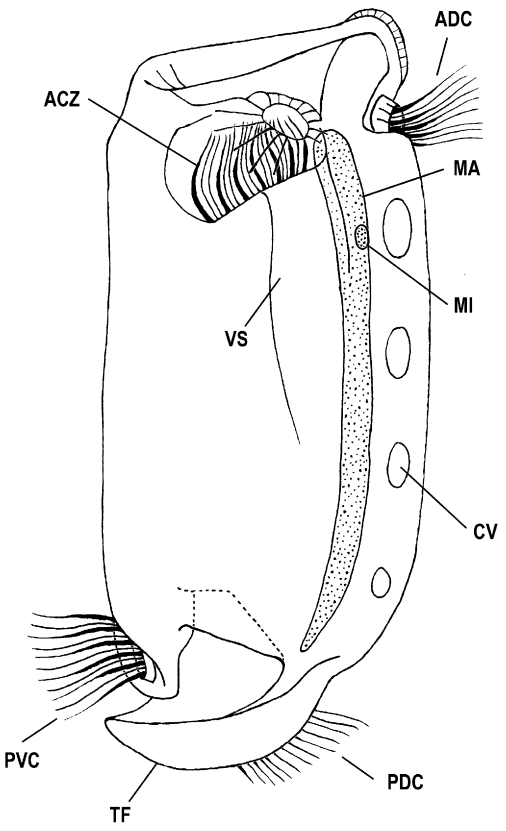
Remarks

T. grypoclunis is distinguished from other *Triplumaria* species by the ventrally curved tail flap and two broad skeletal plates, right and left.

Infraciliature of *T. alluvia* and *T. grypoclunis* (Figs 7–9, 11–13, 18–22, 24–26)

Buccal polybrachykineties and paralabial kineties

T. alluvia and *T. grypoclunis* have an adoral polybrachykinety (AP), a perivestibular polybrachykinety (PVP), and paralabial kineties (PK) in the adoral ciliary zone. Polybrachykinety arrangements are different between *T. alluvia* and *T. grypoclunis*.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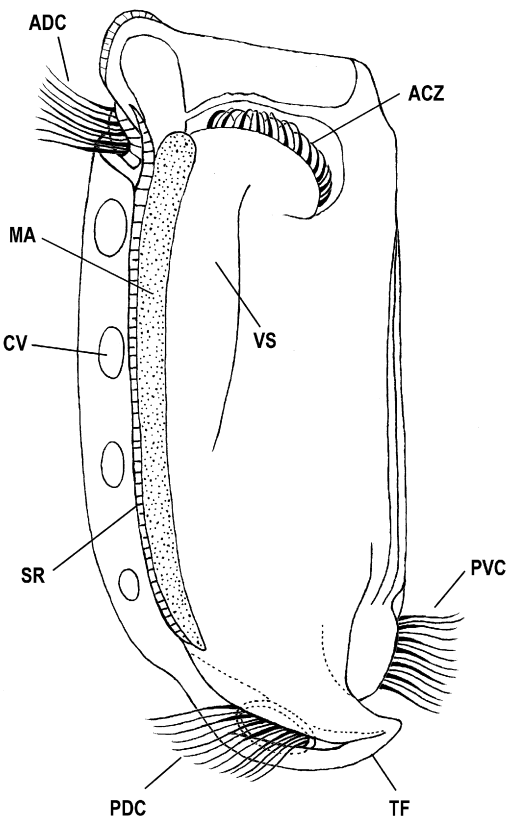
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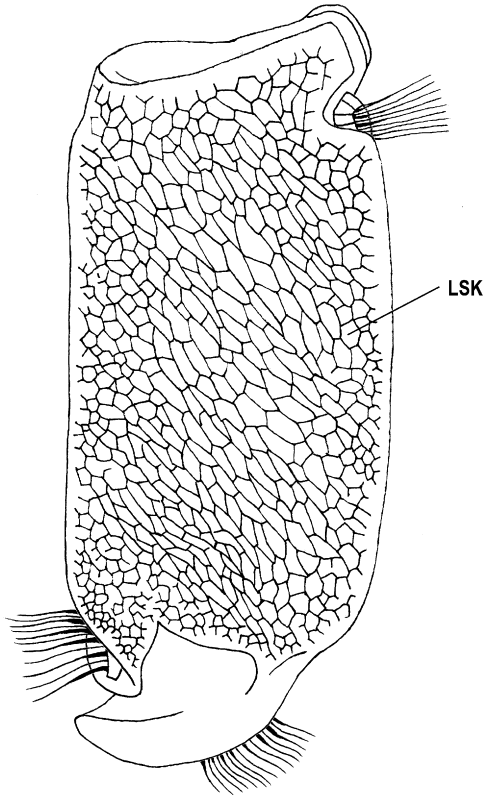
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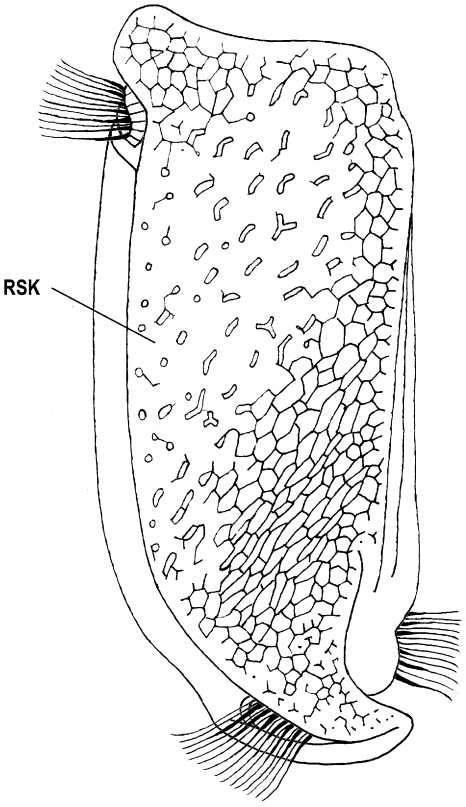
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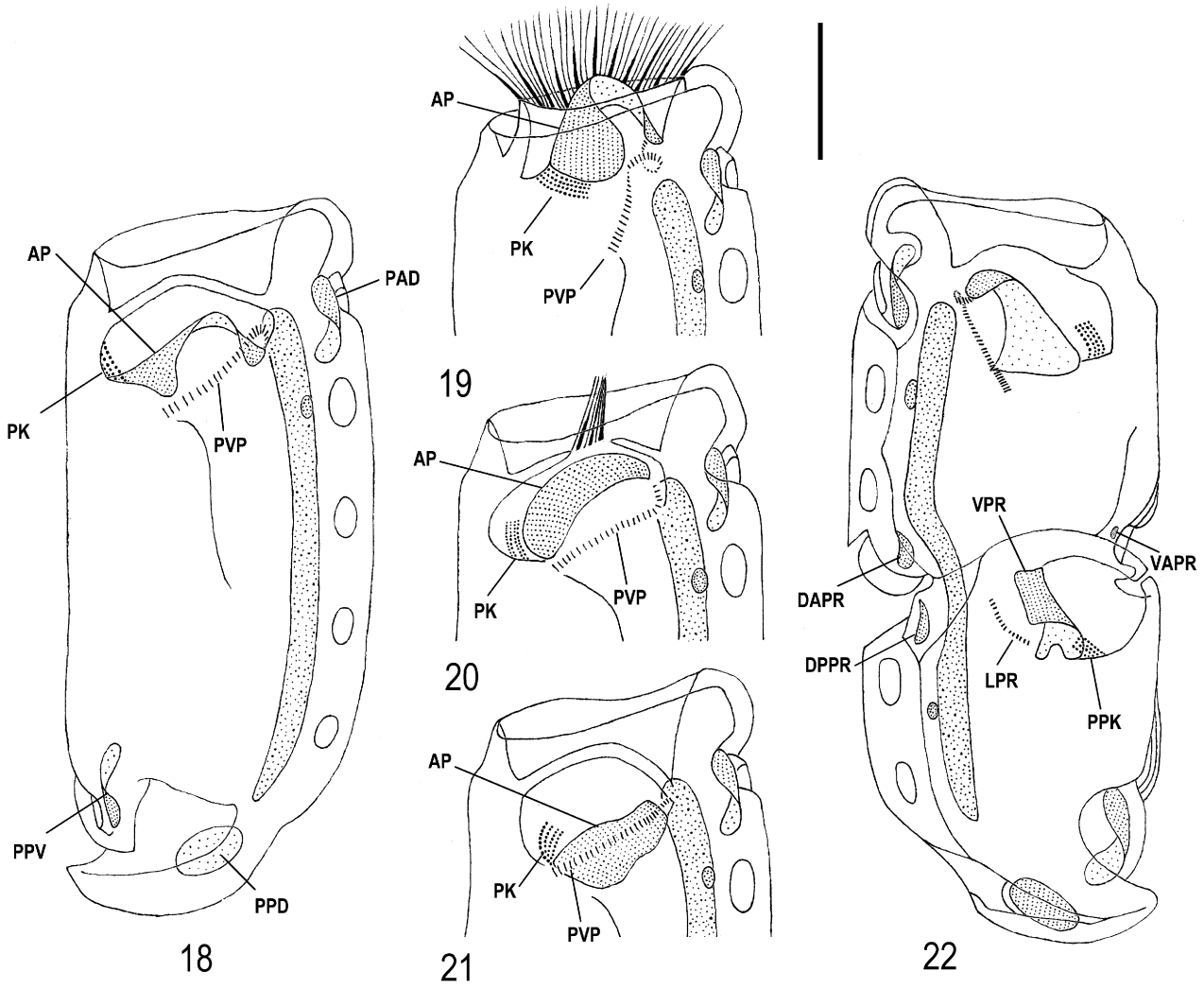


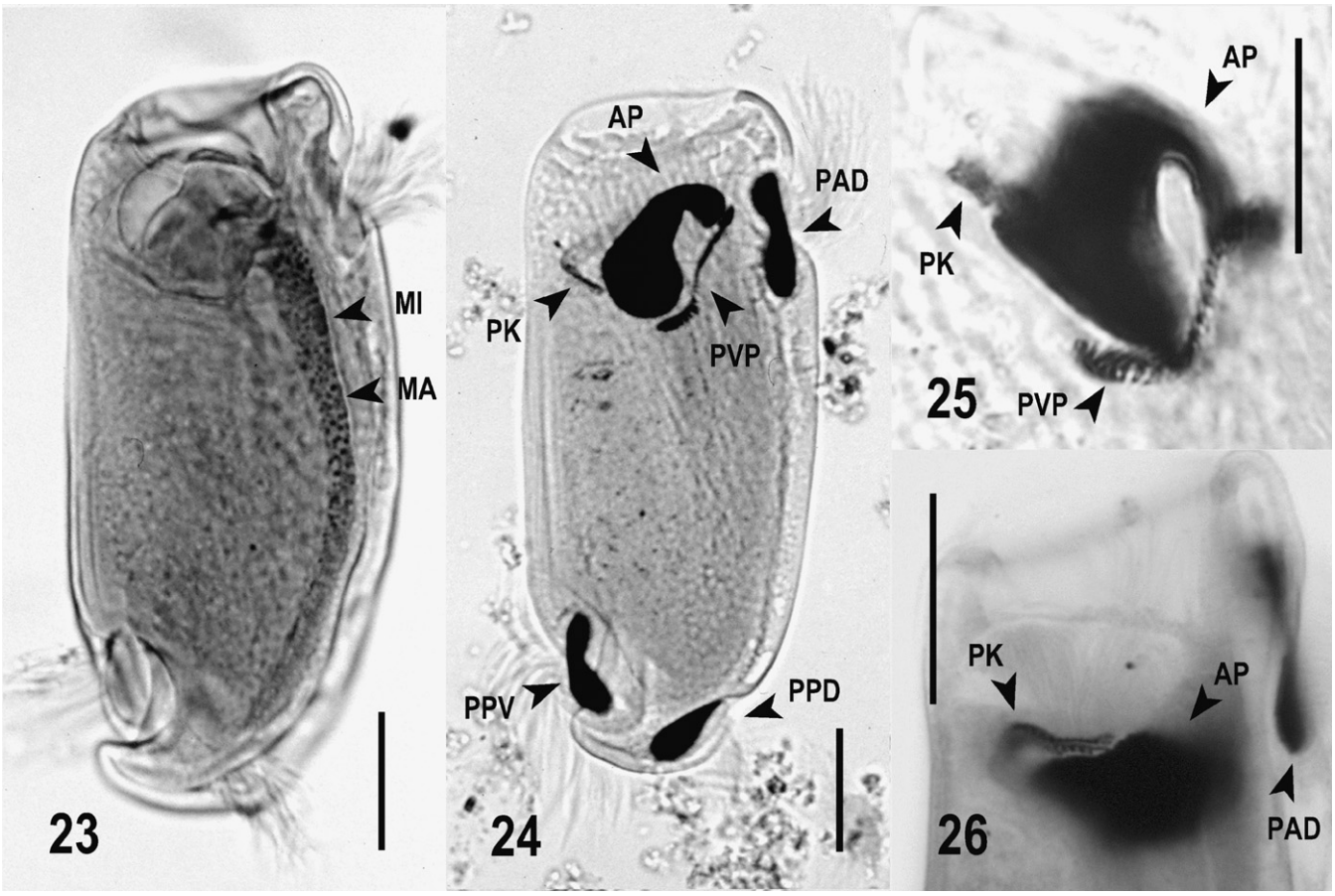
Figs 18–22. *Triplumaria grypoclunis* after silver impregnation: (18) cell from left side, (19–21) anterior part from left side, and (22) cell during binary fission seen from right side. AP, adoral polybrachykinety; DAPR, dorsal anterior primordium; DPPR, dorsal posterior primordium; LPR, left primordium; PAD, polybrachykinety of anterior dorsal caudalium; PK, paralabial kineties; PPD, polybrachykinety of posterior dorsal caudalium; PPK, primordium of paralabial kineties; PPV, polybrachykinety of posterior ventral caudalium; PVP, perivestibular polybrachykinety; VAPR, ventral anterior primordium; and VPR, ventral primordium. Bar = 20 μm.

The AP is wide and extends along the ventral side of the vestibular opening. The AP of *T. alluvia* is C-shaped whereas the AP of *T. grypoclunis* changes its shape according to the movement of the adoral ciliary zone. The AP of *T. grypoclunis* is C-shaped when protruded and retracted (Figs 18, 19, 24, 25) but it extends to be nearly straight when strongly retracted (Figs 20, 21, 26)

The PVP is narrow and extends along the dorsal edge of the vestibular opening. *T. alluvia* has the PVP joining to both the right and left ends of the AP. The AP and the PVP of *T. alluvia* completely surround the vestibular opening. On the other hand with *T. grypoclunis*, the PVP joins to only the right end of the AP. The PVP of *T. grypoclunis* extends from the right end of the AP along the dorsal edge of the vestibular opening and runs down along the dorsal edge of the vestibular slit to the posterior end of the slit.

The PK is composed of more than four short transverse kineties, which extend along the AP. The PK of *T. alluvia* extends along the ventral side of the AP whereas the PK of *T. grypoclunis* extends along the left ventral side of the AP. Kinetids in the

Figs 14–17. Schematic figures of *Triplumaria grypoclunis* n. sp.: (14) cell from left side, (15) cell from right side, (16) left skeletal plate, and (17) right skeletal plate. ACZ, adoral ciliary zone; ADC, anterior dorsal caudalium; CV, contractile vacuole; LSK, left skeletal plate; MA, macronucleus; MI, micronucleus; PDC, posterior dorsal caudalium; PVC, posterior ventral caudalium; RSK, right skeletal plate; SR, skeletal rod; TF, tail flap; and VS, vestibulum. Bar = 20 μm.



Figs 23–26. Micrographs of *Triplumaria grypoclunis* after pyridinated silver carbonate impregnation: (23–26) from left side. AP, adoral polybrachykinety; MA, macronucleus; MI, micronucleus; PAD, polybrachykinety of anterior dorsal caudalium; PK, paralabial kineties; PPD, polybrachykinety of posterior dorsal caudalium; PPV, polybrachykinety of posterior ventral caudalium; and PVP, perivestibular polybrachykinty. Bar = 20 μm.

PK are slightly larger than in kineties in other polybrachykineties.

Somatic infraciliature

T. alluvia and *T. grypoclunis* have infraciliary bands of three caudalia (polybrachykinety of anterior dorsal caudalium (PAD), polybrachykinety of posterior dorsal caudalium (PPD), and polybrachykinety of posterior ventral caudalium (PPV)) on the dorsal and ventral surfaces. The PAD, PPD and PPV are very short and nearly circular in *T. alluvia*, whereas they are longer in *T. grypoclunis*.

Infraciliary primordial

T. alluvia and *T. grypoclunis* show the same pattern of division. During binary fission, six primordia appear. Beneath the middle dorsal surface of the body, two primordia, dorsal anterior primordium (DAPR) and dorsal posterior primordium (DPPR), appear. They are primordia of the PPD of the proter and the PAD of the opisthe, respectively. At the same time, two primordia, one beneath the left surface (LPR) and the other beneath the ventral surface (VPR), appear and develop into the PVP and the AP of the opisthe. Several kineties of the primordium of the paralabial kineties (PPK) arise along the ventral side of the VPR. After arising of these five primordia, one primordium of the PPV of the proter (VAPR) appears beneath the ventral surface immediately anterior to the VPR.

Discussion

Ciliates in the genus *Triplumaria*, order Entodiniomorphida Doflein and Reichenow, 1929, have a retractable adoral ciliary zone and are characterized by the presence of three non-retractable somatic ciliary zones and a large skeletal plate beneath the right, ventral and left sides of the body. The type species of the genus *Triplumaria* is *Triplumaria hamertoni*, Hoare, 1937. *Triplumaria* was classified into the Family Cycloposthiidae Poche 1913 by Hoare (1937) and was transferred from the Cycloposthiidae to the Tripalmariidae (Bonhomme et al. 1989; Grain 1994), when the Family Tripalmariidae Bonhomme et al., 1989 was created. Following the

system of Bonhomme et al., *Triplumaria* belongs to the family Tripalmariidae.

The infraciliature of *Triplumaria* species were first revealed when Latteur et al. (1970) described *Triplumaria selenica*. However, the infraciliary bands of *T. selenica* differ considerably from general patterns of infraciliature in entodiniomorphs and from the two new *Triplumaria* species we describe. In *T. selenica*, according to this description, the AP does not surround the ventral side of the vestibular opening; the PK is very long and circles completely around the AP; the infraciliature along the edge of the vestibular opening is not a polybrachykinety but a single transverse kinety; the AP and the kinety along the vestibular opening are joined by kinetids gathering in a rhomboid; and the three polybrachykineties of the caudalia are ring-shaped. Because of such irregular features, we consider that the infraciliary structure of *T. selenica* described by Latteur et al. (1970) is likely to be incorrect. On the other hand, the infraciliary bands of two new *Triplumaria* species, *T. alluvia* and *T. grypoclunis*, suggest that the genus *Triplumaria* follows the general pattern of the entodiniomorphs.

T. alluvia has the same infraciliary bands as those of *Cycloposthium* and *Monoposthium* species (Fernández-Galiano 1959; Ito and Imai 2000; Ito et al. 2002). The vestibular opening is completely surrounded by the wide C-shaped AP and the slender PVP which joins to both the right and left ends of the AP (Figs 7–9, 12, 13).

T. grypoclunis has characteristic infraciliary bands intermediate between *Cycloposthium* and *Tripalmaria* species. The AP of *T. grypoclunis* is wide and C-shaped when protruded and retracted (Figs 18, 19), but, when strongly retracted, it extends to be straight (Figs 20, 21). Such a distinct morphological change of the AP has not been observed in other entodiniomorphs. The AP is analogous to those of *Cycloposthium*, *Monoposthium* species and *T. alluvia*, whereas the PVP joins to only the right end of the AP. The AP and the PVP of *T. grypoclunis* therefore do not completely surround the vestibular opening and the left end of the AP is a considerable distance from the PVP. The PVP of *T. grypoclunis* is similar to the PVP of *Tripalmaria dogieli*. *T. dogieli* has two PVP with uneven lengths; one PVP is longer, extending dorsally from the right end of the AP to turn ventrally, the other is short and extends dorsally from the left end of the AP (Wolska 1978). The PVP of *T. grypoclunis* is very similar to the longer PVP of *T. dogieli*. The AP and PVP of *T. dogieli* are arranged horizontally, but the PVP of *T. grypoclunis* is considerably inclined.

In spite of such differences in infraciliary bands between *T. alluvia* and *T. grypoclunis*, these two species have a common feature that no infraciliary band runs down inside the vestibulum and the arrangement of their infraciliary bands suggests that the genus *Triplumaria* is closely related to the genera *Cycloposthium*, *Monoposthium* and *Tripalmaria*.

Entodiniomorphs in the genera *Tricaudalia*, *Rhabdothorax*, *Rhinozeta*, and *Gilchristia* have three somatic ciliary zones and skeletal plates as in *Triplumaria* and *Tripalmaria* (Dehority 1986; Grain 1994; Ito et al. 2006; Kornilova 2004; Latteur and Dufey 1967). They have been described based on general morphological features, except for *Gilchristia* whose infraciliary bands were described (Ito et al. 2006). *Gilchristia* species have the vestibular polybrachykinety extending inside the vestibulum which is significantly different from the PVP of *Triplumaria*. Comparisons in future studies on the infraciliature of these genera will lead to valuable knowledge on the taxonomy of entodiniomorphs.

Acknowledgment

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References

Bonhomme, A., Grain, J., Coliet, J.Y., 1989. Etude ultrastructurale de *Troglodytella gorillae*, Cilié de l'intestin des Gorilles. Eur. J. Protistol. 24, 225–237.

Dehority, B.A., 1986. Protozoa of the digestive tract of herbivorous mammals. Insect Sci. Appl. 7, 279–296.

Dogiel, V.A., 1927. Monographie der Familie *Ophryoscolecidae*. Arch. Protistenkd. 59, 1–288.

Eloff, A.K., Van Hoven, W., 1980. Intestinal protozoa of the African elephant *Loxodonta africana* (Blumenbach). S. Afr. J. Zool. 15, 83–90.

Fernández-Galiano, D., 1959. La infraciliación en *Cycloposthium edentatum* Strelkow. Bol. Real. Soc. Esp. Hist. Nat. Biol. 57, 139–150.

Fernández-Galiano, T., Serrano, S., Fernández-Galiano, D., 1985. General morphology and stomatogenesis of two species of the genus *Entodinium* (Ciliophora, Entodiniomorphida). Acta Protozool. 24, 181–186.

Grain, J., 1994. Classe Vestibuliferea de Puytorac et al. 1974. In: Grassé, P.-P. (Ed.), Traité de Zoologie, vol. 2/2. Masson, Paris, pp. 311–379.

Hoare, C.A., 1937. A new cycloposthiid ciliate (*Triplumaria hamertoni* gen. n., sp. n.) parasitic in the Indian rhinoceros. Parasitology 29, 559–569.

Ito, A., Imai, S., 1998. Infraciliary bands in the rumen ophryoscolecid ciliate *Ostracodinium gracile* (Dogiel, 1925), observed by light microscopy. J. Eukaryot. Microbiol. 45, 628–636.

Ito, A., Imai, S., 2000. Ciliates from the cecum of Capybara (*Hydrochoerus hydrochaeris*) in Bolivia 2. The family Cycloposthiidae. Eur. J. Protistol. 36, 169–200.

Ito, A., Miyazaki, Y., Imai, S., 2002. Descriptions of new *Parentodinium* ciliates in the family Parentodiniidae n. fam. from *Hippopotamus amphibius* in comparison with some

entodiniomorphs from horses and cattle. Eur. J. Protistol. 37, 405–426.

Ito, A., Van Hoven, W., Miyazaki, Y., Imai, S., 2006. New entodiniomorphid ciliates from the intestine of the wild African white rhinoceros belong to a new family, the Gilchristidae. Eur. J. Protistol. 42, 297–307.

Kornilova, O.A., 2004. History of Study of Enbiotic Ciliates of Mammalia. TESSA Publ., St-Petersb.

Latteur, B., Dufey, M.M., 1967. Reforme systematique de la famille des Cycloposthiidae Poche, 1913. Acta Zool. Pathol. Antwerp 44, 125–139.

Latteur, B., Tuffrau, M., Wespes, G., 1970. *Triplumaria selenica* n. sp., cilié spirotriche du colon de l'éléphant d'Afrique. Protistologica 6, 319–330.

Timoshenko, O., Imai, S., 1995. Eleven new species of the genus *Triplumaria* (Entodiniomorphida) from Asian elephant, *Elephas maximus* and African elephant, *Loxodonta africana*. J. Protozool. Res. 5, 157–175.

Van Hoven, W., Gilchrist, F.M.C., Stenson, M.O., 1998. Six new ciliated protozoan species of Trichostomatida, Entodiniomorphida and Suctorida from the intestine of wild African rhinoceroses. Acta. Protozool. 37, 113–124.

Wolska, M., 1978. *Tripalmaria dogieli* Gass., 1918 (Ciliata, Entodiniomorphida). Structure and ultrastructure. Part I. Light-microscope investigations. Acta Protozool. 17, 13–20.