

## New entodiniomorphid ciliates from the intestine of the wild African white rhinoceros belong to a new family, the Gilchristidae

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### Abstract

*Gilchristia artemis* n.g., n.sp. and *Digilchristia draconis* n.g., n.sp. in the order Entodiniomorpha are described from the large intestine of the African white rhinoceros, and a new family Gilchristidae is proposed to contain them. These new species have a C-shaped adoral polybrachykinety, a slender vestibular polybrachykinety, and paralabial kineties along the ventral side of the adoral polybrachykinety in their retractable adoral ciliary zone, showing the same arrangement as in the rumen ciliates in the family Ophryoscolecidae. *G. artemis* has two skeletal plates and *D. draconis* one plate. In both species the dorsal skeletal plate is bow-shaped, folded in half longitudinally, twisting in the anterior part, and lying along the dorsal left side of the macronucleus. The second plate of *G. artemis* is slender and lies along the ventral side of the macronucleus. *G. artemis* has three ciliary arches and *D. draconis* has four arches along the dorsal and ventral sides of the body. Their arches are long and non-retractable, closely resembling those of ciliates in the families, Spirodiniidae and Cycloposthiidae, and are not analogous to the single retractable ciliary arch of the rumen ciliates in the family Ophryoscolecidae.

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**Keywords:** African white rhinoceros; *Digilchristia draconis* n.g., n.sp.; Entodiniomorpha; Gilchristidae n.fam.; *Gilchristia artemis* n.g., n.sp.; Infraciliature

### Introduction

Studies on entodiniomorphid ciliates from the digestive tracts of wild African rhinoceroses (Buisson 1923; Van Hoven et al. 1987, 1988, 1998) and other investigated herbivorous mammals (Dehority 1986; Dogiel 1927; Grain 1994; Kornilova 2004; Williams and Coleman 1992), have revealed forms with a C-shaped adoral polybrachykinety and a vestibular polybrachykinety in a retractable adoral ciliary zone

only in rumen ciliates in the family Ophryoscolecidae (Fernández-Galiano 1958; Fernández-Galiano et al. 1985; Ito and Imai 1998, 2003, 2005; Ito et al. 1997, 2001, 2002; Noirot-Timotheé 1960). Such buccal infraciliary bands have never been described from other entodiniomorphid families.

This is the first report concerning entodiniomorphid ciliates from the intestine of non-ruminating herbivorous mammals which have the same buccal infraciliary bands in their retractable adoral ciliary zone as are found in the rumen ophryoscolecids. We describe these two new species found from the large intestine of the wild African white rhinoceros, proposing a new family, Gilchristidae.

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## Materials and methods

Intestinal contents were obtained from a free-living African white rhinoceros (*Ceratotherium simum*) shot by hunters in Pilanesburg Game Reserve in the North West Province of the Republic of South Africa (27°E, 26°S). These intestinal contents were immediately fixed in two times the volume with 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). Macronuclei and micronuclei were stained by protargol silver impregnation, following Ito and Imai (2000). The orientation of ciliates used by Dogiel (1927) when he described *Diplodinium* species in the family Ophryoscollecidae was adopted; the side beneath which the macronucleus lies was termed the right dorsal side. The side towards which the vestibulum inclines as it descends into the body was termed the dorsal side and the opposite one the ventral side; these defined the right and left sides. Cell measurements were made from a sample of 20 fixed cells using a calibrated micrometer. Body length was taken 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. They have the same arrangement of the buccal infraciliary bands in their retractable ciliary zone as in the family Ophryoscollecidae. However, their somatic ciliary arches are non-retractable like those in the families, Spirodiniidae and Cycloposthiidae. It is concluded that these new species do not belong to any existing family and genus, and that a new family and two new genera must be named to accommodate them. The details supporting this conclusion are described below.

### Family Gilchristidae n. fam.

**Diagnosis.** Body is ovoid or rectangular and laterally compressed. Adoral ciliary zone is retractable and encircles the vestibular opening. Vestibular ciliary zone extends down the wall of the vestibulum. Some non-retractable ciliary arches extend transversely on the dorsal and ventral surfaces of the body. Cytoproct is located at or near the posterior end of the body. Macronucleus is rod-shaped, lying beneath the right dorsal surface of the body. Micronucleus is located on

the dorsal side of the macronucleus. Multiple contractile vacuoles lie beneath the dorsal surface of the body. One or two skeletal plates lie alongside the macronucleus. Division is by binary fission. Ciliates in this family are found in the intestine of African white rhinoceros.

**Taxonomical discussion.** Creation of the new family Gilchristidae is proposed. This family belongs to the order Entodiniomorpha Reichenow in Doflein and Reichenow 1933. Species in the family Gilchristidae might be thought to belong to the family Ophryoscollecidae, since gilchristids have one or two skeletal plates alongside the macronucleus as in ophryoscollecids, and the arrangement of the adoral and vestibular ciliary bands is the same as in ophryoscollecids. However, ophryoscollecids have a single retractable somatic ciliary arch whereas gilchristids have non-retractable ciliary arches which closely resemble the ciliary arches of entodiniomorphids in the families, Spirodiniidae and Cycloposthiidae. Therefore, the diagnostic characters of the Gilchristidae are the retractable adoral ciliary zone with a C-shaped adoral ciliary band and a vestibular ciliary band, some non-retractable somatic ciliary arches, and skeletal plates alongside the macronucleus. With such morphological characters, this new family differs markedly from other entodiniomorphid families. The family Gilchristidae includes two genera, *Gilchristia* and *Digilchristia*, each containing a single species found in the African white rhinoceros.

**Type genus.** Genus *Gilchristia* n.g.

### Genus *Gilchristia* n.g.

**Diagnosis.** With characteristics of the family Gilchristidae. Body is ovoid. Macronucleus is rod-shaped and lies beneath the dorsal right surface and micronucleus adheres to the dorsal side of macronucleus. Four contractile vacuoles lie between the dorsal surface and the macronucleus. Two skeletal plates lie alongside the macronucleus, one dorsal and the other ventral to the macronucleus. Three arched ciliary zones, one on the ventral side and the other two on the dorsal side.

**Type species.** *Gilchristia artemis* n.g., n.sp.

**Etymology.** *Gilchristia* is named after Dr. F. M. C. Gilchrist, a famous ciliatologist of the University of Pretoria in South Africa who contributed to the taxonomy of intestinal ciliates of rhinoceroses. Gender: female.

### *Gilchristia artemis* n.g., n.sp.

**Description.** (Table 1, Figs 1–11, 22–25). The body is irregularly ovoid, slightly narrowed in both anterior and posterior parts, and is laterally compressed. The adoral ciliary zone is retractable into the anterior end of the body. There are three non-retractable somatic ciliary

**Table 1.** Measurements ( $\mu\text{m}$ ) and morphometric ratios (mean  $\pm$  S.D. (minimum–maximum);  $n = 20$ ) of *Gilchristia artemis* and *Digilchristia draconis*

|  | <i>Gilchristia artemis</i>     | <i>Digilchristia draconis</i>     |
|--|--------------------------------|-----------------------------------|
| Body length  | 98.7 $\pm$ 3.8<br>(89.3–104.6) | 192.3 $\pm$ 19.7<br>(153.0–224.4) |
| Body width   | 60.3 $\pm$ 6.2<br>(48.5–71.4)  | 102.6 $\pm$ 5.7<br>(91.8–119.9)   |
| Body length/body width   | 1.65 $\pm$ 0.16<br>(1.32–1.84) | 1.87 $\pm$ 0.16<br>(1.64–2.08)    |
| Macronuclear length  | 55.1 $\pm$ 5.0<br>(43.4–63.8)  | 113.2 $\pm$ 16.4<br>(89.3–140.3)  |
| Macronuclear length/body length  | 0.56 $\pm$ 0.04<br>(0.49–0.63) | 0.59 $\pm$ 0.04<br>(0.46–0.65)    |
| Distance from anterior end of the macronucleus to the micronucleus                     | 23.6 $\pm$ 3.0<br>(20.4–30.6)  | 40.3 $\pm$ 4.9<br>(35.7–53.6)     |
| Distance from anterior end of the macronucleus to the micronucleus/macronuclear length | 0.43 $\pm$ 0.03<br>(0.35–0.48) | 0.36 $\pm$ 0.05<br>(0.28–0.44)    |

arches; the anterior and posterior dorsal ciliary arches are short, lying across the dorsal side of the anterior and posterior narrowed parts of the body and the long posterior ventral arch lies opposite the posterior dorsal arch with its left part extending dorsally further than the right part. An irregular-shaped operculum is formed between the adoral lip and the anterior dorsal ciliary arch. The macronucleus is wedge-shaped with a distinct depression in the middle of the dorsal side, lying beneath the right dorsal surface of the body, and varying in shape. The micronucleus is small and ovoid, lying in the dorsal depression of the macronucleus. The vestibulum is long and tubular. The cytoproct is located behind the posterior ventral arch. Two skeletal plates extend along the macronucleus; the ventral skeletal plate is slender, extending obliquely from the middle of the macronucleus to the anterior end of the body; the dorsal skeletal plate is bow-shaped, lying along the dorsal left side of the macronucleus, but is folded in half longitudinally and twists toward the right in the anterior part, and a lobe-like projection is formed by the anterior twisting from the left plane of the plate. Four contractile vacuoles lie beneath the dorsal surface of the body; the three anterior vacuoles are located between the anterior and posterior dorsal arches, along the left dorsal side of the dorsal skeletal plate, and the posterior vacuole is located behind the posterior dorsal arch.

**Habitat, type host and locality.** The large intestine (proximal colon) of the African white rhinoceros (*Ceratotherium simum*) in Pilanesburg Game Reserve, South Africa (27°E, 26°S).

**Etymology.** *Gilchristia artemis* is named after the Greek goddess Artemis with her bow and arrow,

because of the bow-shaped dorsal skeletal plate and the arrow-shaped ventral plate.

**Type material.** Holotype slides (NSMT-Pr187) are deposited in National Science Museum, Tokyo, Japan.

### Genus *Digilchristia* n.g.

**Diagnosis.** With characteristics of the family Gilchristidae. Body is rectangular. Macronucleus is rod-shaped beneath the dorsal right surface and micronucleus adheres to the dorsal side of macronucleus. Eight contractile vacuoles beneath the dorsal surface. Single skeletal plate along the dorsal side of the macronucleus. Four arched ciliary zones, one on the ventral side and the other three on the dorsal side.

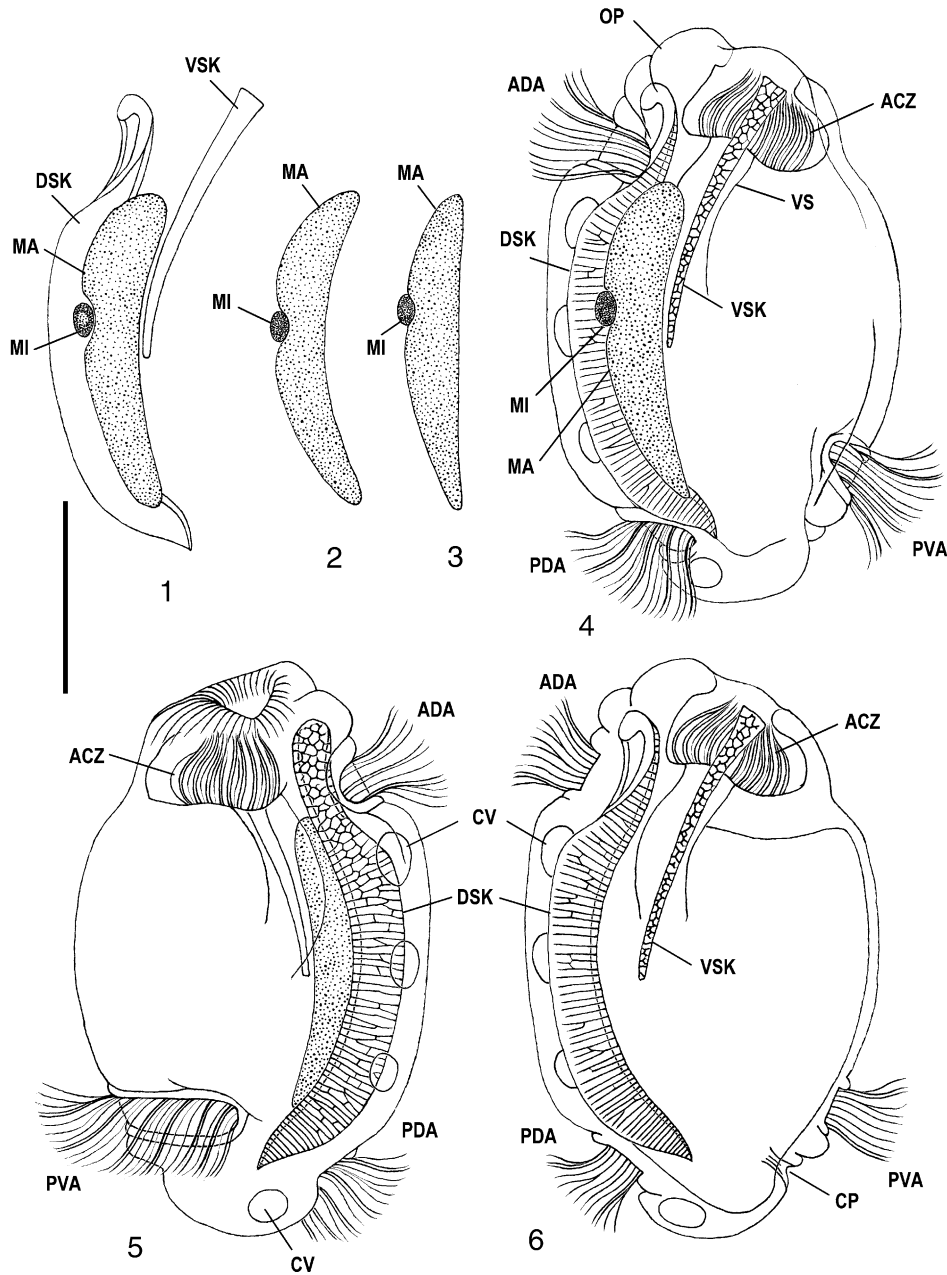
**Taxonomical discussion.** The genus *Digilchristia* is closely related to the genus *Gilchristia*. However, these two genera can be distinguished by the body size, the number of somatic ciliary arches and the number of skeletal plates. The single species of *Digilchristia* named so far is larger than *Gilchristia artemis*, and has four ciliary arches and a single skeletal plate.

**Type species.** *Digilchristia draconis* n.g., n.sp.

**Etymology.** *Di-* and *Gilchristia*. Gender: female.

### *Digilchristia draconis* n. g., n. sp.

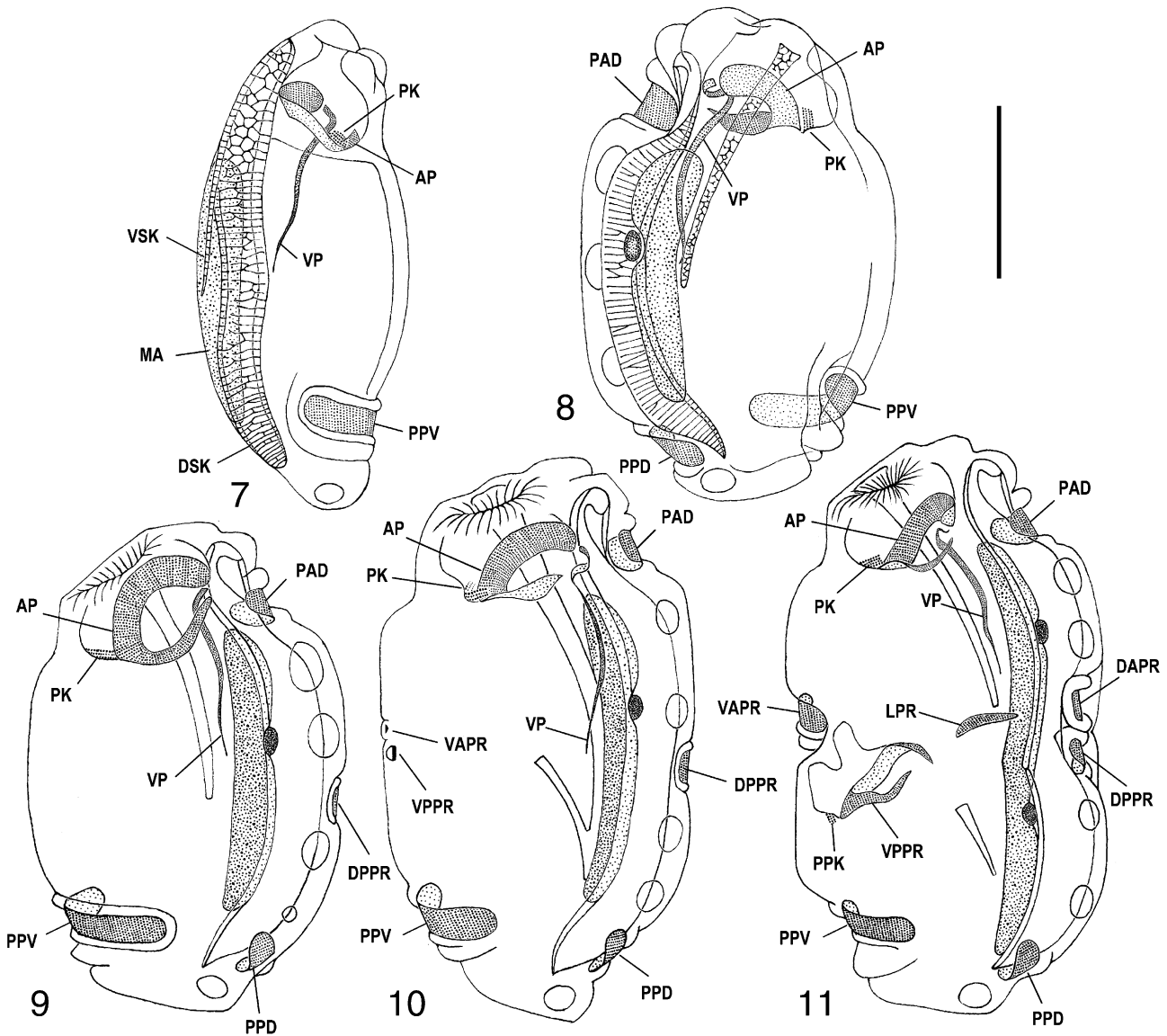
**Description.** (Table 1, Figs 12–21, 26–29). The body is irregularly rectangular, slightly narrowed in both anterior and posterior parts, and is laterally compressed. The adoral ciliary zone is retractable into the anterior end of



**Figs. 1–6.** Schematic figures of *Gilchristia artemis* n.g., n.sp. **1.** Skeletal plates, macronucleus and micronucleus from right side. **2, 3.** Macronucleus and micronucleus from right side. **4.** Cell from right side. **5.** Cell from left side. **6.** Cell from right side omitting macronucleus and micronucleus. ACZ, adoral ciliary zone; ADA, anterior dorsal arch; CP, cytoproct; CV, contractile vacuole; DSK, dorsal skeletal plate; MA, macronucleus; MI, micronucleus; OP, operculum; PDA, posterior dorsal arch; PVA, posterior ventral arch; VS, vestibulum; VSK, ventral skeletal plate. Bar = 30  $\mu$ m.

the body. The four somatic ciliary arches are non-retractable; the anterior and posterior dorsal ciliary arches are relatively long, lying at the dorsal sides of the anterior and posterior narrowed parts of the body; the middle dorsal ciliary arch is short and situated on the dorsal surface just posterior to mid-body; and the posterior ventral arch is very long, lying opposite to the posterior dorsal arch with its left part extending dorsally further than the right part, toward near the left end of the posterior dorsal arch. A round operculum is

formed between the adoral lip and the anterior dorsal ciliary arch. Some parallel grooves extend on the left dorsal surface from the anterior dorsal arch to the posterior dorsal arch; there are four grooves in most cells. A deep groove extends along the dorsal edge of the skeletal plate from the anterior dorsal arch to the posterior dorsal arch. The macronucleus is wedge-shaped with a distinct depression in the middle of the dorsal side, lying beneath the right dorsal surface of the body, and varying in shape. The micronucleus is small

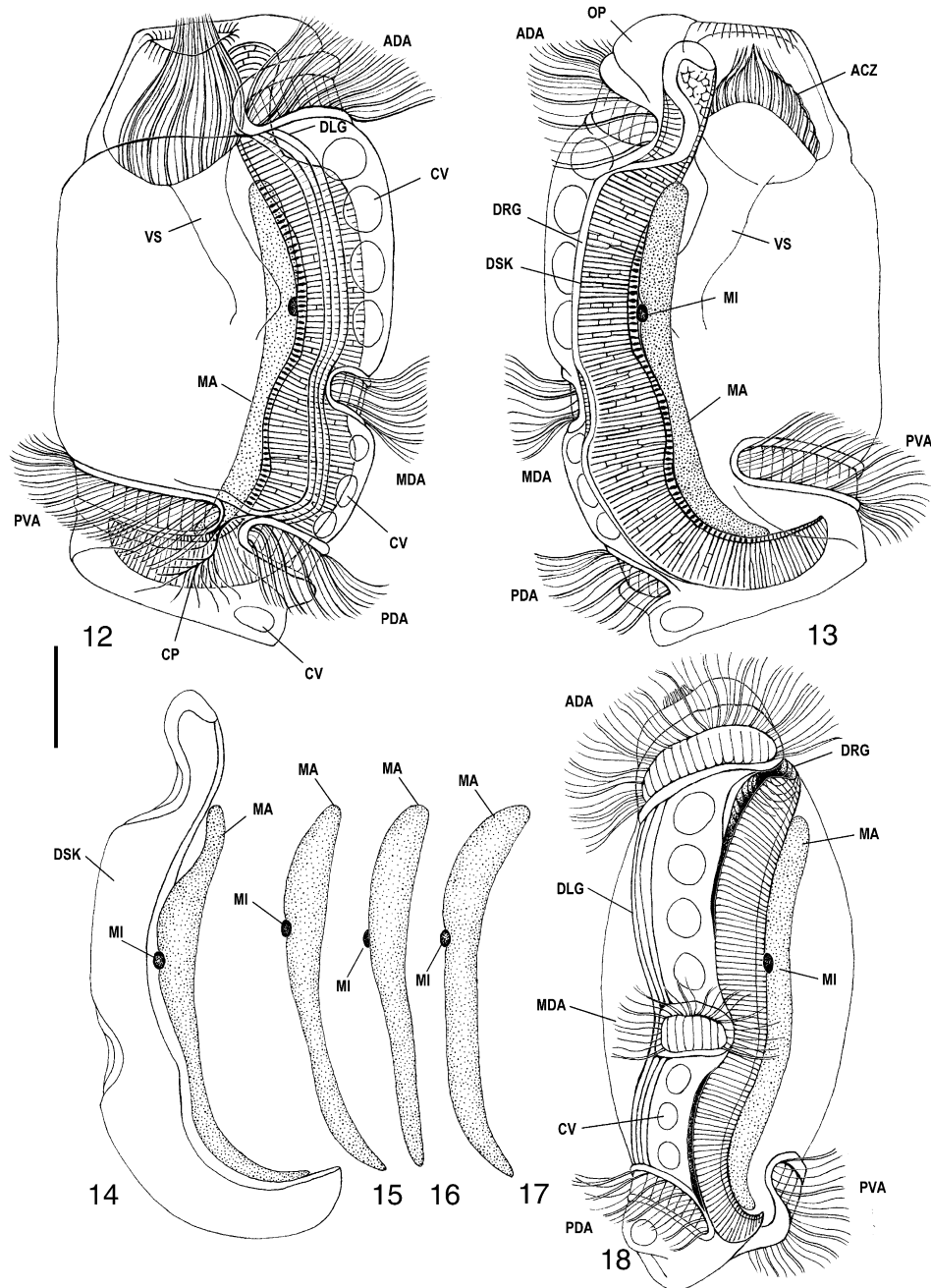


**Figs. 7–11.** *Gilchristia artemis* after silver impregnation. 7. Cell seen from ventral side. 8. Cell from right side. 9–11. Stages during binary fission seen from left side. AP, adoral polybrachykinety; DAPR, dorsal anterior primordium; DPPR, dorsal posterior primordium; DSK, dorsal skeletal plate; LPR, left primordium; MA, macronucleus; PAD, polybrachykinety of anterior dorsal ciliary arch; PK, paralabial kineties; PPD, polybrachykinety of posterior dorsal ciliary arch; PPK, primordium of paralabial kineties; PPV, polybrachykinety of posterior ventral ciliary arch; VAPR, ventral anterior primordium; VP, vestibular polybrachykinety; VPPR, ventral posterior primordium; VSK, ventral skeletal plate. Bar = 30  $\mu$ m.

and ovoid, lying in the dorsal depression of the macronucleus. The vestibulum is long and tubular. The cytoproct is located behind the left end of the posterior ventral arch. The single skeletal plate is bow-shaped, lying along the dorsal left side of the macronucleus, and its posterior part bends and extends considerably toward the ventral side. It is folded in half longitudinally with three lobe-like projections; one lobe-like projection is formed from the right plane of the anterior end of the plate, and two other lobe-like projections are formed from the left plane of the plate beneath the right ends of the anterior and middle dorsal

ciliary arches. Eight contractile vacuoles lie beneath the dorsal surface of the body; the anterior four vacuoles are located between the anterior and middle dorsal arches, along the left dorsal side of the skeletal plate, the middle three vacuoles are located between the middle and posterior dorsal arches, along the left dorsal side of the skeletal plate, and the posterior vacuole is located behind the posterior dorsal arch.

**Habitat, type host and locality.** The large intestine (proximal colon) of African white rhinoceros (*Ceratotherium simum*) in Pilanesburg Game Reserve, South Africa (27°E, 26°S).



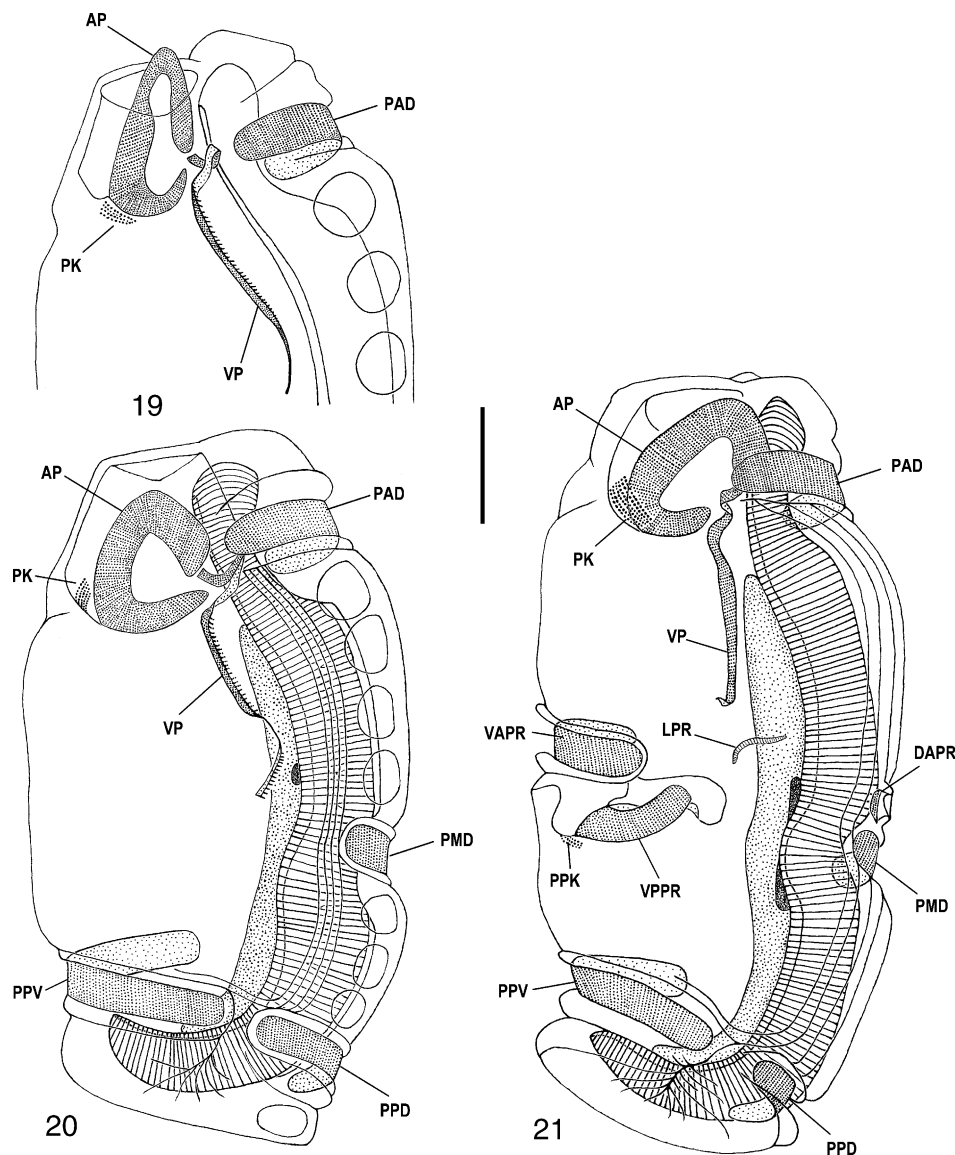
**Figs. 12–18.** Schematic figures of *Digilchristia draconis* n.g., n.sp. **12.** Cell from left side. **13.** Cell from right side. **14.** Skeletal plate, macronucleus and micronucleus from right side. **15–17.** Macronucleus and micronucleus of different cells seen from right side. **18.** Cell from dorsal side. ACZ, adoral ciliary zone; ADA, anterior dorsal arch; CP, cytoproct; CV, contractile vacuole; DLG, dorsal left grooves; DRG, dorsal right groove; DSK, dorsal skeletal plate; MA, macronucleus; MDA, middle dorsal ciliary arch; MI, micronucleus; OP, operculum; PDA, posterior dorsal arch; PVA, posterior ventral arch; VS, vestibulum. Bar = 30  $\mu$ m.

**Etymology.** *Digilchristia draconis* is named after its skeletal plate, which resembles a dragon in shape (*L. draco*, -onis).

**Type material.** Holotype slides (NSMT-Pr188) are deposited in National Science Museum, Tokyo, Japan.

**Remarks.** *Digilchristia draconis* is closely related to *Gilchristia artemis* in body shape, skeletal plate, and somatic ciliary arches. However, *D. draconis* is larger

than *G. artemis*, and has a single skeletal plate along the dorsal side of the macronucleus, four ciliary arches, and eight contractile vacuoles. The skeletal plate of *Digilchristia* is like the dorsal skeletal plate of *Gilchristia*; however, the lobe-like projection of the anterior end arises from the left plane of the plate in *G. artemis*, whereas it arises from the right plane in *D. draconis*. The skeletal plate of *D. draconis* is long; it is constricted at



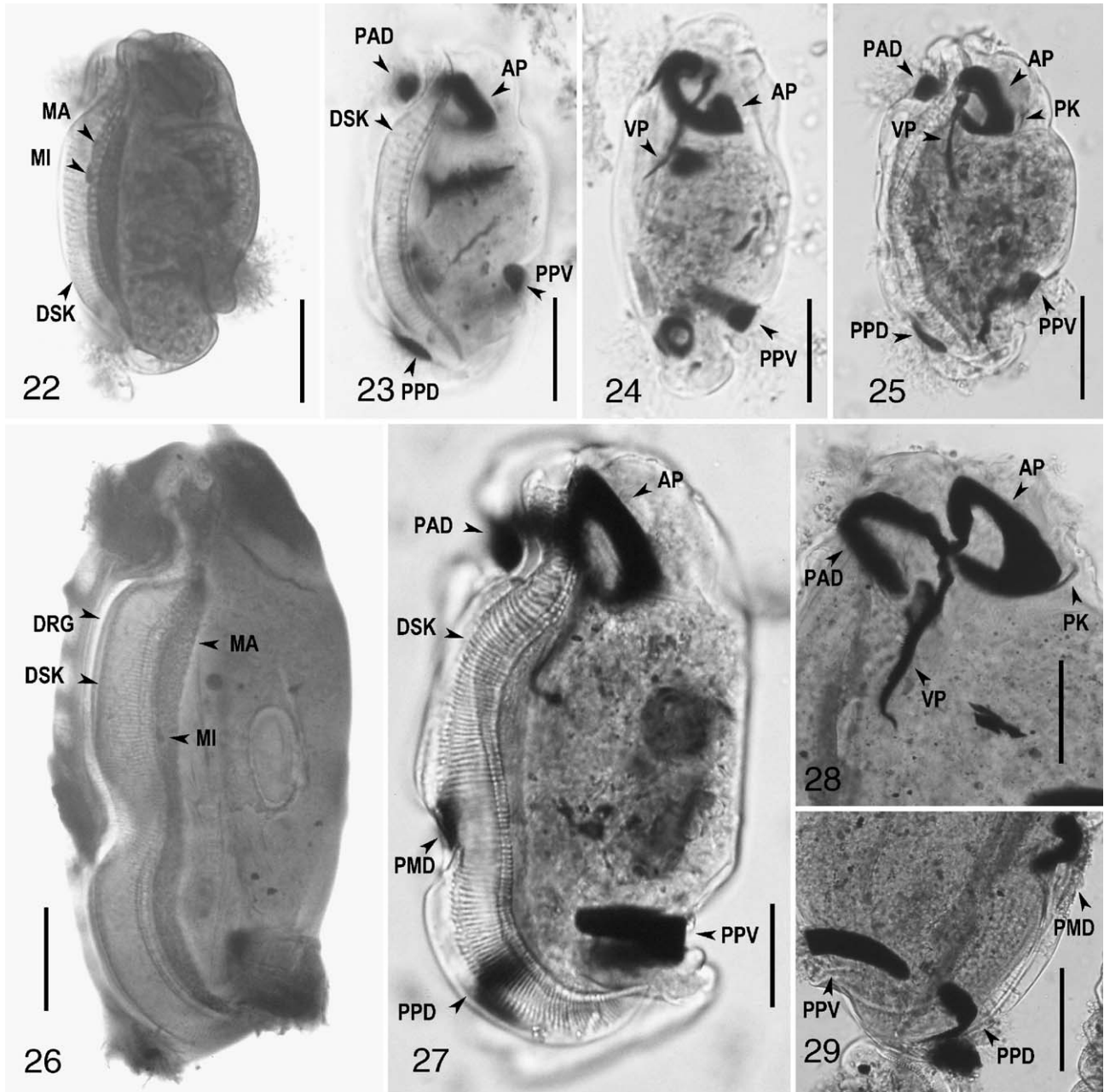
**Figs. 19–21.** *Digilchristia draconis* after silver impregnation. **19.** Anterior half of cell with adoral ciliary zone protruding, seen from left side. **20.** Cell from left side. **21.** Cell during binary fission seen from left side. AP, adoral polybrachykinety; DAPR, dorsal anterior primordium; LPR, left primordium; PAD, polybrachykinety of anterior dorsal ciliary arch; PK, paralabial kineties; PMD, polybrachykinety of middle dorsal ciliary arch; PPD, polybrachykinety of posterior dorsal ciliary arch; PPK, primordium of paralabial kineties; PPV, polybrachykinety of posterior ventral ciliary arch; VAPR, ventral anterior primordium; VP, vestibular polybrachykinety; VPPR, ventral posterior primordium. Bar = 30  $\mu$ m.

the level of the middle dorsal ciliary arch, and its posterior part bends and extends considerably toward the ventral side.

**Infraciliature and infraciliary primordia of gilchristid species.** (Figs 7–11, 19–21).

**Buccal polybrachykineties and paralabial kineties.** *Gilchristia artemis* and *Digilchristia draconis* have an adoral polybrachykinety (AP), a vestibular polybrachykinety (VP), and paralabial kineties (PK) in the adoral ciliary zone. *G. artemis* and *D. draconis* show the same arrangement of buccal infraciliary bands.

The AP extends along the edge of the vestibular opening and the VP extends inside the vestibulum. The AP is wide and C-shaped. The VP extends down the wall of the vestibulum to the level of the micronucleus. The VP is slender and extends from the dorsal extremity of the AP and the anterior part of the VP is bent into an S-shape. The PK is composed of more than four short transverse kineties which extend along the ventral side of the AP. Kinetids in the PK are slightly larger than in kineties in the other polybrachykineties.



**Figs. 22–29.** Micrographs of two species in the family Gilchristidae. **22, 26.** After protargol silver impregnation. **23–25, 27–29.** After pyridinated silver carbonate impregnation. **22–25.** *Gilchristia artemis*. **22, 23, 25.** From right side. **24.** From ventral side. **26–29.** *Digilchristia draconis*. **26–28.** From right side. **29.** From left side. AP, adoral polybrachykinety; DRG, dorsal right groove; DSK, dorsal skeletal plate; MA, macronucleus; MI, micronucleus; PAD, polybrachykinety of anterior dorsal ciliary arch; PK, paralabial kineties; PMD, polybrachykinety of middle dorsal ciliary arch; PPD, polybrachykinety of posterior dorsal ciliary arch; PPV, polybrachykinety of posterior ventral ciliary arch; VP, vestibular polybrachykinety. Bar = 30  $\mu$ m.

**Somatic infraciliature.** *G. artemis* has three infraciliary bands (the polybrachykinety of the anterior dorsal arch (PAD), the polybrachykinety of the posterior dorsal arch (PPD), and the polybrachykinety of the posterior ventral arch (PPV)) on the dorsal and ventral surfaces (Fig. 8). The PAD is short and extends laterally across the dorsal side of the body at the same level as the AP. The PPD is short and extends slightly obliquely across

the dorsal side of the body near the level of the posterior end of the dorsal skeletal plate. The PPV is relatively long and extends obliquely across the ventral side of the body at a level slightly anterior to the PPD. The left part of the PPV extends dorsally further than the right part.

*D. draconis* has four bands (PAD, PPD, PPV and the polybrachykinety of the middle dorsal arch (PMD)) on the body surface (Fig. 20). The PAD is relatively long



and extends laterally across the dorsal side of the body at the same level as the AP. The PMD is short and extends laterally across the dorsal side of the body a little posterior to mid-body. The PPD is relatively long and extends slightly obliquely across the dorsal side of the body at the posterior narrowed part of the body. The PPV is long and extends obliquely across the ventral side of the body at a level slightly anterior to the PPD. The left part of the PPV and the left part of the PPD extend towards one another further than their right parts and nearly meet on the left surface of the body.

**Infraciliary primordia.** *G. artemis* and *D. draconis* show the same pattern of division. Division is by binary fission, perpendicular to the longitudinal axis. In the early stage of preparations for division, before the micronucleus divides, one primordium appears beneath the middle dorsal surface of the body in *G. artemis* (Fig. 9). This primordium is the dorsal posterior primordium (DPPR). Then, two primordia, the ventral anterior primordium (VAPR) and the ventral posterior primordium (VPPR), appear beneath the ventral surface (Fig. 10). When the micronucleus begins to divide, two more primordia appear, one (the LPR) beneath the left surface and the other (the DAPR) beneath the dorsal surface immediately anterior to the DPPR (in *G. artemis*, (Fig. 11)) or the PMD (in *D. draconis*, Fig. 21). Also several kineties, the primordium of the paralarial kineties (PPK), arise along the ventral side of the enlarging VPPR (Figs 11, 21). During cytokinesis, after the micronucleus divides, the cell is constricted in the region of the stomatogenic field, with the ventral extremity of the LPR turning towards the posterior. After binary fission, the VPPR, LPR, PPK, and DPPR in *G. artemis* or PMD in *D. draconis* develop into the AP, VP, PK, and PAD of the opisthe, respectively. And the VAPR and DAPR develop into the PPV and PPD of

the proter, respectively. In *D. draconis*, the PMD of the proter appears between the PAD and DAPR and the PMD of the opisthe between the PMD and PPD.

## Discussion

Entodiniomorphid ciliates inhabit the digestive tract of herbivorous mammals (Dehority 1986; Kopperi 1935; Kornilova 2004; Ogimoto and Imai 1981; Williams and Coleman 1992). They are classified into 14 families based on Cameron et al. (2001), Dehority (1996), Grain (1994), Ito et al. (2002), Lynn and Small (2002), Wolska (1986, 1978), and the present study. The arrangement of infraciliary bands and the retraction of ciliary zones are important characters distinguishing between entodiniomorphid families. A summary of these characters for the 14 entodiniomorphid families is given in Table 2. The arrangement of the buccal infraciliary bands of entodiniomorphids can be classified into four types; AP (adoral polybrachykinety), AP + VK (vestibular kineties extending longitudinally), AP + PVP (perivestibular polybrachykinety extending along the dorsal edge of the vestibular opening), and AP + VP (vestibular polybrachykinety composed of numerous, short, parallel kineties extending inside the vestibulum) types. The pattern of the Spirodiniidae is regarded as an AP + PVP type, based on our unpublished observations that the slit-like vestibular opening is surrounded by the PVP, which disagrees with previous descriptions (Wolska 1980, 1985). The somatic ciliary zone, that is the caudalia, of the Cycloposthiidae is regarded as non-retractable, which differs from the previous description (Grain 1994), because the whole somatic ciliary tufts or arches can not be completely retracted into the caudalia.

**Table 2.** Buccal infraciliary bands, adoral ciliary zone, somatic ciliary zone, and habitat of the entodiniomorphid families

|                    | Buccal infraciliary bands | Adoral ciliary zone | Somatic ciliary zone | Habitat                          |
|--------------------|---------------------------|---------------------|----------------------|----------------------------------|
| Buetschliidae      | AP                        | Non-retractable     | Non-retractable      | Foregut and hindgut              |
| Macropodiniidae    | AP + VK                   | Non-retractable     | Non-retractable      | Foregut                          |
| Blepharocorythidae | AP + VK or VP             | Non-retractable     | Non-retractable      | Foregut and hindgut              |
| Parentodiniidae    | AP + VK                   | Retractable         | Non-retractable      | Foregut                          |
| Pseudoentodiniidae | AP + VK                   | Retractable         | None                 | Hindgut                          |
| Ophryoscolecidae   | AP + VP                   | Retractable         | Retractable          | Foregut (and hindgut)            |
| Gilchristidae      | AP + VP                   | Retractable         | Non-retractable      | Hindgut                          |
| Spirodiniidae      | AP + PVP                  | Non-retractable     | Non-retractable      | Hindgut                          |
| Cycloposthiidae    | AP + PVP                  | Retractable         | Non-retractable      | Hindgut and hippopotamus stomach |
| Triplalmariidae    | AP + PVP                  | Retractable         | Non-retractable      | Hindgut                          |
| Polydiniellidae    | Unknown                   | Non-retractable     | Non-retractable      | Hindgut                          |
| Prototapirellidae  | Unknown                   | Retractable         | Non-retractable      | Hindgut                          |
| Rhinozetidae       | Unknown                   | Retractable         | Non-retractable      | Hindgut                          |
| Troglodytellidae   | Unknown                   | Retractable         | Non-retractable      | Hindgut                          |

AP, adoral polybrachykinety; VK, vestibular kineties arranged longitudinally; VP, vestibular polybrachykinety with short parallel kineties; PVP, perivestibular polybrachykinety along dorsal edge of vestibular opening.

*Gilchristia artemis* and *Digilchristia draconis* in the Gilchristidae have a retractable adoral ciliary zone with the AP+VP type arrangement of the buccal infraciliary bands and their somatic ciliary arches are long and non-retractable.

In entodiniomorphid families classified on their infraciliary bands, only the two families, Ophryoscolecidae and Gilchristidae, have AP+VP type infraciliary bands and a retractable adoral ciliary zone (Fernández-Galiano 1958; Fernández-Galiano et al. 1985; Ito et al. 1997, 2001, 2002; Ito and Imai 1998, 2003, 2005; Noiro-Timothee 1960). In the Ophryoscolecidae, *Entodinium* and *Epidinium* species have buccal infraciliary bands similar to those of gilchristids. *Entodinium* has the VP extending from the dorsal extremity of the AP as in gilchristids, but the AP and the VP of *Entodinium* have the same width (Ito and Imai 2003; Ito et al. 2002). *Epidinium* has a slender VP as in gilchristids, but it extends from the inner edge of the AP (Noiro-Timothee 1960). In addition, ophryoscolecids and gilchristids have some general morphological features in common; the laterally compressed body, the operculum at the anterior end of the body, the macronucleus beneath the right dorsal body surface, and skeletal plates along the macronucleus.

In spite of the similarities to the Ophryoscolecidae, *Gilchristia artemis* and *Digilchristia draconis* in the Gilchristidae have non-retractable somatic ciliary arches, not analogous to the retractable arch of the Ophryoscolecidae, and their ciliary arches closely resemble those of entodiniomorphids in the families, Spirodiniidae and Cycloposthiidae. However, ciliates in the Spirodiniidae and the Cycloposthiidae have the AP+PVP type arrangement of the buccal infraciliary bands (Fernández-Galiano 1959; Wolska 1980, 1981). The adoral ciliary zone of the Cycloposthiidae is retractable as in the families, Gilchristidae and Ophryoscolecidae. The adoral ciliary zone of the Spirodiniidae is incompletely retractable, because the PVP is retractable and the AP is non-retractable. Therefore ciliates in the Gilchristidae have intermediate features between the families, Ophryoscolecidae, Spirodiniidae and Cycloposthiidae. These four entodiniomorphid families may be closely related to each other. The taxonomic problem of which families are near relatives of the Ophryoscolecidae has been discussed and entodiniomorphids in the families, Buetschliidae, Blepharocorythidae, Pseudoentodiniidae and Parentodiniidae, are classified as closely related to the Ophryoscolecidae by some workers (Ito et al. 2002; Wolska 1971, 1986). The Gilchristidae will be added as one of the relations of the Ophryoscolecidae.

Ophryoscolecids inhabit the foregut of ruminants and camelids, except for *Lavierella*, *Endoralium*, and *Cunhaia* species found in the hindgut; but species in these three genera are taxonomically unstable (Buisson 1923; Dogiel 1927; Eloff and van Hoven 1980; Van Hoven et al. 1998). In addition, some *Entodinium* species in the

Ophryoscolecidae are found in the intestine of rodents (Kopperi 1935), but the infraciliatures of these ophryoscolecid species found in the hindgut have not been examined. On the other hand, entodiniomorphids in the families, Spirodiniidae and Cycloposthiidae, inhabit the hindgut of horses, donkeys, and capybaras (Dehority 1986; Grain 1994; Hsiung 1930; Kornilova 2004). Although *Monoposthium*, found in the hippopotamus stomach, has been classified in the Cycloposthiidae, (Thurston and Noiro-Timothee 1973), its infraciliary bands have not been described.

It was thought that entodiniomorphids with AP+VP type infraciliary bands were found in the foregut whereas those with the AP+PVP type inhabited the hindgut. This view must now be modified since gilchristids are found in the hindgut of non-ruminating herbivorous mammals, and will lead to interesting comparisons in future studies on ciliates in other entodiniomorphid families found in the hindgut, the Troglodytelliidae, Prototapirellidae, Polydiniellidae, and Rhinozetidae. Of these families, ciliates in the Troglodytelliidae have a retractable adoral ciliary zone, long and non-retractable ciliary arches on the body surface, and skeletal plates along the macronucleus (Latteur and Dufey 1967), which are similar to those of the Gilchristidae. However, infraciliary bands in these families have not been described in detail.

The basic form of morphogenesis of the ciliature is similar in all entodiniomorphids examined to date, including the family Gilchristidae, with the pair of the VPPR and the LPR developing equatorially, expanding and separating to give the oral ciliature typical of the species (Ito et al. 2002; Noiro-Timothee 1960). But, gilchristid species have some signature patterns in their division morphogenesis in comparison with those of rumen ophryoscolecids. In ophryoscolecids, before the micronucleus divides, all the primordia appear and expand equatorially. On the other hand, the primordia of dorsal and ventral ciliary arches of gilchristids appear before the micronucleus divides whereas the development of the VPPR and the LPR is considerably delayed. Thus, without the silver impregnation, it would be difficult to prove that *G. artemis* with four or five ciliary arches and *D. draconis* with five ciliary arches are their dividing forms in early stage.

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