

## Two new *Tursiocola* species (Bacillariophyta) epizoic on green turtles (*Chelonia mydas*) in French Guiana and Eastern Caribbean

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**Abstract:** Scrapings from several juvenile *Chelonia mydas* LINNAEUS (green turtle) from Eastern Caribbean and adults from French Guiana allowed for the description of two small and relatively rare epizoic *Tursiocola* species (Bacillariophyta). Differences with the other eight *Tursiocola* species previously described are discussed here. Particular attention is given to the cingulum of the two new species and a conceptual key is proposed for *Tursiocola* species, based on the cingulum structure.

**Key words:** Epizoic diatoms, sea turtles, *Tursiocola*, morphology, autecology

### INTRODUCTION

Between 1920 and 1958 (BENNET 1920; HART 1935; OMURA 1950; HUSTEDT 1952; OKUNO 1954 and NEMOTO 1956, 1958) and more intensively during the last three decades, increasing interest was given to epizoic diatoms (see HOLMES 1985; VAN BONN & DENYS 1997; DENYS 1997; FRANKOVICH et al. 2015a). This has led to the description of several genera exclusive to this type of habitat. The first detailed taxonomic investigations concerned diatoms known to colonize whales, with the creation of two new achnantheid genera *Bennettella* R.W.HOLMES and *Epipellis* R.W.HOLMES (e.g., HOLMES 1985; HOLMES & NAGASAWA 1995; DENYS & VAN BONN 2001; DENYS & DE SMET 2010). Until now, both genera were only known in association with cetaceans. After 1985, several new diatom genera and species were discovered and shown to colonize metazoans (e.g., molluscs, holothurians; see TOTTI et al. 2011; RIAUX–GOBIN & WITKOWSKI 2012) and vertebrates (e.g., cetaceans, turtles and manatees) from contrasting environments (HOLMES et al. 1993a,b; VAN BONN & DENYS 1997; DENYS 1997; FRANKOVICH et al. 2015a,b; PAVLOV et al. 2016; RIAUX–GOBIN et al. 2017), including the new genera *Plumosigma* T.NEMOTO (NEMOTO 1956), *Epiphalaina* R.W.HOLMES, NAGASAWA & TAKANO (HOL-

MES et al. 1993a), *Tursiocola* R.W.HOLMES, NAGASAWA et TAKANO (HOLMES et al. 1993a), *Tripterion* R.W.HOLMES, NAGASAWA et TAKANO (HOLMES et al. 1993a), *Chelonicola* MAJEWSKA, DE STEFANO et VAN DE VIJVER (MAJEWSKA et al. 2015), *Poulinea* MAJEWSKA, DE STEFANO et VAN DE VIJVER (MAJEWSKA et al. 2015) and *Medlinella* FRANKOVICH, ASHWORTH et M.J.SULLIVAN (FRANKOVICH et al. 2016).

The species within the genera *Epiphalaina* and *Tursiocola* show very similar morphologies and are characterized by their small size, an acicular shape and the presence of pseudosepta. They mainly differ through an internal butterfly-shaped structure in *Tursiocola* (HOLMES et al. 1993a: p. 7). Eight *Tursiocola* species are currently reported in the literature, with *T. podocnemicola* C.E.WETZEL, VAN DE VIJVER et ECTOR from a freshwater turtle *Podocnemis erythrocephala* SPIX (WETZEL et al. 2012), *T. denysii* FRANKOVICH et M.J.SULLIVAN from a Loggerhead sea turtle *Caretta caretta* (LINNAEUS) (FRANKOVICH et al. 2015b), three taxa from a manatee from Florida Bay (FRANKOVICH et al. 2015a), and the other taxa from whales and Dall's porpoises (NEMOTO 1956; HOLMES et al. 1993a,b; DENYS 1997). Only three *Epiphalaina* taxa have been described thus far, and all were from cetaceans (NEMOTO 1956; HOLMES et al. 1993a,b; DENYS 1997). As reported by

WETZEL et al. (2012), BRADY (2010) also found an unidentified *Epiphialina* or *Tursiocola* species on several species of marine turtles from the Gulf of Mexico. Recently, ROBINSON et al. (2016) illustrated a *Tursiocola* sp. (ref. cit. fig. 2,D).

Scrapings from several juvenile *Chelonia mydas* LINNAEUS (green turtle) from the Lesser Antilles (Eastern Caribbean) and adults from French Guiana allowed for the description of two additional species that can be assigned to *Tursiocola* due to their characteristic internal structure. Differences with the eight previously described *Tursiocola* species are discussed here. Particular attention is given to the cingulum complex of both new species.

## MATERIAL AND METHODS

Materials used in this study were derived from two sources: 1) scrapings from the carapace of several wild juvenile *Chelonia mydas* from Martinique Island (Grande Anse d'Arlet 14°30'10.95"N, 61°05'13.00"E and Anse du Bourg 14°29'13.43"N, 61°04'58.88"E, 12–13 October 2015, CNRS–IPHC survey), 2) scrapings from the carapace of several nesting adult *Chelonia mydas* in Aztèque, French Guiana (48° 51' 45.81"N, 2° 17' 15.331"E, 07 April 2016). The latter samplings were carried out by D.C. during ANTIDOT surveys (CNRS–IPHC program) which investigated the migratory behavior of several species of marine turtles, using genomic, Capture–Mark–Recapture (CMR) and satellite tracking to understand the migration behavior of the different turtle species.

The samples were all very small and the described taxa are relatively scarce. For light microscope (LM) examination, the samples were washed with distilled water to remove salts, treated with 30% H<sub>2</sub>O<sub>2</sub> for 2 h at 70 °C to remove organic matter, rinsed several times in distilled water, alcohol–desiccated and mounted on glass slides using Naphrax®. Diatom slides were examined with a Zeiss Axiophot 200, with differential interference contrast (DIC) optics and photographed with a Canon PowerShot G6 digital camera (CRIOBE–USR 3278, Perpignan, France). For SEM examination, the samples were filtered through 1 µm Nuclepore® filters and rinsed twice with deionised (milliQ) water to remove salts. Filters were air–dried and mounted onto aluminium stubs before coating with gold–palladium alloy (EM–SCOP SC 500 sputter coater) and examined with a Hitachi S–4500 SEM operated at 5 kV, calibrated with a Silicon grating TGX01 (C2M, Perpignan, France).

'Foot pole' (F) and 'head pole' (H) refer to 'the length between the apex of the valve (foot or head) and the middle of the central area of the valve'. The ratio (F/H ± σ) is an approximate measure of the degree of heteropolarity of the cell. **Terminology and abbreviations.** For the description of the frustule and its parts, terminology follows ANONYMOUS (1975), ROSS et al. (1979) and ROUND et al. (1990).

## RESULTS

In addition to *Chelonicola* spp. and *Tripterion* spp.,

several of the turtle scrapings revealed two rare and acicular taxa from the genus *Tursiocola*. One very small sample ('31CM' from a nesting *Chelonia mydas* from Aztèque, see Mat. & Meth.) was found to have both *Tursiocola* species, one of which is very rare. In LM it is almost impossible to separate and correctly discriminate the two latter taxa.

**Light microscopy (Figs 1–12):** A total of 12 frustules or valves were photographed (Figs 1–12). Pseudosepta are visible on each image. The striation of the valves is barely visible and difficult to resolve. Figs 7–12 likely illustrate the dominant species (*Tursiocola yin–yangii* sp. nov., see below), which is slightly more acicular and smaller than the second taxon (*T. guyanensis* sp. nov.) possibly illustrated in Fig. 6 (see SEM description of each taxon). In cingular view (Figs 1–5) it is almost impossible to distinguish between the two taxa, as their stria densities are very similar (see below).

**Taxonomic notes:** In LM, *Tursiocola yin–yangii* sp. nov. and *T. guyanensis* sp. nov. are most similar to *Tursiocola staurolineata* DENYS (DENYS 1997, figs 42a–52) and *T. olympica* (HUSTEDT) HOLMES, NAGASAWA et TAKANO (DENYS 1997, figs 65–75; FRANKOVICH 2015b, figs 31–33), except for their average smaller size. However, these two species are also similar to *Epiphialina aleutica* var. *aleutica* (HOLMES et al. 1993a, figs 1–2; DENYS 1997, figs 1–10) and to *E. aleutica* var. *lineata* (DENYS 1997, figs 24–39, no SEM). SEM was therefore essential for a full description of the new taxa.

### *Tursiocola yin–yangii* RIAUX–GOBIN et WITOWSKI sp. nov. (Figs 7–12, 13–29)

**Description:** Valves small, acicular, with acute apices [n = 66 (SEM); 8.7–14.5 µm (mean ± σ 11.7 ± 1.4) long; 1.32–1.37 µm wide; 36–42.8 striae in 10 µm (mean ± σ 39.7 ± 1.6); length/width 7.8 (Table 1)]. Frustule biraphid, isovalvar, rectangular in girdle view. Valve face not cuneate. Very slight difference between the length of the raphe branches (see SEM morphology). Pseudosepta present. Complex cingulum composed of numerous narrow copulae (see SEM). Never in chains. Found as epizoic on *Chelonia mydas*.

### SEM morphology

**Externally:** Pole showing the valvocopula closed pole [with a second short row of puncta (see below)] is just slightly longer than the other pole (n = 27, ratio F/H ± σ 0.99 ± 0.05), e.g., the specimen illustrated in Fig. 16 has a head pole that is significantly longer than the foot pole and a slightly cuneate shape in girdle view (foot pole slightly narrower). Valve margins straight to slightly undulate. Striae uniseriate, parallel to slightly radiate, denser on both apices. Each stria is composed of four areolae externally ornamented by an 'S'-shaped opening resembling a yin–yang symbol (Figs 14–15, 17, 19–22). Each areola is internally closed by a round and domed hymenate pore occlusion (Fig. 28, position

of slits or perforations is not apparent in SEM). Presence of a relatively wide and slightly bow-tied stauros (the striae delineating the central area are divergent, Figs 14–15), void of areolae up to the margins and externally thickened (Figs 14–15). Raphe filiform and straight. Proximal raphe endings well separate, spatulate and straight-coaxial (Fig. 14). Terminal raphe fissures hooked on the same side and terminating in a sort of contorted areola (Fig. 17). Small apical area void of areolae (Fig. 17 arrow). Cingulum composed of up to 5 copulae on each valve. The valvocopula appears to be open and narrow, with one advalvar row of round puncta which are slightly denser than the striae, and a supplementary short row of scarce and larger puncta on its closed pole. These supplementary puncta, symmetrically positioned on both valves (Fig. 21 arrowheads), give a polarity to the frustule. The pole with the supplementary puncta (on the closed pole of the valvocopulae) may correspond to the head pole of the frustule (Fig. 23 arrowhead). The other copulae (up to four) are open, narrow and show only one row of puncta (Figs 19–22).

**Internally:** A pseudoseptum extends from the apices as siliceous plates covering ca. one-fourth of the valve length on each apex (Figs 24–26). The pseudoseptum continues as very narrow strips along the valve margins (Fig. 28 arrows), and widens into two concave wings in the central area, creating a relatively narrow ‘butterfly-like’ structure (Figs 24, 28). The raphe lies, more or less symmetrically, on the top of a siliceous rib (Fig. 28). One unique pearl-like knob lies in between the proximal raphe endings (Fig. 28). On cleaned mate-

rial, it is possible to see the ‘S-shaped’ external opening of each areola (Fig. 27 arrow) through the internal corroded hymenes (Fig. 27 arrowhead shows the periphery of the corroded hymen).

**Holotype:** Specimen on the SEM stub BM001231764 (National History Museum, London, U.K.) illustrated in Fig. 13.

**Isotypes (Here designated):** Slide BM 101 856 (National History Museum, London, UK), Slide SZCZ 24042 in collection A. WITKOWSKI (The Faculty of Geosciences, Szczecin, Poland).

**Type locality:** Nesting *Chelonia mydas* from French Guiana (Site ‘Azteque’) (48°51'45.81"N, 2°17'15.331"E). Sample named ‘31CM’. Sampling date: 07 04 2016. Collector: Damien CHEVALLIER.

**Etymology:** The epithet was given in reference to the ‘S’-shaped external opening of the areolae, resembling a yin–yang symbol.

**Habitat:** Wild nesting *Chelonia mydas* in French Guiana.

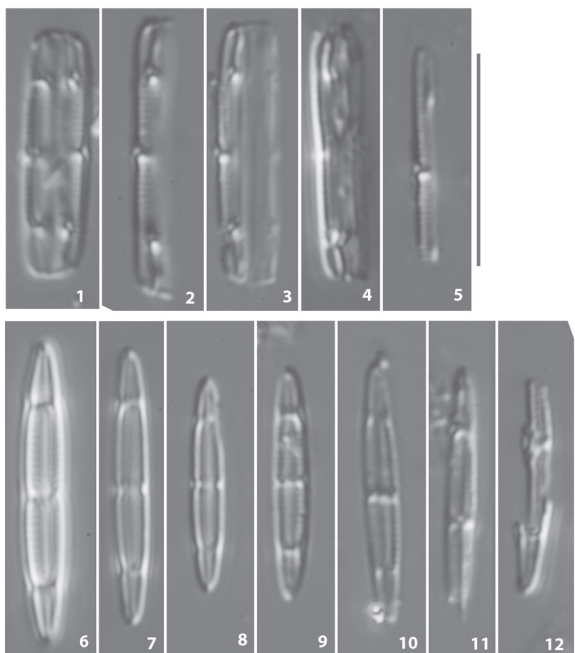
**Taxonomic notes:** Characterized by its small size, narrow and linear shape, complex cingulum and unique external areola opening, this taxon is relatively easy to distinguish from other *Tursiocola* species (Table 1).

***Tursiocola guyanensis* RIAUX–GOBIN et WITOWSKI sp. nov., Figs 30–44.**

**Description:** Very rare, valves small, narrow–elliptical to acicular, with round apices [ $n = 21$  (SEM); 12–13.9  $\mu\text{m}$  (mean  $\pm \sigma$  12.9  $\pm$  0.7) long; 1.70  $\mu\text{m}$  wide; 33–40.6 striae in 10  $\mu\text{m}$  (mean  $\pm \sigma$  37.9  $\pm$  1.7); length/width ca. 7.8]. Frustule biraphid, isovalvar, rectangular in girdle view. Very slightly heteropolar, though the difference between the length of the raphe branches is difficult to discern (see SEM morphology). Pseudosepta present. Complex and wide cingulum composed of numerous copulae (see SEM). Never found in chains in fresh material. Epizoic on *Chelonia mydas*.

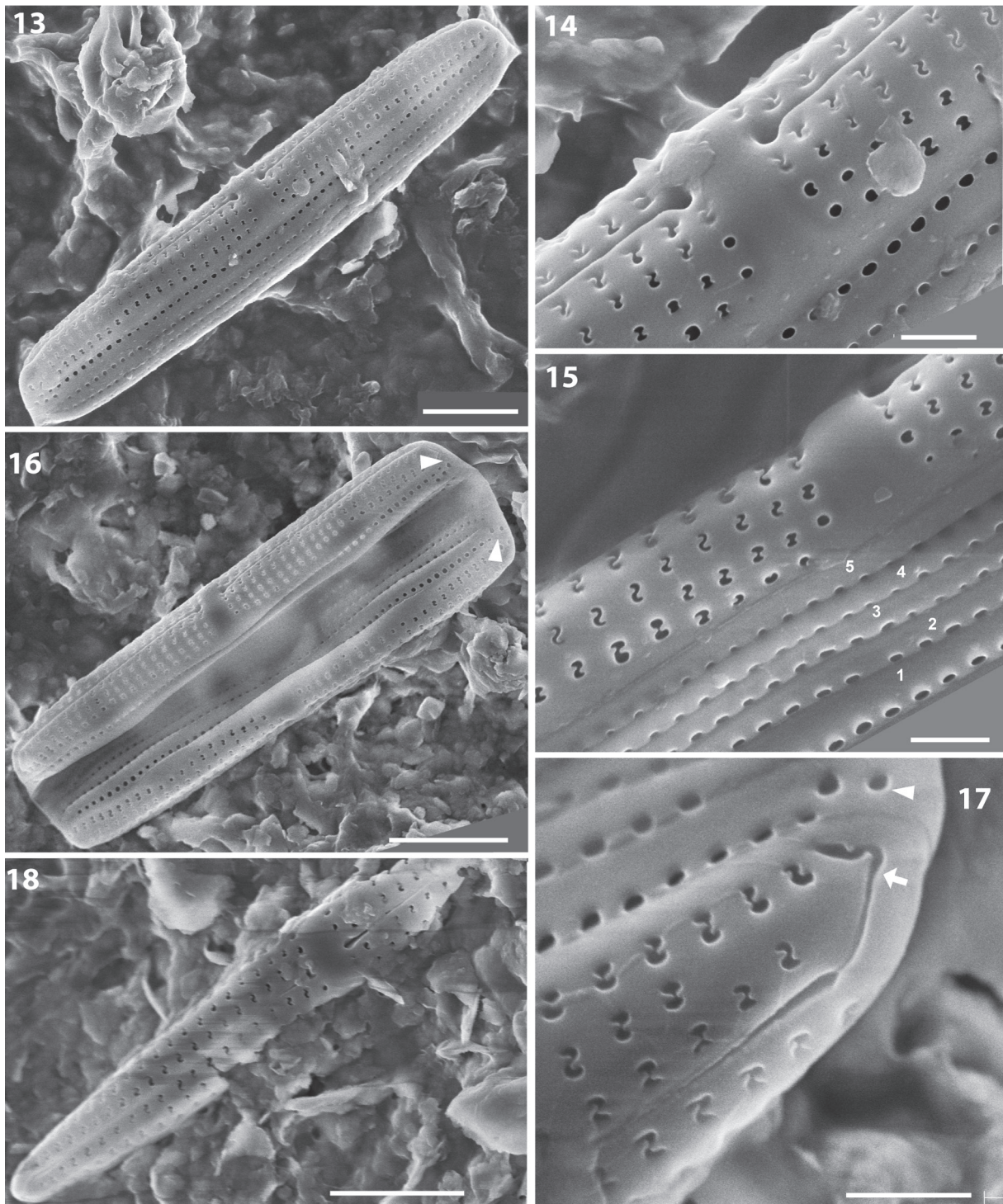
**SEM morphology**

**Externally:** Valve margins with three undulations (Fig. 30). Pole of the frustule showing the closed pole of the copula n°2 (see below), very slightly longer than the other pole. Striae uniseriate, in higher densities on both apices, parallel to very slightly divergent on mid–valve, composed most often of four oblong transapically elongate areolae that are constricted two to three times along their length (Fig. 31 arrowhead). Areolae close to the raphe are diamond-like or quadrangular (Figs 33 arrowhead). Areolae of the apex, near the mantle, often vermiform or with three (or more) constrictions (Fig. 34 arrowhead). Areolae internally closed by domed hymenes (Fig. 44, arrowhead, slits or perforations not apparent in SEM). Relatively large stauros (slightly bow-tied, Fig. 32), void of areolae up to the margins, externally thickened. Raphe straight.



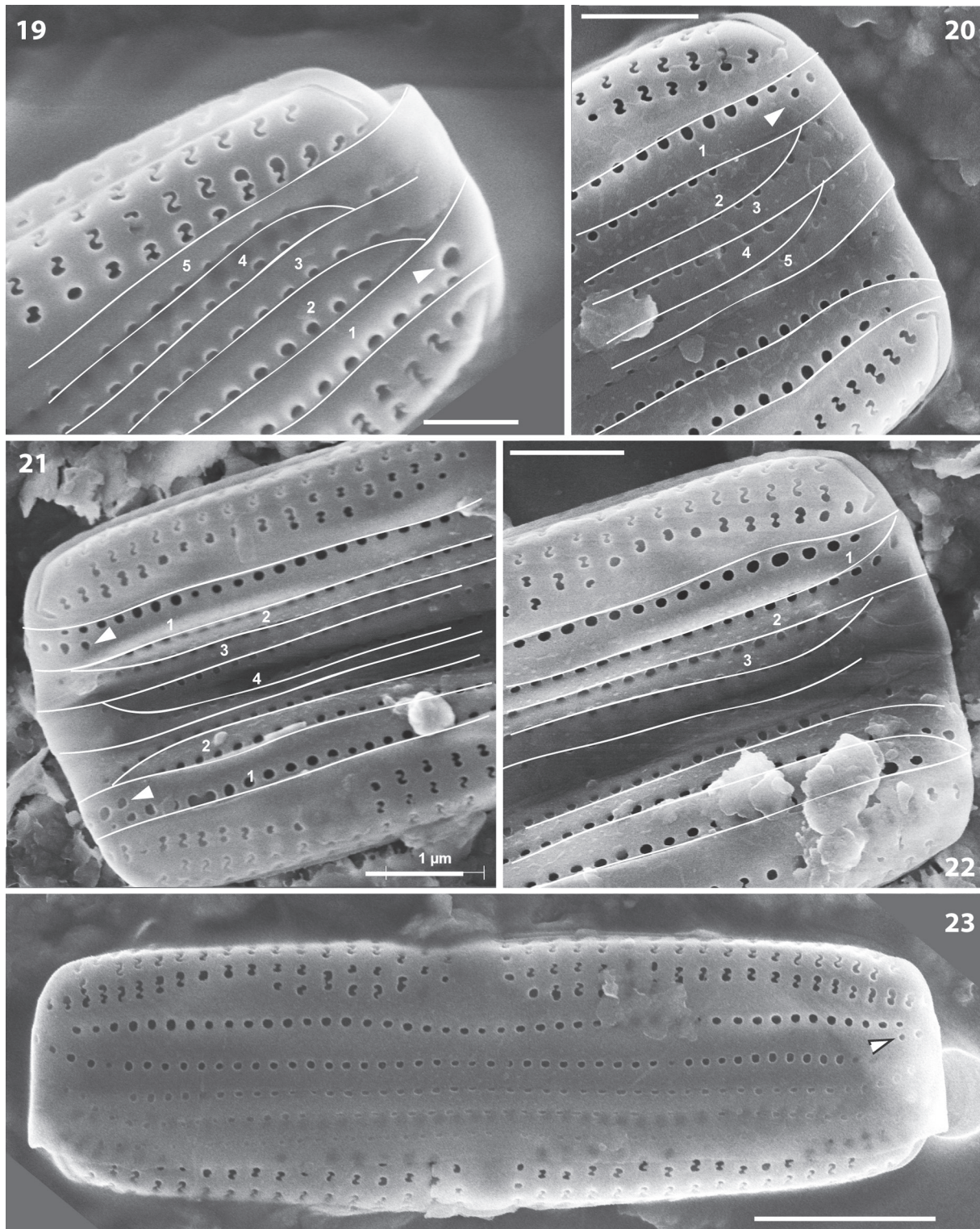
Figs 1–12 (LM): (7–12) *Tursiocola yin-yangii* sp. nov.; (6) *Tursiocola guyanensis* sp. nov. Note the pseudoseptae and the barely visible valve striation. In girdle view (Figs 1–5) it is almost impossible to distinguish between the two taxa. Scale bars 10  $\mu\text{m}$ .





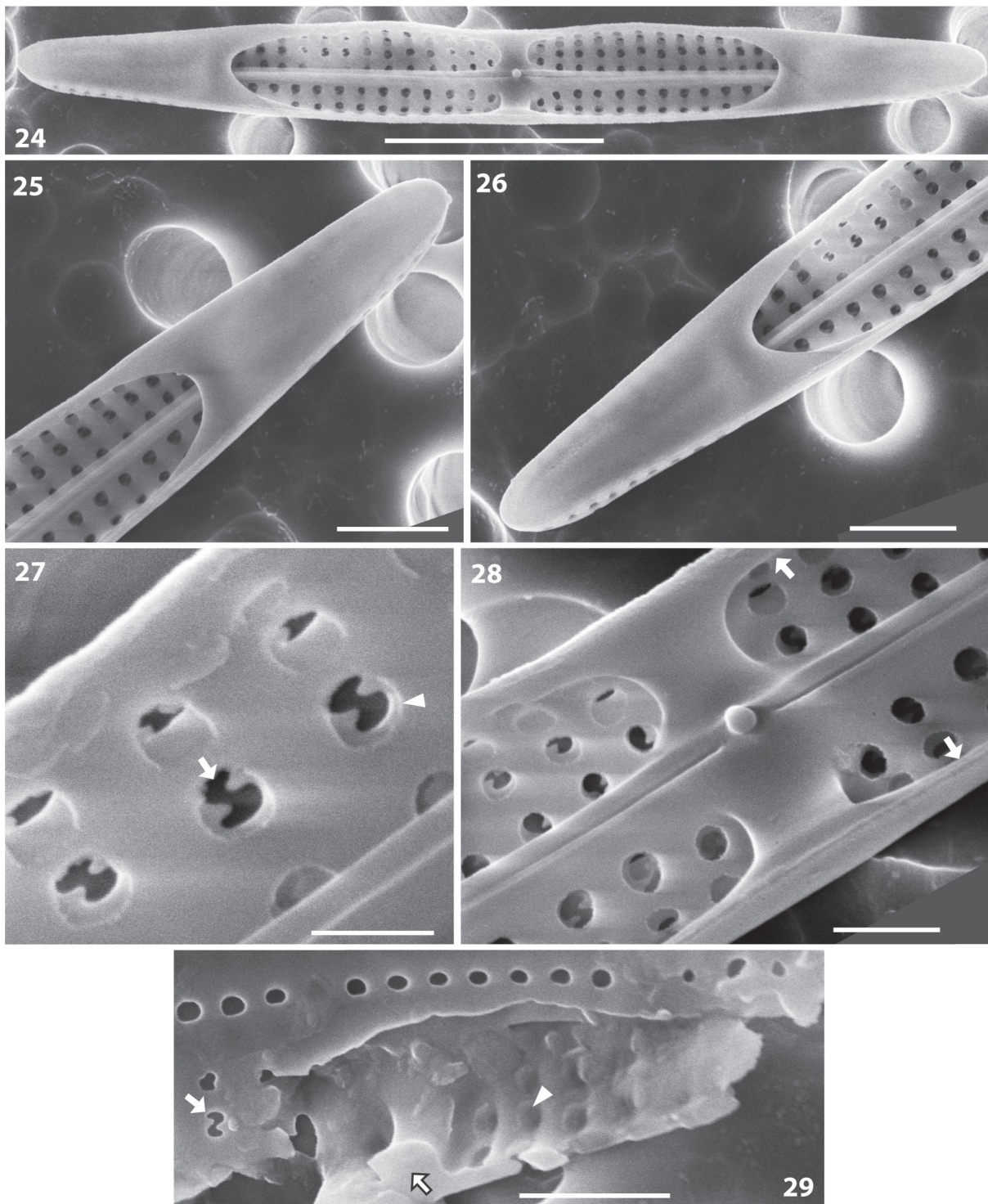
Figs 13–18 (SEM external views), *Tursiocola yin-yangii* sp. nov.: (13) holotype specimen; (13, 16) entire frustules, (16) specimen slightly wedge-shaped in girdle view, foot pole slightly narrower, and head pole with the valvocopulae closed poles (arrowheads); (15) striae composed of two rows of sigmoid areolae; (14) central area with a domed fascia and spatulate proximal raphe endings; (15) striae composed of three to four areolae; (16) somewhat spaced slightly narrower near both poles; (18) frustule acicular in valve view; (17) detail of apex (head pole) with a terminal raphe ending terminating in a contorted areola (arrow) and the valvocopula showing one row of round puncta and a supplementary row of scattered larger puncta (arrowhead). Scale bars 3  $\mu$ m (16); 2  $\mu$ m (13, 18); 500 nm (14, 15, 17).





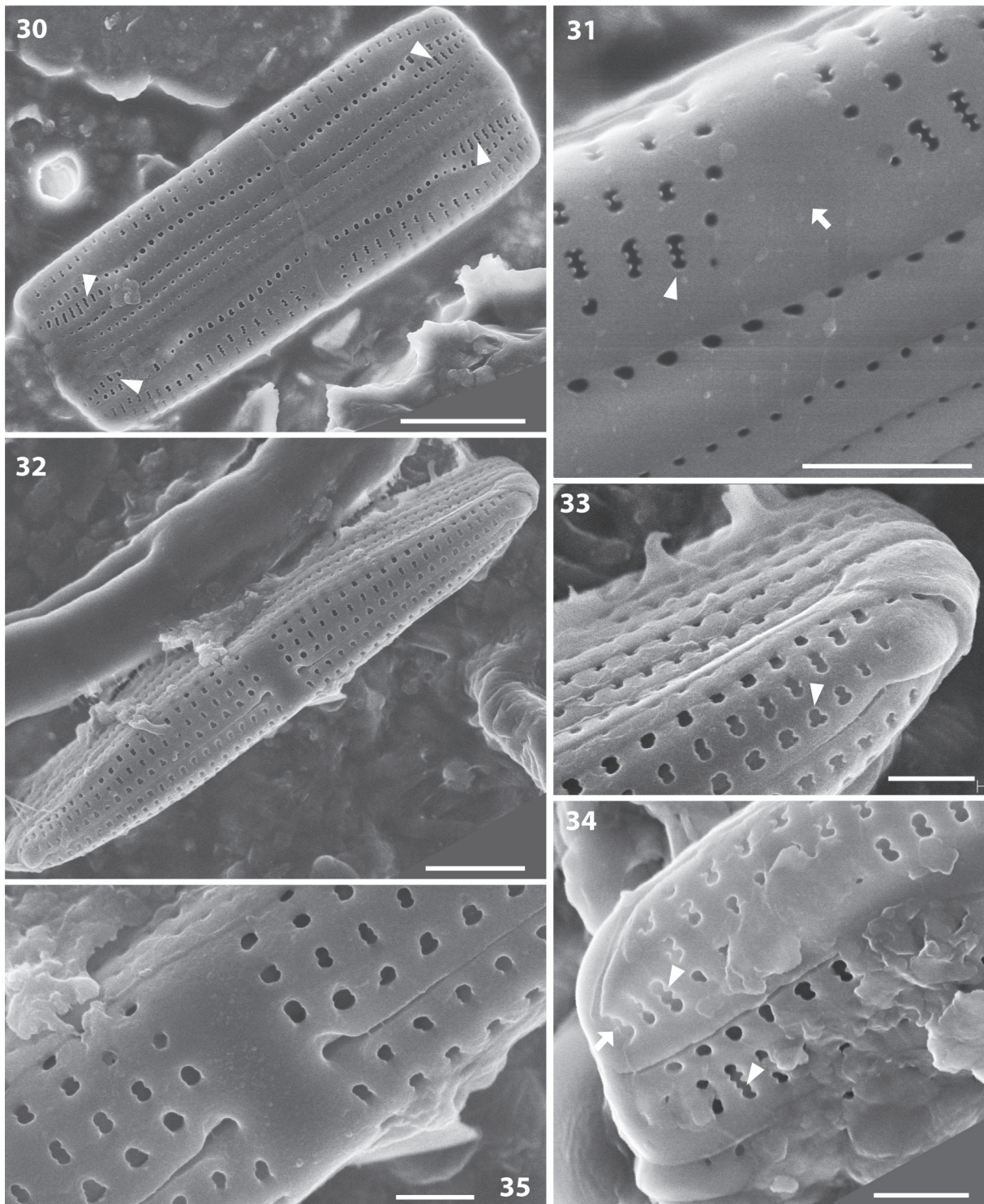
Figs 19–23 (SEM, external views). *Tursiocola yin-yangii* sp. nov.: (23) entire frustule in girdle view with the head pole showing the closed pole of the valvocopula (framed arrowhead); (19–22) the margins of the cingular bands are demarcated; (19–21) details of the frustule head pole with the closed apex of the valvocopula (arrowheads, copulae annotated ‘1’) and open supplementary copulae (annotated ‘2’ to ‘5’); (22) detail of the frustule foot pole with the open apex of the valvocopula; (21, 22) the same frustule. Scale bars 2  $\mu\text{m}$  (23); 1  $\mu\text{m}$  (20–22); 500 nm (19).





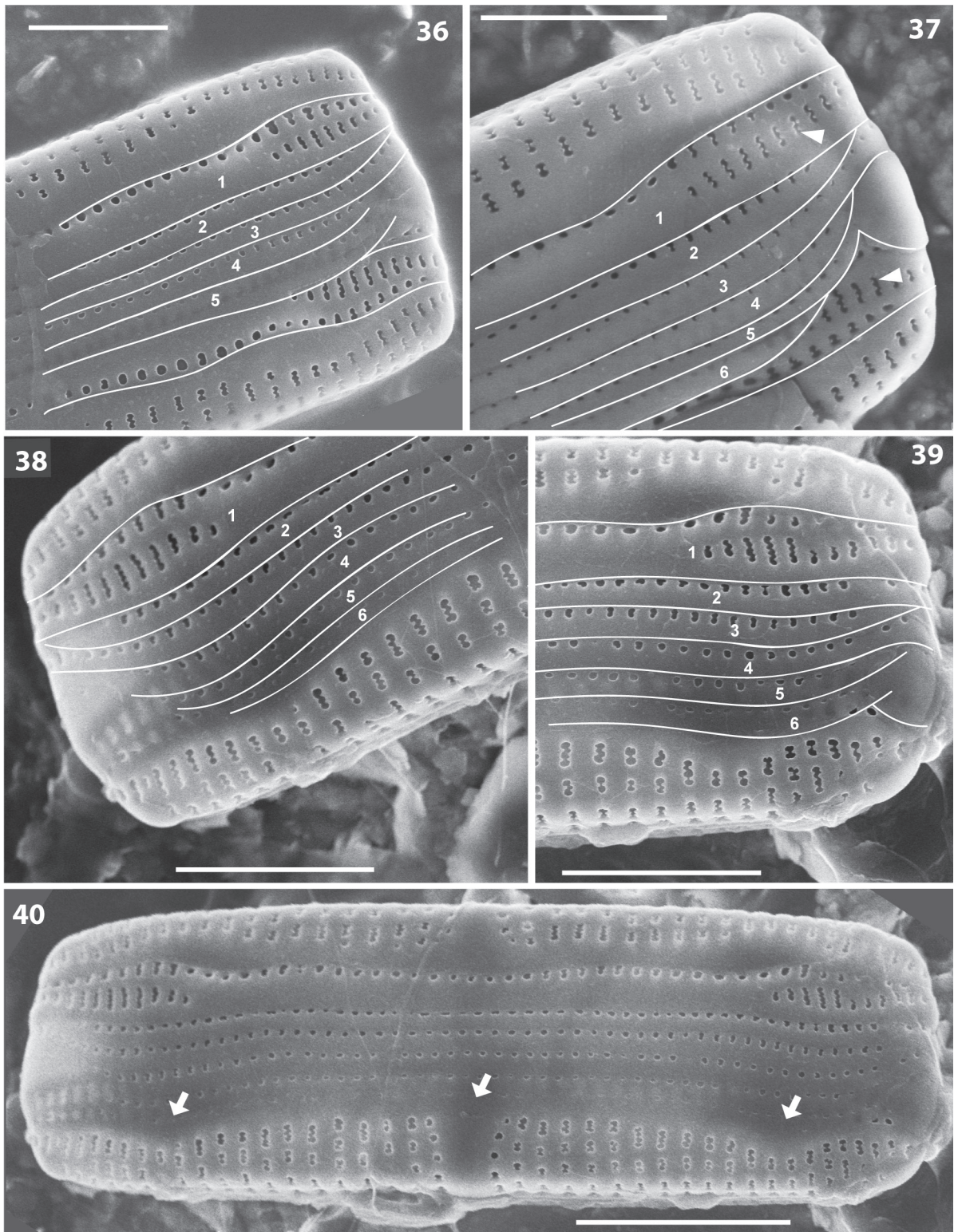
Figs 24–29 (SEM, internal views), *Tursiocola yin-yangii* sp. nov.: (24) entire valve; (25–26) details with the pseudoseptum extending on both apices and continuing as narrow strips along the margins; (27) striae are parallel. Detail of the areolae with corroded hymenes (arrowhead) and underlying sigmoid areola apertures (arrow). (28) detail of the central area with the butterfly structure, raphe slightly lateral and a single knob in between the proximal raphe endings, note the narrow strips along the valve margins; (29) broken frustule with the sigmoid external areola apertures (arrow), the section of the butterfly structure (framed arrowhead) and the internally domed areola hymenes (arrowhead). Scale bars 3  $\mu$ m (24), 1  $\mu$ m (25–26), 700 nm (29), 400 nm (28), 200 nm (27).





Figs 30–35 (SEM). *Tursiocola guyanensis* sp. nov.: (32) frustule in valve view; (30) frustule in girdle view, valvocopula apices with vermiform puncta (arrowheads); (35) central area with a fascia and spatulate proximal raphe endings; (31) fascia in girdle view (arrow), areolae vermiform with two to three constrictions (arrowhead); (33) detail of the valve apex with a reinforcement in one side of the terminal raphe ending, and diamond-like areolae near the raphe (arrowhead); (34) apex in girdle view with the raphe ending terminating in a contorted areola (arrow), areolae vermiform on the mantle and on the valvocopula apex (arrowheads). Scale bars 3  $\mu\text{m}$  (30), 2  $\mu\text{m}$  (32), 1  $\mu\text{m}$  (31), 500 nm (33–35).





Figs 36–40 (SEM, external views), *Tursiocola guyanensis* sp. nov.: (40) entire frustule in girdle view with black shadows corresponding to the siliceous internal septa and central structures (arrows); (36–39) detail of the frustule apex (the margins of the cingular bands are demarcated) with vermiform puncta on the pole of the valvocopulae (annotated ‘1’, Fig. 37 arrowheads), with the presence of 5 supplementary open copulae (annotated ‘5’ to ‘6’). Figs 39 (valve head pole, with the closed pole of the 2<sup>nd</sup> copula) and Fig. 38 (valve foot pole) of the frustule illustrated in Fig. 40. Scale bars 3  $\mu$ m (40); 2  $\mu$ m (36–39).



Proximal raphe endings coaxial, well separated and spatulate. Terminal raphe fissures strongly hooked on the same side, externally ending in a vermiform areola (Fig. 34 arrow). Small apical area void of areolae (Fig. 33). Wide cingulum, composed of numerous copulae (up to 6 per valve). The valvocopula is wide, with an abvalvar row of round to oblong poroids and a second, abvalvar, apical row (1/4 of the valve length, on each apex) with transapically elongate and vermiform poroids (Fig. 37 arrowheads) with more or less the same density as the striae. The supplementary copulae have only one row of puncta that decrease in diameter from the 2<sup>nd</sup> to the 6<sup>th</sup> copula (e.g., in Figs 37, 38). The 5<sup>th</sup> and 6<sup>th</sup> copulae are weakly silicified and narrow. Frustule head pole and foot pole are very similar and hard to distinguish. The closed pole of the 2<sup>nd</sup> copula points out of the head pole of the cell.

The transapically elongate and vermiform puncta on both apices of the valvocopulae are very similar to the areolae on the valve apices (Figs 37–38 arrowheads). These vermiform puncta are present symmetrically on both apices of the valvocopula. The valvocopulae appear to be closed and present tabs standing on the wings of the central structure (Fig. 43). In cleaned material we found no free valvocopula, only narrow open cingular bands, impossible to be attributed to *T. yin-yangii* sp. nov. more than to *T. guyanensis* sp. nov. More observations are needed to clearly describe each cingular band.

**Internally:** Presence of a transverse, narrow and thickened structure (Figs 41–44), with lateral expansions reminiscent of a butterfly (Fig. 43 arrows). A picture of the entire pseudoseptum has not yet been obtained. In Fig. 40 (arrows) the black shadows at each pole of the frustule and on the central part, mark the presence of siliceous internal thickenings corresponding to the septa and central structures.

**Holotype:** Specimen on the SEM stub BM001231764 (National History Museum, London, U.K.) illustrated in Fig. 32.

**Isotypes (Here designated):** Slide BM 101 856 (Natural History Museum, London, UK), Slide SZCZ 24042 in collection A. WITKOWSKI (The Faculty of Geosciences, Szczecin, Poland).

**Type locality:** Nesting *Chelonia mydas* in French Guiana from ‘Aztèque’ (48°51'45.81"N, 2°17'15.331"E). Sampling date: 07 04 2016. Collector: Damien CHEVALLIER. Also present, as rare, on juvenile *Chelonia mydas* at ‘Anse du Bourg’, Lesser Antilles (14° 29' 13.43"N, 61°44 58.886"W).

**Etymology:** The epithet *guyanensis* was given in reference to French Guiana (Guyane), the location where the species was first found.

**Habitat:** Nesting wild adult *Chelonia mydas* from French Guiana, host *Tursiocola guyanensis* sp. nov. In sample ‘31 CM’, this latter taxon is rare, while *T. yin-yangii* sp. nov. is more abundant. *T. guyanensis*

was also observed as very rare on 3 juvenile specimens of *Chelonia mydas* from Martinique Islands (Lesser Antilles). Until now, this species was not observed in samples taken from *Chelonia mydas* from the South Pacific, nor were they found on *Dermochelys coriacea* (leatherback turtle), or *Lepidochelys olivacea* (olive Ridley turtle) from Guiana.

**Taxonomic notes:** The internal central area of *Tursiocola guyanensis* (Figs 40–43), with a narrow silicified transverse structure and semblance of wings (Fig. 42 arrows), are characteristic of the genus *Tursiocola*. There are similarities between our taxon and *T. denysii* FRANKOVICH et M.J.SULLIVAN in FRANKOVICH et al. (2015b). *T. denysii*, like our taxon, has closed valvocopulae ornamented on their apices by a second row of puncta simply referred to as ‘abvalvar pores’ (ref. cit., fig. 28 arrow). In *T. guyanensis* sp. nov., the poroids are long and vermiform. Furthermore the valvocopulae in *T. guyanensis* sp. nov. are wide whereas those in *T. denysii* seem more narrow. Additional features that differentiate the two taxa include the following: *T. guyanensis* sp. nov. is more acicular than *T. denysii* (length/width = ca. 7 in *T. guyanensis* versus ca. 4.8 in *T. denysii*), with striae less strongly radiate, less areolae per stria and most likely a more complex cingulum. Furthermore, the areolae in *T. denysii* are described as ‘transapically elongated’ but not constricted in their middle.

A taxon presented as *Tursiocola* sp. from a leatherback turtle in ROBINSON et al. (2016, fig. 2–D), with an internal narrow transapical structure with lateral triangular wing-shaped expansions, and valvocopulae showing apices with a double row of puncta, may be similar to our new taxon.

## DISCUSSION

Several studies have attempted to find reliable characters to distinguish *Tursiocola* from *Epiphallaina*. HOLMES et al. (1993a) suggested that the butterfly-like internal structure could serve to discriminate between the two latter genera (Table 1). Thereafter, WETZEL et al. (2012) pointed that the copulae in *Tursiocola* systematically have two rows of puncta versus only one in *Epiphallaina*. It is now clear that the latter does not hold and that the ornamentation of the copulae varies among *Tursiocola* species (FRANKOVICH et al. 2015a,b and present study, Table 1): For example, *Tursiocola costata* FRANKOVICH et M.J.SULLIVAN has copulae with only one row of punctae (Table 1).

The two taxa reported here belong to *Tursiocola*, and species from this genus may be differentiated ultrastructurally, particularly by the: 1) shape of the external areola opening, 2) external shape and width of the stauros, 3) proximal raphe endings shape, 4) complexity of the cingulum (simple or composed of

Table 1. Morphometrics and characteristic features of *Tursiocola* and *Epiphthalina* published species [(+) present, (–) absent, (nd) not specified, ( ) = as observed from the original illustration and/or from recent bibliographic references].

	Heteropolarity	Frustule outline	Length (µm)	Width (µm)	Striae in 10 µm and position	Areola shape	Proximal raphe endings	Valvocopulae	Copulae	Stauros in external side	Internal central knob(s)	Internal butterfly structure
<i>Tursiocola yin-yangii</i> sp. nov. Present study	difficult to observe	acicular, acute apices	8.7–14.5	1.32–1.37	36–42.8 parallel, slightly radiate on mid-valve	'S'-shaped	round to spatulate, coaxial	open, narrow, 1 row of round puncta, and short row of bigger puncta at foot pole	multiple	relatively wide, bow-tie shaped	1 knob	+
<i>Tursiocola guyanensis</i> sp. nov. Present study	very slight	narrow-elliptical to acicular	12–13.9	1.7	33–40.6 parallel, slightly radiate on mid-valve	oblong with constrictions	round to spatulate, coaxial	closed, wide, 1 row of round to elongate puncta, and a partial row of vermiform puncta at both apices	multiple	relatively wide, bow-tie shaped	nd	+(remains to be fully illustrated)
<i>Tursiocola omurai</i> (T.NEMOTO) DENYS in DENYS1997 (no SEM) Basionym: <i>Stauroneis omurai</i> NEMOTO in NEMOTO 1956	nd	median sub-constriction of the valves, rostrate ends	20–30	4–5	30 parallel	indistinctly punctate (round to rectangular)	nd	closed, 2 rows of round puncta	nd	central area very narrow, linear fascia	one hump	+
<i>Tursiocola staurilineata</i> DENYS in DENYS1997	slight	linear to very narrowly lanceolate, constricted at the pseudo-septa	15–32.9	2.2–3.2	34–36 parallel in mid-valve, slightly radiate on apices	more or less round	coaxial, slightly enlarged	closed girdle bands, 2 per frustule, aligned round poroids	normally	narrow, straight, small roundish central area	2 knobs	+
<i>Tursiocola olympica</i> (HUSTEDT) R. W. HOLMES, NAGASAWA et TAKANO in HOLMES et al. 1993a Basionym: <i>Stauroneis olympica</i> HUSTEDT 1952	nd	linear lanceolate, round apices	15–35	1.5–4	28–32 (almost parallel)	round (0.16–0.22 µm in diameter)	(slightly deflected)	closed girdle bands, double row of puncta		(fairly broad, rectangular to bow-tie shaped)	1 knob	+



Table 1 Cont.

<i>Tursiocola podocnemicola</i> C.E. WEITZEL, B.VAN DE VUIVER et L. ECTOR in WEITZEL et al. 2012	nd	narrow lanceo- late, acute apices, slightly constricted	15–26	1.5–2	30–35 parallel	round, oval to rectan- gular	strongly curved unilaterally	2–3 open bands, 1 row of large pores and a short row of smaller pores (or 2 rows, unclear)	rectangular stauros	2 knobs	+
<i>Tursiocola ziemani</i> FRANKO- VICH et M.J.SULLIVAN in FRANKOVICH et al. 2015a	–	narrow lanceo- late, rostrate apices	20–61	2.4–5.2	22–25 convergent in mid- valve, paral- lel on apices	oval	doubly hooked unilaterally	only 2, open, 2 rows elongate pores	diamond-shaped central area, + stauros	2 knobs	+
<i>Tursiocola costata</i> FRANKO- VICH et M.J.SULLIVAN in FRANKOVICH et al. 2015a	+	lanceolate, drawn out ros- trate apices	17–29	2.5–3.9	22–29, onvergent, raised virgae	circular to oval to irre- gular	strongly deflected on one side	2 copulae, open, 1 row of circular to oval pores	diamond-shaped central area, narrow stauros	2 knobs	+
<i>Tursiocola variocopi- lifera</i> FRANKOVICH et M.J.SULLIVAN in FRANKOVICH et al. 2015a	–	narrow, constricted in the middle in girdle view	31–57	2.9–4.7	25–28 slightly convergent in mid- valve, paral- lel on apices	oval, fine slit-like elongate areolae	straight expanded	coarsely striated, open, 2 rows of linear pores	diamond-shaped central area, nar- row rectangular stauros	2 knobs	+
<i>Tursiocola denysii</i> Franko- vich & M.J.Sullivan in Frankovich et al. 2015b	slight	narrowly lanceolate, change in curvature to juncture of pseudoseptum	10–20	2–2.9	2 6 – 3 4 , radiate, to parallel on apices: 37– 43	elongated	straight, asymme- tric spathulate	closed, 1 row of ovoid pores, a second abvalvar row of similarly spaced pores	wide bow-tie stauros	1 knob	+
<i>Epiphthalina aleutica</i> (T.Nemoto) R.W.Holmes, S.Nagasawa & Takano in Holmes et al. 1993a, see also Denys 1997	+	lanceolate	15–26 (28–42 in Ne- moto)	1.75–3	30–32 more or less parallel, radiate on apices	approximate- ly circular, 0.15 µm in diameter	(coaxial, not enlar- ged)	open, 1 row of round to rectan- gular perfora- tions	fairly broad, rectangular to butterfly-shaped	1 knob- like struc- ture	–
<i>Epiphthalina aleutica</i> var. <i>lineata</i> Denys in Denys 1997 (no SEM)	nd	slightly constricted or concave in mid-valve		<2.5	more slender form (on Dall's porpoise only) other structures identical to <i>E. aleutica</i>						
<i>Epiphthalina radiata</i> R.W.Holmes, S.Nagasawa & Takano in Holmes et al. 1993b	+	slightly constricted or concave in mid-valve	17–31	2.4–4	26–28, radiate (5–6 puncta, except in the central area)	round to oval	(coaxial, not enlar- ged)	open, 1 row of elongate pores	(narrow, bow-tie shaped)	(possibly 1 knob)	–

multiple bands), 5) valvocopula and supplementary copula ornamentation (the puncta, sometimes identical in form and dimensions to the valve areolae, are not perforations and are closed by domed internal hymenes), 6) closed or open valvocopulae and copulae.

The valvocopula structure, and the presence of a short supplementary row of puncta at both poles (or at a single pole) of the valvocopulae and copulae, may be particularly good criteria to differentiate species. Unfortunately, all published taxa have not been examined with the same degree of EM accuracy, so some details concerning the number of copulae or their ornamentation, is not yet known.

Following FRANKOVICH et al. (2015a,b) and their ‘artificial’ key based on diverse morphological features, *Tursiocola denysii* FRANKOVICH et M.J.SULLIVAN and *T. variocopulifera* FRANKOVICH et M.J.SULLIVAN have valvocopulae that are different from their abvalvar copulae. *T. guyanensis* sp. nov. and *T. yin–yangii* sp. nov. also belong to this category. Nevertheless, some *Tursiocola* species are described as having only 2 cingular bands (valvocopulae with no supplementary bands), that are difficult to be qualified as ‘undifferentiated’.

The following key primerly uses the structure of the cingulum and its diverse degree of complexity, to separate all currently known *Tursiocola* taxa.

Provisional key to *Tursiocola* species, mainly based on the cingulum:

- 1 more than 4 copulae per valve and valvocopulae different from abvalvar copulae.....2
- only 2 or 3 cingular bands per frustule.....6
- 2 valvocopulae closed, with 1 row + short segment of ovoid puncta.....*T. denysii*
- different valvocopula ornamentation.....3
- 3 valvocopulae broad, with 1 row of round puncta + large segment of vermiform puncta .....*T. guyanensis*
- valvocopulae otherwise.....4
- 4 valvocopulae open, 2 complete rows of linear puncta.....*T. variocopulifera*
- different valvocopula ornamentation.....5
- 5 1 row of round puncta + short segment of scarce round puncta.....*T. yin–yangii*
- 6 2 to 3 bands, open, 1 row + segment of smaller puncta.....*T. podocnemicola*
- cingulum with only 2 bands.....7
- 7 valvocopulae open, 1 row of oval puncta.....*T. costata*
- different valvocopula ornamentation.....8
- 8 2 rows of elongate puncta.....*T. ziemanii*
- valvocopulae otherwise.....9
- 9 valvocopulae closed, 2 rows of round puncta.....*T. staurolineata*
- Idem, with broader and more rhombic stauros.....*T. olympica*
- Idem, with distinct median constriction and somewhat rostrated ends.....*T. omurai*

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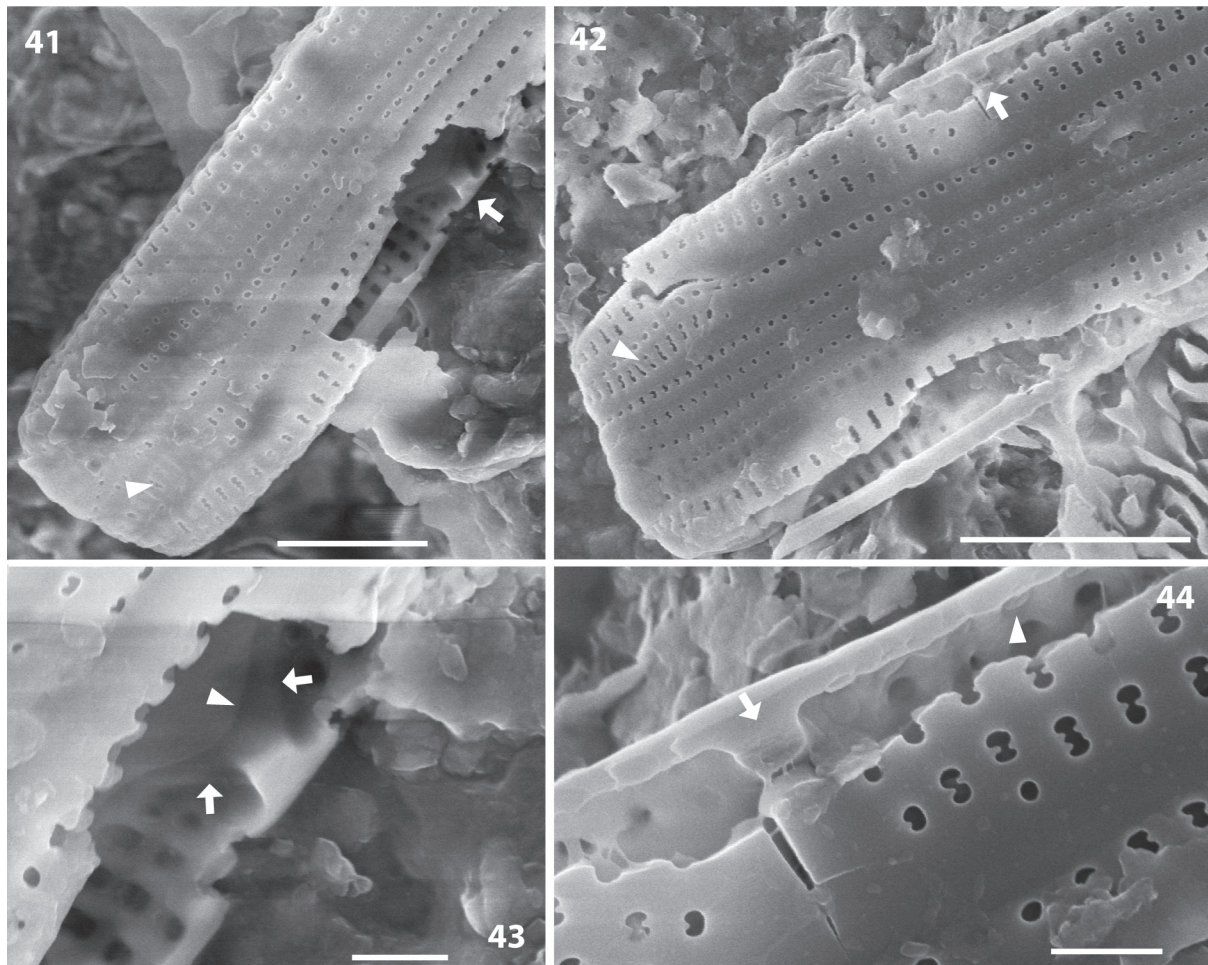
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Figs 41–44 (SEM, internal views), *Tursiocola guyanensis* sp. nov.: (41) broken frustule showing the internal central structure (arrow) and the vermiform puncta on the pole of the valvocopula (arrowhead); (43) detail of Fig. 41 with the valvocopula median tab (arrowhead) standing on the lateral expansions of the butterfly structure (arrows); (42) broken frustule showing the internal central structure (arrow) and the vermiform puncta on the pole of the valvocopula (arrowhead); (44) detail of Fig. 42 with the broken and narrow butterfly structure (arrow) and the internally domed areola hymenes (arrowhead). Scale bars 3  $\mu\text{m}$  (42); 2  $\mu\text{m}$  (41); 500 nm (43–44).

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