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Kataplana celeretrix n. sp. (Platyhelminthes: Proseriata: Otoplanidae) from the Coast of North Carolina, USA

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Kataplana celeretrix is described as a new species of proseriate flatworm belonging to the Otoplanidae. This species was found in low-tide-level surface sediments at two high-energy beach sites in North Carolina and is unique among described Otoplanidae in possessing post-pharyngeal germaria. In addition, we consider the intermediate taxonomic position that this new species occupies between Parotoplaninae and Otoplaninae, and point to the utility of confocal microscopy in routine species descriptions.

Sediment was collected in October 2010 from the lower mid-tide level (LMTL 0 to 15 cm sample depth), resurgence ("shiny" 0 to 10 cm) and retention (0 to 15 cm) zones of a high-energy beach near Emerald Isle (EI), North Carolina (within 10 m of N34°38'41"; W77°5'22.8"—Type Locality), and from the shiny, swash, and shallow subtidal at a beach near Oak Island (OI), North Carolina (within 10 m of N33°54'46"; W78°13'1.5") in June 2011. Specimens were extracted from the sand samples, photographed and drawn, processed for Confocal Laser-Scanning Microscopy (CLSM) and resin-embedment/serial-sectioning (Whitson *et al.* 2011). DNA was extracted from single specimens as described in Whitson *et al*. (2010) and an approximately 400bp fragment of the 18s rDNA gene was recovered by PCR and sequenced using "universal" 18s primers (Fonseca *et al.* 2010). Material examined included 5 sets of serial sections, photographs and/or drawings of 15 living, squeezed specimens, and CLSM stacks of 18 whole-mounted specimens. CLSM stacks were rendered with the following colors: Blue: Hoechst 33342 for DNA; Green: Alexa488 phalloidin for muscles; Red: Anti-phosphoH3 for mitotic cells (and artefactual binding to certain glandular secretions); Yellow: Anti-acetylated Tubulin for cilia and flagella. Positions along the longitudinal body axis are expressed as percentage of the body length and given as "U-values", where U0 is the anterior tip of the body and U100 is the posterior end.

***Kataplana celeretrix* n. sp.**

The specific epithet (L. "swift harlot") refers to the extremely rapid movement and bivaginate condition of the organism. Since the early 1970s, this species has been known from the EI site as "OtoStumpf" (Rieger, unpublished). Type material was deposited at the Smithsonian National Museum of Natural History (NMNH) as follows: Holotype—a whole-mounted specimen from EI (NMNH 1156977); Paratypes: two serially-sectioned specimens from OI (NMNH 1156978-1156979). A 412-base sequence from the 18s rDNA gene of a specimen from OI was deposited at GenBank under accession number JQ180234; blastn returned the sequence from *Parotoplana renatae* at an E-value of $2e^{-171}$, placing our specimens firmly into Parotoplaninae.

In transmitted light, the free-swimming animal was golden brown with a peripheral translucent zone; the body shape was an elongated oval with a slightly pointed tail and blunt head (Fig. A). *Kataplana celeretrix* were exceptionally fast-moving, darting from grain to grain in a fast stop-and-go pattern by means of ciliary gliding. The animals were also remarkably adhesive.

Kataplana celeretrix possessed intraepithelial nuclei throughout, with rare mitoses (detectible by anti-phosphoH3 staining) always beneath the body-wall muscle (CLSM data not shown). The head region had numerous sensory bristles with two pairs of larger bristles arising laterally from regions of ciliation found on either side of the head (Fig. B). Smaller bristle groups were spaced along the sides of the body running to the posterior end (Figs. B, F). A pair of photoreceptors ("Sehkolben"), slightly pink in life, occurred in the posterior part of the brain (Fig. B). Pericerebral ciliary aggregates, visible only with CLSM, were anterior to the brain dorsally underneath the epidermis (Fig. C). The statocyst

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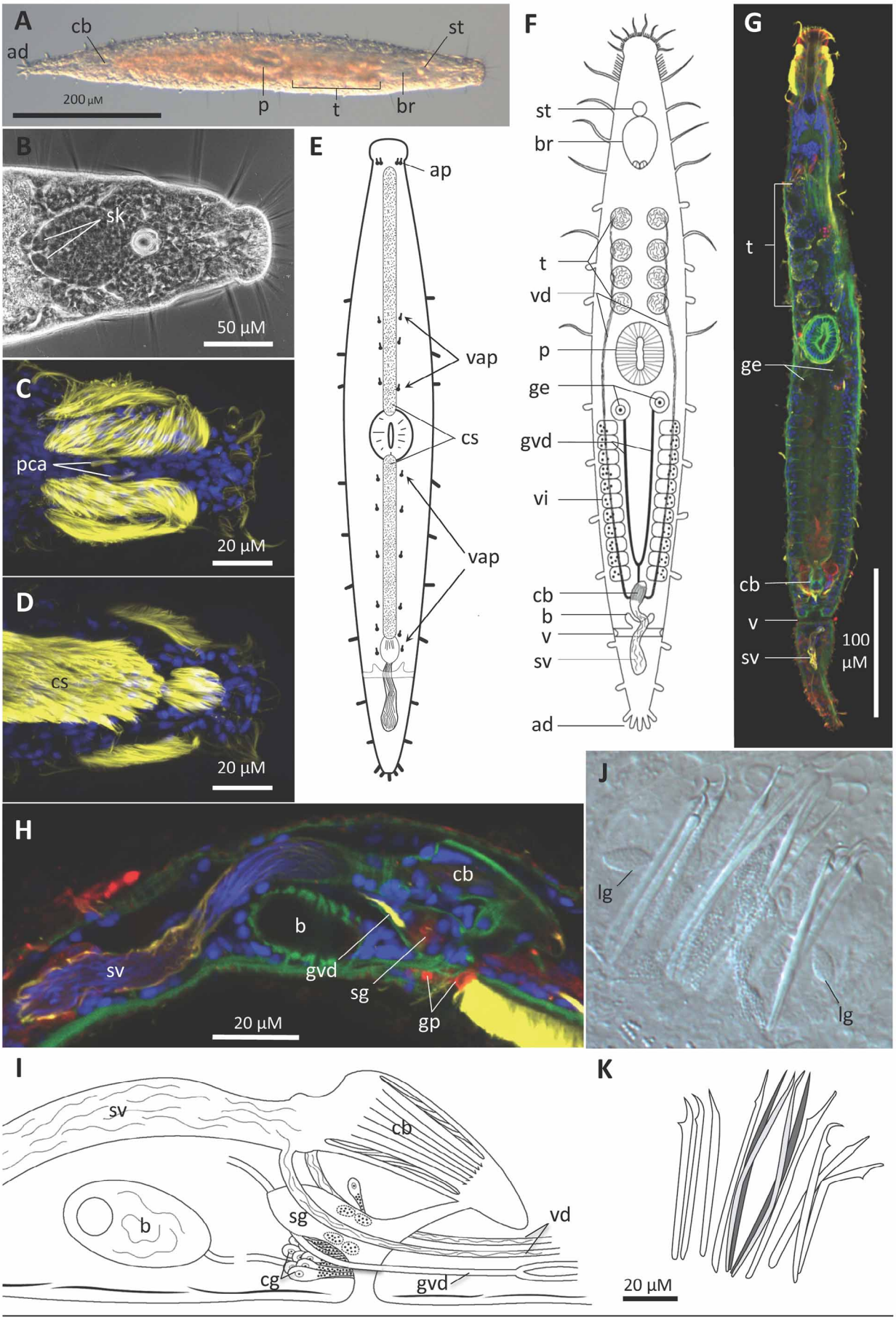
possessed 8 accessory cells (“Nebensteinchen”; see Ehlers & Sopott-Ehlers, 1990). Ventrally, there was a small patch of cilia on the underside of the head, and the “creeping sole” (an English translation of the German “Kriechsohle” that we prefer to the more awkward “creepsole”) originated just posterior to this and ended just anterior to the male pore (Figs. D, E). The creeping sole was interrupted by a cilium-free zone at the level of the mouth. Five adhesive papillae were grouped into a tailplate, and others occurred along the margin of the body, in two dorsal rows, and ventrally along either side of the creeping sole. The anterior-most ventral papillae comprised a pair (rarely three) on either side of the midline at the anterior end of the creeping sole (Figs. E, F).

Kataplana celeretrix had a vertically directed pharynx plicatus at U44 with both internal and external ciliation. The pharynx was oval in cross-section in living and fixed specimens, measuring 54 µm in the anterio-posterior axis and 34 µm transversely. The musculature of the pharynx comprised outer longitudinal and inner circular fibers along both the inner and outer walls. Pharyngeal glands occurred in the parenchyma circumferentially around the pharynx. Their gland necks entered the pharynx dorsally and opened around the ventral tip of the pharynx. The gut possessed an extremely thin epithelium separating it from dorsally-located chordoid tissue. A pre-cerebral diverticulum was not visible in living material or in our sections; however, it is possible that an anterior extension of the gut with such a thin epithelium could be invisible in living material and could not be distinguished from chordoid tissue in our sections.

Germaria were located posteriorly to the pharynx (Figs. F, G). Allosperm were occasionally observed by CLSM at the proximal ends of the germovitelloducts, in association with the germaria. Paired germo-vitelloducts ran posteriorly, combining into a single duct on the right side of the body which passed around the genital atrium just before entering the shell-gland region (Figs. F, H, I). The duct exited the glandular region before it seemingly disappeared just ventrally to the seminal vesicle (Fig. H, I). Follicular vitellaria occurred laterally from just posterior to the germaria to just anterior to the male copulatory organ (Fig. F). Two vaginae opened from either side of the body into a bursa, located ventrally to the seminal vesicle and posteriorly to the common genital atrium (Figs. E-G). Vaginae and the bursa were underlain by strong circular muscles (Fig. H) and lined by a thin epithelium. The bursa possessed two anterior wing-like extensions running towards the common genital atrium (Fig. F), and a single DIC micrograph showed these each continuing anteriorly into what looked like an unciliated duct. We could not detect connections between the bursa and the rest of the genital system.

Four to five pairs of testes were visible in the anterior portion of the body with two vasa deferentia running parallel from the testes and opening into the anteriormost portion of the seminal vesicle from either side (Figs. F-I). The entire copulatory bulb was 38 µm in length and the elongated sac-like seminal vesicle was 105 µm in length. Sperm were observed within the seminal vesicle and throughout the vasa deferentia of fully mature specimens. The copulatory bulb contained a wreath-like stylet apparatus comprising 12 stylets of four different types in three separate groupings (Figs. J, K). These were: Group A: two sets of three hooked stylets (30 µm long), on either side of a central set of six stylets, comprising: Group B: two lateral hooks (37 µm) and four central bow-shaped stylets (Group C: outer pair—39 µm and Group D: inner pair—40 µm with outwardly-turned tips). Three pairs of prominent muscles were found within the bulb dorsally, laterally, and ventrally; the relationship of these six muscles to the stylets in group A was not ascertained. Prostate glands were found outside the copulatory bulb laterally, and sent gland-necks bearing granular secretions into the bulb. A small pair of glandular sacs was present within the copulatory bulb on both sides of the stylet apparatus (Fig. J). In our CLSM material, the common gonopore was surrounded by glandular material (Fig. H), although these glands were not unequivocally observed in our living, squeezed specimens.

Kataplana celeretrix belongs in the Parotoplaninae (Ax 1956) based on the vertically oriented pharynx plicatus, the possession of a shell-gland (“Schalendrüsen”) combined with the lack (at least in our living material) of a ring of cement glands (“Kittdrüsen”) around the gonopore. Within Parotoplaninae, the combination of a bursa separate from and posterior to the genital atrium (found in *Kataplana, Otoplanidia*), a vesicula granulorum incorporated into the copulatory bulb and a well-developed shell-gland region in a posterior pocket of the genital atrium (found in *Kataplana, Parotoplanina, Praebursoplana, Triporoplana*), and Sehkolben (found in *Kataplana, Triporoplana, Parotoplana*) places our species into *Kataplana* (Ax 1956). Within *Kataplana*, *K. celeretrix* differs from the three described species in number and shape of the copulatory stylets and in the possession of paired vaginae, rather than a single (or presumed single) vagina (*cf. K. germanica—sensu* Ax 1951; *K. mesopharynx*—Ax 1956; *K. arcuata*—Sopott-Ehlers 1976). The male organ of *K. arcuata* is most similar to that of *K. celeretrix*, sharing a similar bow-shaped central stylet group and lateral gland cells incorporated into the copulatory bulb (Sopott-Ehlers 1976, her Fig. 12B). However, the number of stylets (12 in *K. celeretrix*, 14 in *K. arcuata*), the number of types of stylets (4 in *K. celeretrix*, 3 in *K. arcuata*), and the post-pharyngeal germaria and the presence of 2 vaginae in *K. celeretrix* make it extremely unlikely that our species is identical with *K. arcuata*. Unfortunately, *K. arcuata* was described only from living material, and no type material exists.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Outside the Parotoplaninae, a bivaginate condition is also found in the genus *Kata*. In addition, *Kata* and *Kataplana* have very similar connections of the germovitelloduct to the bursa. Ax (1956, p.154) dismisses these similarities as convergent, as *Kata* and *Kataplana* belong to separate subfamilies (Otoplaninae *vs* Parotoplaninae). Because the orientation of the pharynx is highly diagnostic in otoplanid taxonomy, any species with a vertically oriented pharynx plicatus will be almost automatically assigned to Parotoplaninae. Accordingly, a possible alternative explanation for the similarities between *Kata* and *Kataplana* might be that our species and others now assigned to *Kataplana* represent "miniaturised" members of the genus *Kata*—all known *Kata* species are several millimeters in length, whereas the 4 known species of *Kataplana* are smaller than one millimeter. Evolution for small body size could be coupled with shortening and vertical re-orientation of the pharynx (with *Kataplana mesopharynx* as an intermediate form). Ax (1956, his Fig. 2) carefully considered this change in pharyngeal orientation, but he assumed that it has only occurred once. We suggest that it has occurred at least twice, as we agree with Ax (1956, p 74) that *K. mesopharynx* is unlikely to represent the primitive member of the Parotoplaninae. This change in the orientation of the pharynx might also be coupled with the post-pharyngeal position of the germaria, and in this context, it is important to note that Ax (1956) describes the position of the germaria as "lateral to the pharynx" in *K. mesopharynx*. Furthermore, the other diagnostic character of the Otoplaninae (shell-glands around the gonopore) may or not be present in *Kata* (shell-glands not shown nor mentioned for *K. evelinae*, and drawn, but not labeled for *K. leroda*—Marcus 1949, 1950; "not observed in our serial sections" for *K. galapagoensis*—Ax & Ax 1974; drawn but not mentioned for *K. galeae*—Ax & Sopott-Ehlers 1987). In summary, we suggest that careful ultrastructural and molecular studies of members of these two genera might prove fruitful.

Although the use of CLSM with fluorescent phalloidin staining of muscles has become commonplace in routine species descriptions, it is likely to hold additional untapped potential when combined with fluorescent antibody staining. The anti-acetylated tubulin antibody used here reveals both external and internal cilia and sperm-tail flagella well, making visible details not otherwise readily seen. In particular, the ciliated part of the germovitelloduct, allosperm in association with the germaria, pericerebral ciliary aggregates, and head ciliation that comprises separate patches (instead of a single field) were all observed using this technique, and could not be observed in either living specimens or our sectioned material.

JRB carried out the majority of live observations and of the CLSM work, drafted the manuscript and composed the plate. JSIII contributed DIC micrographs, prepared the serial sections, made initial CLSM observations, drew figures E and K and edited the draft manuscript. MKL sequenced material for 18 rDNA. The authors are grateful Dr. Stephen Fegley and the UNC-IMS for laboratory space during the 2010 and 2011 field seasons, to Ms. Nicole Dallin for drawing figures F and I, and to Dr. Gunde Rieger and the estate of the late Dr. R.M. Rieger for permission to use his unpublished work on "OtoStumpf", including the micrograph in figure B. All three authors reviewed and approved the final manuscript before submission. Research support for JSIII was provided by the Molecular Biomedical Research Initiative (MBRI). This publication was made possible in part by NIH Grant Number 2P20RR016461-10 from the National Center for Research Resources. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH

FIGURE 1. *Kataplana celeretrix* **n. sp.** Anterior end is to the right in Figs A-D, H & I, and to the top in the remaining figures. **A.** Dorsal view of live animal, unsqueezed; ad, adhesive papillae of tailplate; br, brain; cb, copulatory bulb; p, pharynx; st, statocyst; t, testes. **B.** Phase-contrast micrograph of head region; paired Sehkolben (sk) are embedded in the posterior margin of the brain. **C.** CLSM stack of head, dorsal view; head ciliation is shown along with a pair of pericerebral ciliary aggregates (pca). **D.** CLSM stack of head, ventral view; head ciliation is shown along with the anterior part of the creeping sole (cs). **E** Schematic ventral view of animal, showing anterior pair of adhesive papillae (ap); ciliated creeping sole and ventral row of adhesive papillae (vap). **F.** Dorsal-view reconstruction of internal anatomy; b, bursa; ge, germaria; gvd, germo-vitelloducts; sv, seminal vesicle; v, paired vaginal openings; vd, vasa deferentia; vi, vitellaria. **G.** CLSM slice through entire organism, mid-frontal plane. **H.** Partial sagittal CLSM stack of reproductive system, showing seminal vesicle, muscles around bursa, posterior extension of germo-vitelloduct, shell-gland (sg), and glandular material at genital pore (gp). **I.** Reconstruction of reproductive system; note possible cement glands (cg) opening into posterior side of genital pore**. J.** Copulatory bulb; note small lateral glands (lg) in addition to stylets and granular prostatic material. **K.** Half-schematic drawing of stylet arrangement from a heavily-squeezed specimen; central stylets are shaded for clarity: Group C—dark grey; Group D—light grey.

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