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NOTE

Yellow-stained oikopleurid appendicularians are caused by bacterial parasitism

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ABSTRACT: While planktonic tunicates of the genus *Oikopleura* (Appendicularia) are generally quite colorless and transparent, specimens with an opaque, strong yellow colour have been reported occasionally for more than a century. Light and electron microscopy of 2 such yellow individuals; one *O. vanhoeffeni*, and one *O. dioica*, revealed large concentrations of rod-shaped bacteria in all interstitial and blood-filled spaces. In otherwise identical controls these spaces were filled by a completely transparent and acellular fluid. The 2 yellow appendicularians were actively feeding inside their jelly-like houses and expanded new houses in captivity. Thus they behaved like their healthy colorless congeners. The functional and pathological significance of this bacterial parasitism remains obscure.

Appendicularians in general are completely colorless and transparent animals (Lohmann 1933). However, the existence of individual oikopleurids with brilliant yellow, opaque tails and trunks has been reported occasionally. Fol (1872, pp. 457 and 470) described a few such individuals and believed their coloration was due to myriads of globular parasitic organisms, with a diameter of 30 µm, spread throughout the blood lacunae, tissue spaces and genital cavity. Lohmann (1933, p. 22), without giving more details, confirmed the presence of innumerable 'smallest parasites' in the body fluids of such yellow-stained specimens.

Recently I obtained 2 such yellow-stained appendicularians alive in their houses. These specimens were of different species and collected thousands of miles apart. Yet the cause of their unusual coloration seemed to be similar and is reported herein.

Material and methods. One yellow-stained appendicularian, an *Oikopleura vanhoeffeni* of 1.3 mm trunk length (including gonad) and 5 mm total length, was collected in a 500 ml jar by SCUBA diving at Logy Bay near St. John's, Newfoundland, Canada, on 8 June 1989. The water temperature was between 1 and 5 °C. The other yellow-stained appendicularian, an *Oikopleura dioica* of 0.8 mm trunk length and 3.3 mm total length, was scooped out of surface waters at Oksen Island near Bergen, Norway, on 24 July 1989. The water temperature was 12 °C. Hundreds of normal looking appendicularians of the same species were collected in parallel to the mentioned ones. Some of these were used for comparison.

The specimens were examined in their houses under a Wild M420 photomacroscope or a Zeiss dissection microscope equipped with a phototube. Dilute sepia ink was added to the seawater that was filtered through the houses to visualize their architecture (Flood et al. 1990). Later, the houses and appendicularians were fixed in 2 % glutaraldehyde in 0.1M sodium cacodylate. For the *O. vanhoeffeni* 0.6M sucrose, and for the *O. dioica* 60 % seawater, were added to make the fixative isosmotic. Both yellow-stained and normal looking specimens of the 2 species were photographed through a Zeiss Axiophot microscope before being divided into 2 segments for further processing. The trunks and anterior halves of the tail were rinsed in isotonic buffer comparable to that of the primary fixative, postfixed in 1 % osmium tetroxide in 0.1M sodium cacodylate, rinsed in distilled water, dehydrated in 70 to 100 % ethanol and embedding in epoxy plastic for examination by light- and transmission electron microscopy. Semithin plastic sections were stained by 1 % toluidine blue in 0.1M borax solution or by the periodic acid Schiff technique. Ultrathin sections were stained by uranyl acetate and lead citrate and examined in a Philips EM 300 transmission electron microscope. The posterior half of the tails was used for direct light microscopy. Body fluids contained in that segment were pipetted onto object slides and studied unstained by differential interference contrast microscopy.

Results. The yellow appendicularians appeared normal with respect to tailbeating within the house, feeding behaviour, and expansion of new house. The field

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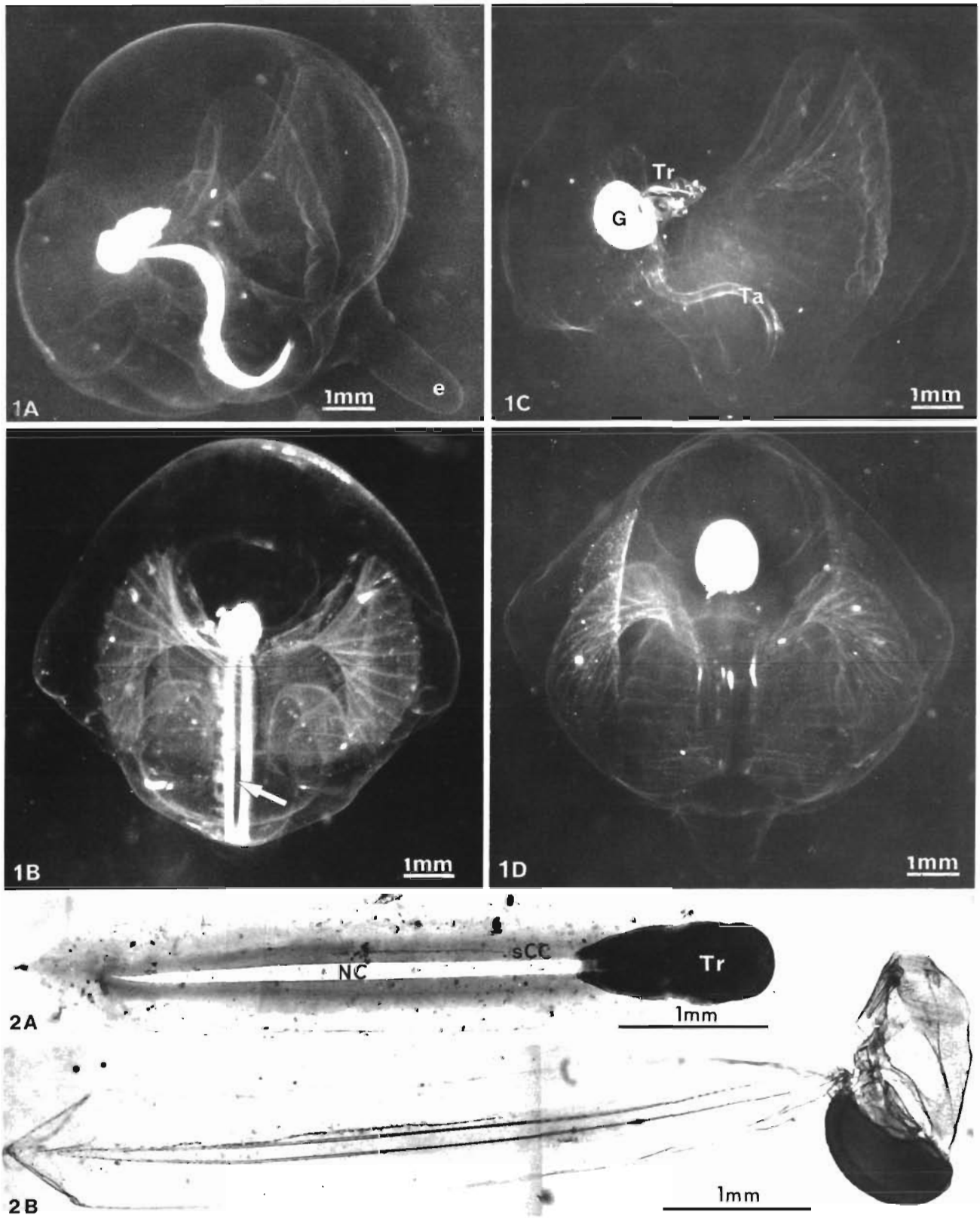
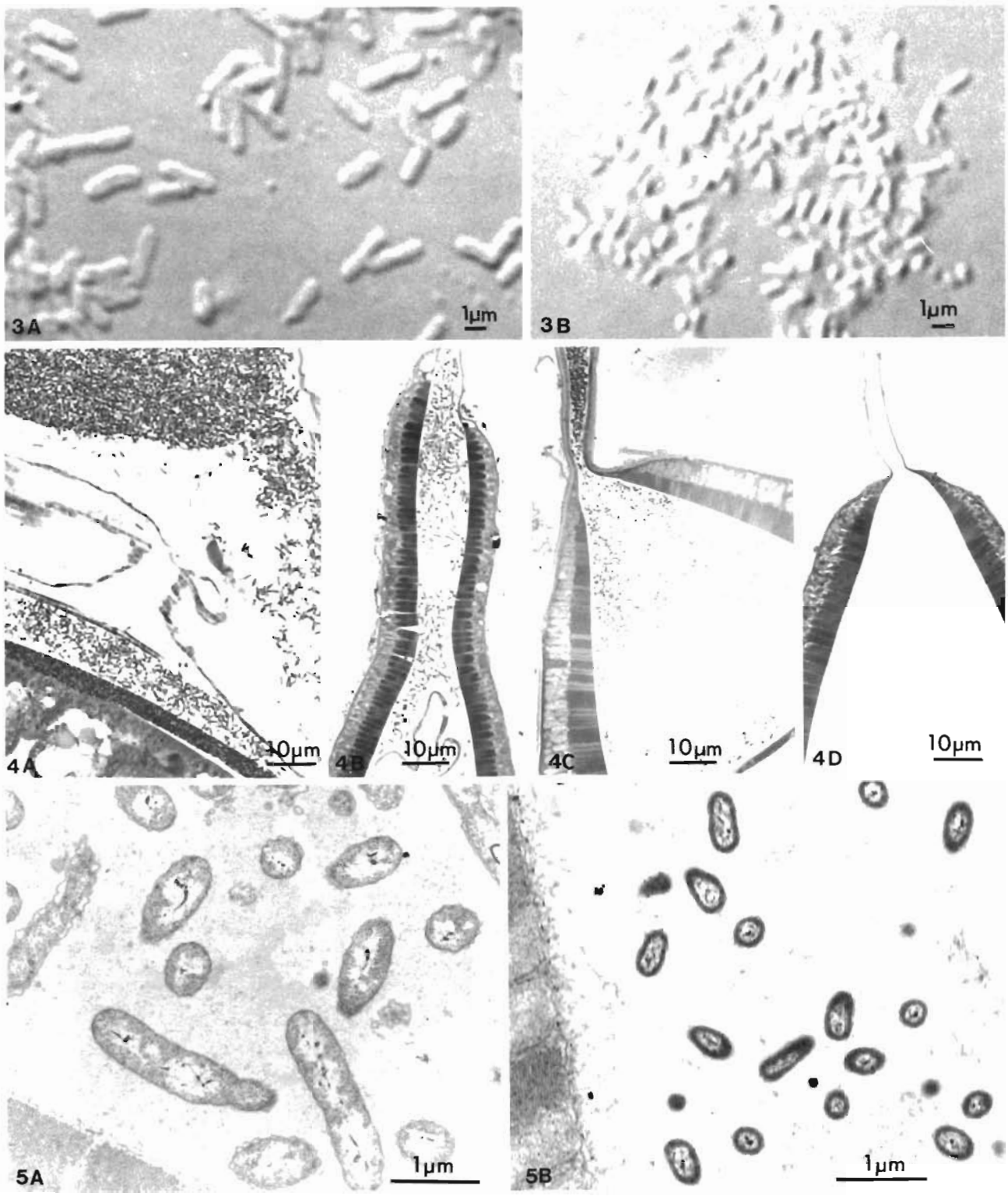


Figs. 1 & 2. *Oikopleura dioica* and *O. vanhoeffeni*. Fig. 1. (A, B) Yellow-stained *O. dioica* in its house as visualized by sepia ink and dark field illumination. (A) The house as seen from the side with the individual actively pumping. Note the extended exit spout (e). (B) As seen from the front with the individual in its resting position. Note the transparent line (arrow) corresponding to the notochord of the tail. Fig. 1. (C, D) Control *O. dioica* in its house, visualized and oriented in the same ways as (A) and (B). This individual was a sexually mature male with a prominent yellow gonad (G). However, the rest of the trunk (Tr) as well as the entire tail (Ta) was quite transparent. Fig. 2. (A) Bright field micrograph of the yellow-stained *O. vanhoeffeni*. Note the diffuse opaqueness of the tail dorsal and ventral to the notochord (NC), the presence of a continous row of subchordal cells (sCC), and the opaqueness of the entire trunk (Tr). (B) Similar to (A) but from a normal *O. vanhoeffeni*. Note the transparency of both trunk and tail



Figs. 3 to 5. *Oikopleura dioica* and *O. vanhoeffeni*. Fig. 3. Direct mounts of opaque body fluid isolated from tail segments of the yellow-stained *O. dioica* (A) and *O. vanhoeffeni* (B) as seen by differential interference contrast. Fig. 4. Toluidine blue stained semithin sections through the trunks and tails of the yellow-stained *O. dioica* (A, B) and *O. vanhoeffeni* (C) and a control *O. dioica* (D). Note the presence of variable amounts of bacteria-like granules between the cellular tissues in (A) to (C). Fig. 5. Transmission electron micrographs of the tails of the yellow-stained *O. dioica* (A) and *O. vanhoeffeni* (B) demonstrating the difference in morphology between the bacteria found in the 2 specimens

houses, as well as houses expanded in captivity, looked normal in architecture as visualized by dilute sepia ink (Fig. 1).

The tails and trunks of the 2 yellow specimens were mostly opaque (Fig. 2A). Only a medial line along the entire tail, corresponding to the notochord, remained transparent (Figs. 1A, B & 2A). The control specimens, on the other hand, looked quite transparent across their entire tails and most of their trunks (Fig. 1C, D & 2B).

All interstitial tissue spaces and blood lacunae within the trunks and tails of the yellow specimens were filled by an opaque liquid packed with rod-shaped bodies, comparable to bacteria in size. The rods isolated from the yellow *Oikopleura dioica* (Fig. 3A) were almost twice as big as those isolated from the yellow *O. vanhoeffeni* (Fig. 3B). The concentration of such bodies varied between distinct parts of the individuals and was generally higher in *O. dioica* than *O. vanhoeffeni* (Fig. 4). In the controls the comparable spaces always lacked bacterium-like bodies and appeared to contain a completely structureless and acellular fluid (Figs. 4E, F).

Electron micrographs revealed the rod-shaped bodies to be typical bacteria. Those present in the yellow *Oikopleura dioica* were ca 0.6 µm wide (Fig. 5A), whereas those present in the yellow *O. vanhoeffeni* never exceeded 0.4 µm in diameter (Fig. 5B). Beyond the difference in size and a higher electron density in the peripheral cytoplasm of the bacteria found in *O. vanhoeffeni*, no other difference was noted between the 2 samples. The bacteria did not invade the cellular tissues of these yellow specimens, and no signs of damage to the cells or tissues were observed.

Discussion. The 2 yellow oikopleurans reported here had massive microbial invasion of their blood- or fluid-filled interstitial spaces in both trunks and tails. This is in agreement with earlier reports of similarly yellow-stained oikopleurans (Fol 1872, Lohmann 1933).

This condition of parasitism seems to be rather rare, but affects several species of *Oikopleura*. Occasional yellow chaetognaths and whitish salps may possibly have a similar parasitic origin (anonymous reviewer's comment). In the present material the yellow appearance occured in less than 1 % of both *O. vanhoeffeni* and *O. dioica* caught by visual identification in the sea. Since yellow-stained specimens are probably easier to discover in the field than their unstained congeners (pers. obs.), an even lower frequency may be more realistic. Very little further information on their incidence seems to exist. Fol (1872) found his lemon-yellow *O. albicans* (at that time called *O. cophocerca*) 'from time to time' during the late spring and early summer in the Strait of Messina. Lohmann (1933), who studied Appendicularia from all over the world for decades, found yellow-stained oikopleurans 'here and there', but mentioned no species names, seasons or localities. A 15 mm long oikopleuran with opaque, yellow colour beyond the notochord, but of undetermined species, was collected in the Atlantic Ocean (7° N, 35° W) at 17 m depth and surface sea temperature of 28 °C in late July 1983 (C. Mills pers. comm.).

In the present study rod-shaped bacteria of distinct sizes were found to be responsible for the yellow-staining of 2 specimens, suggesting that 2 distinct bacterial species may be involved. Fol (1872) described membrane-enclosed globular corpuscles of 30 µm diameter, with internal green granules, as the parasite in his yellow-stained oikopleurans. Fenaux (1963), in addition to describing various dinoflagellate ectoparasites to both *Oikopleuridae* and *Fritillaridae*, also found 25 µm wide globular cells, containing yellow-orange droplets, as endoparasites to *Oikopleura albicans*. The latter parasites, however, were confined to the genital cavity in the trunk, and apparently did not affect the colour of the tail.

Since appendicularians have no vascular endothelium or macrophages, bacteria admitted to their interstitial tissue spaces have easy access to the blood and will spread readily throughout their body. Accordingly, it is difficult to point out the entrance port(s) of the reported bacterial infections. The tail seems to be one likely site. This appendage is covered by a thin single-layered squamous epithelium and moves vigorously within the close-fitting tail chamber of the house (Alldredge 1977, Deibel 1986, Fenaux 1986). On several occasions I have seen specimens with scars along their tail edge, probably caused by abrasion of epithelial cells by particles suspended in the sea.

The significance of the bacterial parasitism for the well-being of the host remains uncertain. The 2 massively infested specimens reported here behaved for hours after their capture in exactly the same way as their uninfected congeners. This may indicate that the microorganisms multiply slowly and that they have a low toxicity to the hosts. On the other hand, the low incidence of yellow-stained individuals, among functionally intract ones, may indicate that infested specimens are rapidly excluded from the population. In this context it should be remembered that the generation time, at least of *Oikopleura dioica*, is in the range of only 2 to 3 wk (Paffenhöfer 1973).

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