RESEARCH NOTE

SUB-TEGUMENTAL VENTRAL FIBRES SURROUNDING THE VAGINAL OPENING OF *ENTOBDELLA SOLEAE* (VAN BENEDEN ET HESSE, 1864) JOHNSTON, 1929 (PLATYHELMINTHES: MONOGENEA: CAPSALIDAE)

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Abstract. The distribution of F-actin in the monogenean *Entobdella soleae* (van Beneden et Hesse, 1864) Johnston, 1929 (Platyhelminthes: Capsalidae) was revealed by staining paraformaldehyde-fixed specimens with FITC-labelled phalloidin. On the ventral surface on the left side of the body, just posterior to the pharynx, a concentrically arranged array of fluorescent fibres was observed, following a circular path around the tiny ventral opening of the vagina. It is assumed that this asymmetrically placed array of actin fibres is contractile and the possible role of these fibres in the assimilation of sperm from an attached spermatophore into the vagina is discussed.

Entobdella soleae (van Beneden et Hesse, 1864) Johnston, 1929 is a capsalid monogenean parasite inhabiting the body surface of a teleost flatfish, the common sole, *Solea solea* (L.) (Pleuronectiformes: Soleidae). To reveal the extent and pattern of musculature of this parasite, specimens were fixed in 4% paraformaldehyde in phosphate-buffered saline for at least 30 min. After rinsing in fresh buffered saline, the specimens were stained with fluorescein (FITC)-labelled phalloidin at a concentration of 5 $\mu g \cdot ml^{-1}$ for about 45 min at 25°C. After further rinsing with buffered saline the specimens were mounted in an appropriate mounting medium and viewed with a Nikon Eclipse 800 microscope equipped with epifluorescence optics. The FITC-labelled phalloidin was observed using a BP 465–495 excitation filter and a Ba 420 emission filter.

Intense fluorescence, indicating high concentrations of F-actin, was observed as expected in the muscular haptor (not shown) and pharynx (Fig. 1). The rest of the body stained less intensely, but sub-tegumental fibres were prominent, especially those running in an antero-posterior direction near the ventral surface (Fig. 1). A striking feature was an asymmetrically arranged pattern of concentric circular fibres, lying immediately beneath the ventral tegument on the left side of the body, postero-lateral to the pharynx (Figs. 1, 2). The fibres were well spaced and occupied an area substantially larger than that of the pharynx. In the centre of this array in some specimens a short and very narrow fluorescent tube was observed (Fig. 1). This corresponded to the small opening of the vagina on the ventral surface and the first short section of the narrow vaginal duct.

Since the circularly arranged fibres stain positively for F-actin, it seems likely that they are contractile. The asymmetrical arrangement of the fibre pattern, with the vaginal opening occupying a central position in relation to the fibre array, suggests that the fibres play a part in vaginal function. The penis of *E. soleae* is far too large to enter the tiny vagina; sperm is exchanged between hermaphrodite individuals by way of jelly-like spermatophores, each of which contains a central mass of sperm (Kearn 1970). In a brief mating interaction a spermatophore is implanted on the left side of the ventral surface of each parasite, i.e. with the centre of the spermatophore located near the vaginal pore. Soon after the individuals separate the antero-lateral region of the body of each parasite contracts and thereby adopts a cone shape, with the wide base of the cone directed ventrally (see Kearn 1970, text-fig. 4). The sperm mass in the centre of the spermatophore is located at the apex of the cone, adjacent to the vaginal pore. It seems highly likely that this vigorous distortion of the body is brought about by contraction of the circular array of actin fibres and it is assumed that the sperm lying in the centre of the spermatophore is forced into the vagina while these body contractions are taking place.

The narrow vaginal pore leads to a short section of vaginal duct, the wall of which fluoresces, indicating the presence of F-actin. If this distal section of vaginal duct is contractile, as seems likely, it may serve to close the vagina after sperm assimilation is completed, preventing loss of newly acquired sperm.

In *Neoentobdella diadema* and *N. apiocolpos* each spermatophore is enclosed in a thin outer case (Kearn et al. 2006) and assimilation of sperm from these presumably more rigid spermatophores may take place in a different way. It is not yet known whether sub-tegumental actin fibres are associated with the vaginal openings of these parasites.

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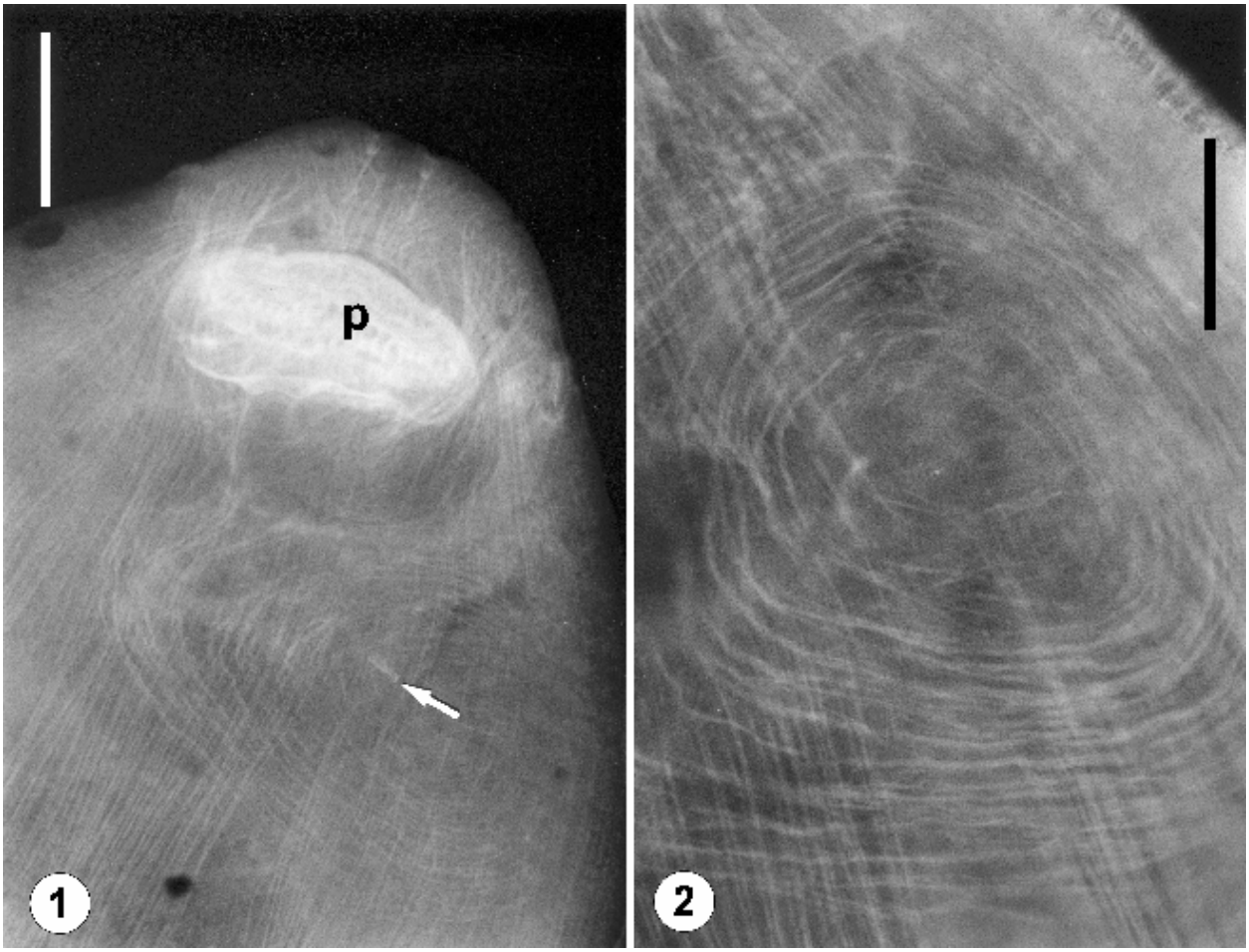

Fig. 1. Fluorescence microscope photograph of an adult specimen of *Entobdella soleae* stained with FITC-labelled phalloidin in ventral view. Note fibres arranged in a circular pattern postero-lateral to the pharynx (p) and surrounding the vaginal opening (arrow). **Fig. 2.** Fluorescence microscope photograph of circular array of fibres on the ventral surface of another adult specimen of *E. soleae*. Scale bars: Fig. 1 = approx. 300 μm; Fig. 2 = approx. 150 μm.