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# Can spawning marks on females of the spined loach (*Cobitis* sp., Teleostei) be caused by scratches from males during mating?

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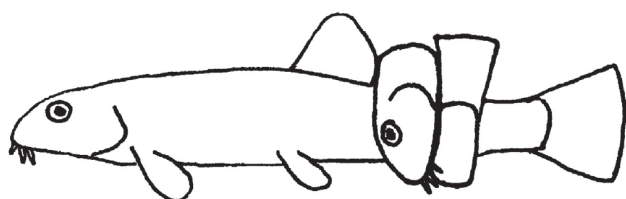
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**Abstract.** The skin histology from the Danubian spined loach *Cobitis elongatoides* disputes the notion that the spawning marks (lighter spots on the body sides of the females) are the result of physical damage to the epidermis by a male during spawning. Physiological changes that induce a specific local decrease in lateral pigmentation in some individuals appear to be a more likely source.

**Key words:** *Cobitis elongatoides*, epidermis, spawning

## Introduction

Despite the recent emergence of many new publications concerning members of the genus *Cobitis*, there are still some areas where our knowledge is rather limited. The reproduction biology of spined loaches and the related physiological and morphological changes during spawning is one of these areas. All of the species of the spined loach investigated up until this time share the same specialised mating behaviour: the male follows the female into vegetation and forms a complete ring around the female (Lodi & Malacarne 1990, Bohlen 2000) (Fig. 1).



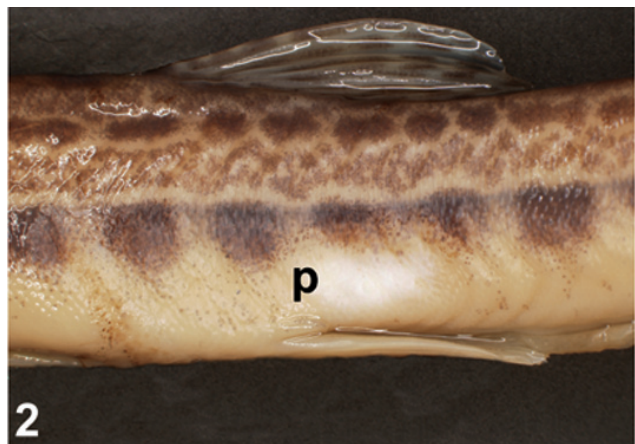
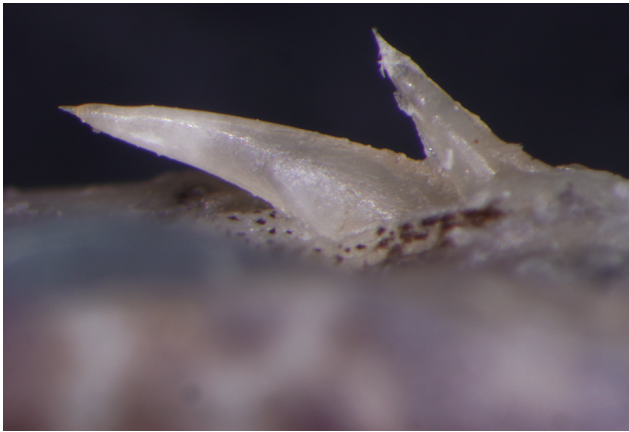
**Fig. 1.** Spined loaches mate with the male forming a complete circle around the female. The male is located behind the base of dorsal fin of the female, its head or pectoral fin base are close to the spot where the spawning marks can be seen after spawning of the female's sides (Bohlen 2008).

In the Northern spined loach *C. taenia* and with a lower intensity in *C. elongatoides* and the Siberian spined loach *C. melanoleuca*, the mating events leave spawning marks on the body of the females, and this can be used to estimate the beginning of the spawning period (Bohlen 2008). These whitish areas are located under the caudal part of the dorsal fin at the same level as the edge of the large dark blotches of the 4<sup>th</sup> Gambetta zone. They give an impression of possible scratched areas, but no cases of infection in these areas have occurred. Bohlen (2008) sees the most probable cause of the appearance of these “scratched areas” is the skin coming into contact with the hard surface structures of the male: the suborbital spine and/or the *lamina circularis* (Fig. 2). The aim of this study is to confirm or reject the hypothesis that the appearance of these spawning marks in *C. elongatoides* females is a result of grazing by males while attached to the female.

## Material and Methods

Female specimens of *C. elongatoides* were collected in Nová Říše Reservoir (49°10' N, 15°32' E, River Řečice, Morava basin) in May (spawning period) and in July (post-spawning period). At both times, individuals with spawning marks of variable intensity on both body sides were observed (Fig. 3).

For each period, five females with distinct spawning

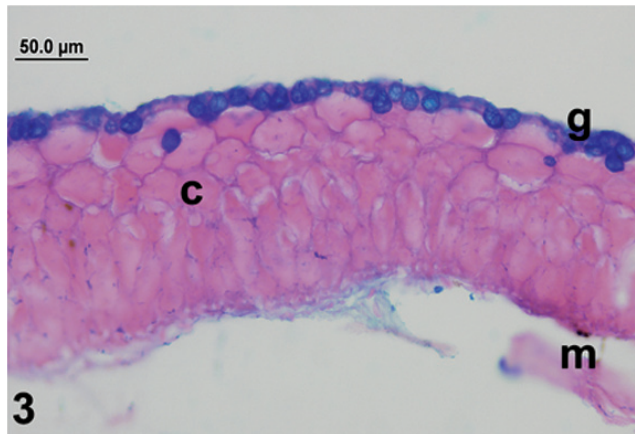


**Fig. 2.** The source of the supposed damage to the epidermis – the suborbital spine (upper part) and the lamina circularis on the basis of male's pelvic fin (lower part).

marks and five females lacking spawning marks were chosen for histological analysis. The fish were killed by an overdose of anaesthetic (2-phenoxyethanol) and preserved in 4% formaldehyde. Skin samples of approximately 5 x 5 mm were taken from two spots: at the side of the body at the level of the end of the 4<sup>th</sup> Gambetta zone between the pectoral and pelvic fins, and from the side of the body at the same level under the base of the dorsal fin (the place where spawning marks occur). After being embedded in paraffin wax, 7µm-thick sections were prepared, perpendicular to the surface of the skin. The sections were stained by the trichromatic method according to Mallory and with Alcian blue at a pH of 2.5-eosin.

## Results

The outer layer of the integument (the epidermis) was on average approximately 100 µm thick. Near the surface there was a row of goblet, strongly alcianophilic, secretory cells, with the middle part dominated by secretory club cells. Dermis contains



**Fig. 3.** The side of the body (2) and a cross-section of the female's epidermis *Cobitis elongatoides* without (1), and with pronounced spawning marks (3); g – goblet cell, c – club cells, s – scale, M – melanophores, m – melanophore with aggregated pigment granules, p – spawning mark; Alcian blue at pH 2.5-eosin.

scales, whose posterior edges partially permeate into the epidermis, causing an undulation of the line between the epidermis and dermis. Pigment cells (melanophores) concentrate mainly in the proximity of this line, and do not occur in the epidermis.

The structure of the epidermis in the spawning marks was not significantly different from the epidermis from other areas of the same individual, nor was it different from the epidermis samples from individuals without spawning marks. The tissue was compact, without signs of injury. Possible differences could be seen in the form of dermis pigmentation of a lower intensity in skin samples taken from the spawning marks, where the pigment is aggregated towards the centre of the melanophores.

## Discussion

In most Cypriniformes, apart from common epithelial cells, there are two types of secretory cells which form the basic elements of the epidermis: the goblet cells, which participate significantly in the producing a layer of a mucus on the body surface, and the club cells, which contain alarm substances that are released when the fish is wounded, thus informing the surrounding area of possible danger. Behavioural experiments on many species (including *Cobitis taenia*; Kasumyan & Ponomarsy 1986) have demonstrated that these substances work not only conspecific, but also interspecific. The melanophores, which are normally situated in the dermis under the epidermis, can occur in a small number of fish species in the epidermis as well. The melanophores have the ability to translocate the pigment inside their centres, resulting in an apparent change of colour. It was demonstrated that the process can be under both hormonal and/or neuronal control (Whitear 1986, Knoz & Halačka 1991).

In Cyprinidae, the secretory cells are usually dispersed in the entire middle layer (goblet and club cells), and eventually the superficial (goblet cells) layer of the epidermis. Characteristically, the structure of the epidermis in Cobitidae is slightly different. Goblet

cells form a continuous line in close proximity to the surface of the epidermis. Below these lies a compact layer of club cells, occupying up to 80% of the total epidermis volume. None of the loaches studied by us (*C. taenia*, *C. elongatoides*, *C. tanaitica*, *Misgurnus fossilis*, *Sabanejewia balcanica*, *Barbatula barbatula*) carries melanophores in the epidermis (black caudal spots at *Cobitis* and *Sabanejewia* sp. are of dermal origin too; data not shown).

The assumption that the spawning marks in loaches appear as a result of skin grazing from contact with males (Bohlen 2008) can be disputed based on following reasons:

- If the spawning marks are caused by abrasion (from males), the consequences would, apart from possible subsequent infection, also include the release of alarm substances from club cells, which might disrupt spawning or even increase the attraction of predators.
- The pigment cells (melanophores) in the integument of *Cobitis* are located only in the dermis, therefore any damage that does not destroy the epidermis can not cause a discolouration of the dermis.

Based on the aforementioned points, we can suppose that, at least in *C. elongatoides*, the appearance of the spawning marks is caused by concurrence of physiological (hormonal) changes in the female organism during spawning, and by the mechanical (but not destructive, i. e. grazing) stimulation of the epidermis by the male during this period.

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