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# *Gyrodactylus longipes* n. sp. (Monogenea: Gyrodactylidae) from farmed gilthead seabream (*Sparus aurata* L.) from the Mediterranean

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ABSTRACT

*Gyrodactylus longipes* n. sp. (Monogenea, Gyrodactylidae) is described from the gills of farmed juvenile gilthead seabream (*Sparus aurata* L.) from two sites located in Italy and Bosnia–Herzegovina and represents the second species of *Gyrodactylus* to be described from *S. aurata*. *Gyrodactylus orecchiae* Paladini, Cable, Fioravanti, Faria, Di Cave *et* Shinn, 2009 was the first gyrodactylid to be described from *S. aurata*, from populations cultured in Albania and Croatia. In the current study, *G. longipes* was found in a mixed infection with *G. orecchiae* on fish maintained in Latina Province, Italy, thus extending the reported distribution of the latter throughout the Mediterranean. The morphology of the opisthaptoral hard parts of *G. longipes* is compared to those of *G. orecchiae*, using light and scanning electron microscopy. *Gyrodactylus longipes* is characterised by having larger, elongated ventral bar processes and long, triangular-shaped toe region to their marginal hook sickles which, by comparison, are rhomboid in *G. orecchiae*. The marginal hook sickles of *G. longipes* are almost double the size of *G. orecchiae* which allows for their rapid discrimination from each other in mixed infections. A comparison of the DNA sequence of the ribosomal internal transcribed spacer 1 and 2 regions (ITS1 and ITS2) of *G. longipes* with the corresponding sequence from *G. orecchiae* and with those available in GenBank, supports the separate species status of *G. longipes*. Part of this study necessitated an overview of the existing *Gyrodactylus* fauna from Italy and Bosnia–Herzegovina; a summary from each country is provided here to assist future investigations.



## 1. Introduction

Gilthead seabream (*Sparus aurata* L.) (Sparidae) is ranked among the most important fish species farmed in the Mediterranean with annual production now exceeding 125,000 tonnes. The largest producer in the Mediterranean is Greece (49%), followed by Turkey (15%), Spain (14%) and Italy (6%) [1].

As the production of *S. aurata* throughout the Mediterranean has increased, commercial enterprises have placed a greater emphasis on health management. If infections by Monogenea only are considered, then the most commonly encountered species infecting the gills of *S. aurata* are: the microcotylid *Sparicotyle chrysophrii* (van Beneden *et* Hesse, 1863) Mamaev, 1984, which can cause anaemia and high mortality at low intensities (8–10 parasites/gill arch) in fish weighing 10–300 g; and, the diplectanid *Furnestinia echeneis* (Wagener, 1857) Euzet *et* Audouin, 1959, which although common, does not represent a significant threat. Most recently, *Gyrodactylus orecchiae* Paladini, Cable, Fioravanti, Faria, Di Cave *et* Shinn, 2009, the first species of *Gyrodactylus* to be described from *S. aurata*, was found to be responsible for a ~10% mortality in juvenile stock [2]. This latter material obtained from Albania and Croatia was infected with a single species of *Gyrodactylus*, parasitising the skin, fins, eyes and gills in high numbers (1000+ parasites/fish) [2]. Additional *S. aurata* samples subsequently received from a farm site located on the Tyrrhenian coast of Italy and from a second farm site from Bosnia–Herzegovina, in addition to harbouring *G. orecchiae*, were found to have a second species of *Gyrodactylus*. This study provides a morphological description of the new species using light and scanning electron microscopy (SEM), which is supplemented with a reference DNA sequence of the internal transcribed spacer region.

## 2. Materials and methods

### 2.1. Specimens collection

Two samples of *S. aurata*, submitted as part of each farm's routine health assessment of stock, were processed by the fish health diagnostics team in the Department of Veterinary Medical Sciences, Univesity of Bologna, Italy. The first sample came from a marine cage site in Latina Province, located on the Tyrrhenian coast of Italy (41°13′

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36.45″N; 13°34′23.62″E), and the second sample from a marine hatchery located at Neum, Bosnia–Herzegovina (42°55′00.07″N; 17°36'59.91"E). A small sub-sample of both collections was fixed in 70% ethanol to preserve any ectoparasites present. The Italian sample, collected in January 2007, consisted of three fish weighing ~25 g, whilst the second sample of five fish weighing ~50 g was collected in May 2007 from Bosnia–Herzegovina.

### 2.2. Specimens preparation for morphological analysis

Individual gyrodactylids, principally infecting the gills, were removed from the fixed hosts and then rinsed in distilled water. Specimens were prepared either as whole mounts in ammonium picrate glycerine according to the method detailed by Malmberg [3] or had their opisthaptors excised and then subjected to proteolytic digestion. The alcohol-fixed body corresponding to each digested opisthaptor was subsequently transferred to 95% ethanol for molecular characterisation. For digestion, individual opisthaptors were placed on a glass slide and the tissues enclosing the attachment hooks were removed using 3 μl of digestion solution [4]. The digestion of each specimen was continuously monitored under a ×4 objective on an Olympus SZ30 dissecting microscope. Tissue digestion was then arrested by the addition of 2 μl of a 1:1 formaldehyde : glycerine mix. A glass coverslip (18×18 mm, "0" thickness, VWR International, Lutterworth, UK) was then placed over the hook preparation and the edges sealed with nail varnish. For specimens prepared for scanning electron microscopy (SEM), individual opisthaptors were digested on 13 mm diameter glass coverslips (Chance Propper Ltd., Warley, UK), rinsed several times with distilled water, air-dried, sputter-coated with gold and then examined using a JEOL JSM5200 scanning electron microscope operating at an accelerating voltage of 10 kV.

For the morphological study, the opisthaptoral hard parts were studied from images captured using a Zeiss AxioCam MRc digital camera mounted on top of an Olympus BH2 compound microscope using a ×0.75 interfacing lens. Images of the opisthaptoral hard parts were captured using ×40 and ×100 oil immersion objectives and MRGrab 1.0.0.4 (Carl Zeiss Vision GmbH, 2001) software. A total of 27 point-to-point morphometric measurements were made on the opisthaptoral hooks of each specimen from images captured using a JVC KY-F30B 3CCD (JVC, Yokohama, Japan) video camera mounted on

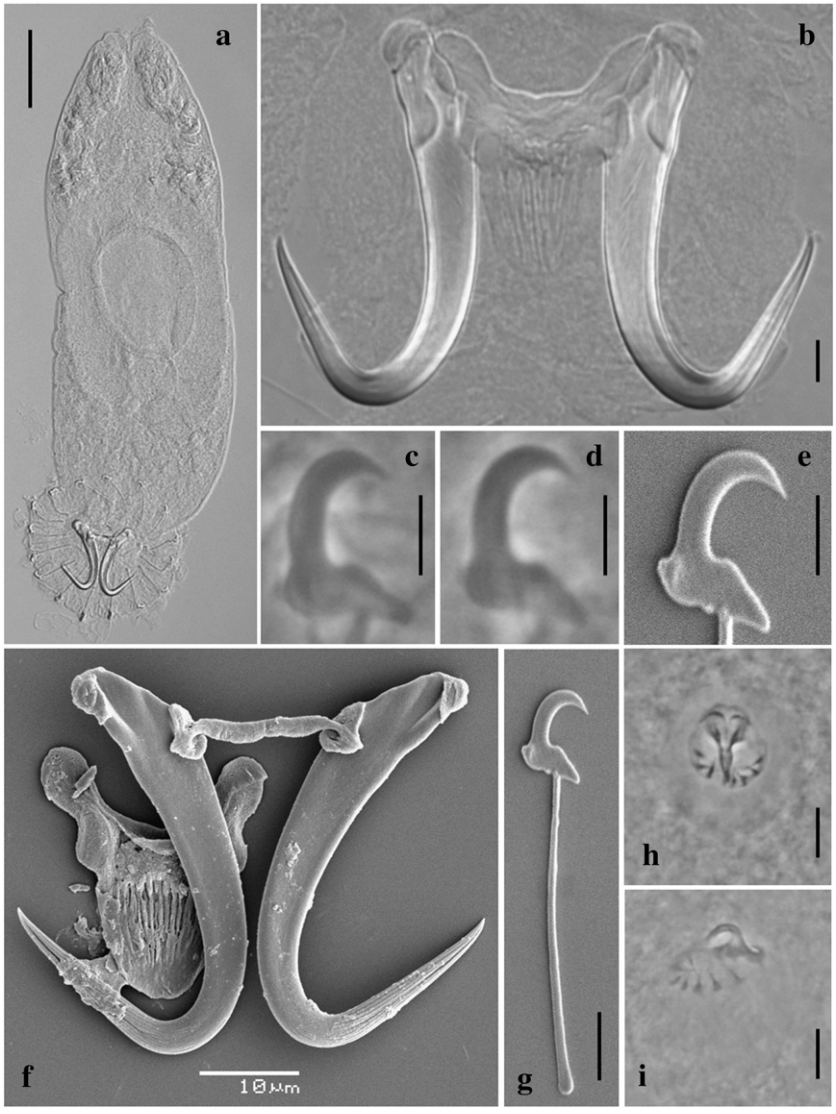


**Fig. 1.** *Gyrodactylus longipes* n. sp. from gilthead seabream (*Sparus aurata* L.) from Latina Province, located on the Tyrrhenian coast of Italy (type-locality). a – holotype, whole parasite in ventral view; b – light micrographs of the opisthaptoral central hook complex showing the hamuli, the dorsal and the ventral bar (ventral view); c – light micrograph of a marginal hook sickle; d – light micrograph of a marginal hook sickle from *G. longipes* from Neum, Bosnia-Herzegovina; e – scanning electron micrograph of the marginal hook sickle. f – scanning electron micrograph of the opisthaptoral central hook complex (dorsal view); g – scanning electron micrograph of a marginal hook; h – male copulatory organ (MCO); i – MCO of *G. longipes* from Neum, Bosnia-Herzegovina. Scale bars: a = 50 μm; b, g-i = 5 μm; c-e = 3 μm.